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ORIGINAL REPORT

Randomized Trial of the Combination of Lomeguatrib and Temozolomide Compared With Temozolomide Alone in Chemotherapy Naive Patients With Metastatic Cutaneous Melanoma

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ABSTRACT

Purpose
To evaluate tumor response, pharmacodynamic effects, and safety of a combination of lomeguatrib (LM), an O^6-methylguanine DNA-methyltransferase (MGMT) inactivator, and temozolomide (TMZ), TMZ alone, and LM/TMZ after disease progression on TMZ alone in patients with advanced melanoma.

Patients and Methods
Patients with unresectable stage III or IV cutaneous melanoma who had no prior systemic chemotherapy were randomly assigned to receive either 40 to 80 mg LM and 125 mg/m^2 TMZ or 200 mg/m^2 TMZ on days 1 through 5 of each 28-day treatment cycle. Drugs were administered orally for up to six cycles of treatment. Patients on TMZ alone were offered LM/TMZ at progression, if fit enough to receive treatment.

Results
One hundred four patients were enrolled, with 52 in each trial arm. Twenty-seven TMZ-treated patients received LM/TMZ after progression on TMZ. Unexpectedly, analysis of tumor biopsies showed rapid recovery of MGMT after LM/TMZ with 40 mg/d LM. Therefore, doses of LM were escalated to 60 then 80 mg/d. Tumor response rates were 13.5% with LM/TMZ and 17.3% with TMZ alone. No patient responded to LM/TMZ having progressed through TMZ. Median time to disease progression was 65.5 days for LM/TMZ and 68 days for TMZ. All treatments were well tolerated, although hematologic and gastrointestinal adverse events were common. A higher incidence of hematological adverse events was observed in the LM/TMZ combination arm.

Conclusion
The efficacy of LM and TMZ in the current dosing schedule is similar to that of TMZ alone. To maintain MGMT depletion in tumor dosing of LM needs to be continued beyond that of TMZ.

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INTRODUCTION

Temozolomide (TMZ) is an oral methylating agent used in the treatment of primary CNS tumors and melanoma. Well-tolerated, it has been shown to be at least as active as dacarbazine (DTIC), which remains the standard systemic treatment for advanced melanoma.[1,2] The cytotoxicity of TMZ and DTIC is mediated principally through methylation of DNA at the O^6 position of guanine.[3-5] Repair of the lesion by O^6-methylguanine-DNA methyltransferase (MGMT) and/or tolerance based on deficiencies in mismatch repair or resistance to apoptosis have been identified as important aspects of resistance to DTIC and temozolomide.[6-8] Base excision repair also may bear on chemosensitivity, as inhibition of polyadenosine diphosphate ribose polymerase has been shown to enhance cell killing by temozolomide.[9]

Tumor cells frequently express high levels of MGMT, and preclinical studies identify the protein as the key determinant of cell survival.[5,6] Inactivation of MGMT before dosing with an O^6-alkylating agent considerably enhances the antitumor activity of the latter drug in vitro and in animal tumor models.[6,10] Lomeguatrib (LM)—6-[4-bromo-2-thienyl]methoxy)purin-2-amine—is a nontoxic low molecular weight pseudosubstrate that inactivates

MGMT. Given in combination with temozolomide, LM has been shown to sensitize human melanoma xenografts more than can be achieved by escalation of TMZ dose alone.[11]

A phase I dose escalation study conducted using the LM/TMZ drug combination showed that intravenous (IV) administration of 10 mg/m^2 LM decreased levels of MGMT in peripheral blood mononuclear cells (PBMCs) and tumors by more than 97% and 92%, respectively, 4 hours after dosing. Oral administration of the same dose was less depleting, but 40 or 80 mg orally showed 100% MGMT depletion in PBMCs in all patients tested.[12] Based on these data and tolerability, we identified 40 mg/d LM with 125 mg/m^2 TMZ given on days 1 through 5 of each 28-day cycle as the regimen for use in phase II trials.

The present study aimed to evaluate the combination of LM and TMZ to test whether LM attenuation of MGMT activity might increase the efficacy of TMZ in patients with advanced melanoma. Besides determining tumor response rate to LM/TMZ, we aimed to test whether the combination could produce tumor shrinkage in patients progressing on TMZ alone. Secondary objectives included assessments of drug pharmacokinetics, MGMT depletion, safety, time to progression, and survival.

PATIENTS AND METHODS

Inclusion and Exclusion Criteria

Patients with histologically proven cutaneous melanoma or unknown primary melanoma with metastases were eligible for the study, provided they had not received systemic chemotherapy previously. Other requirements included stage III or IV measurable disease, age older than 18 years, Eastern Cooperative Oncology Group performance status 0 or 1, life expectancy of more than 12 weeks, adequate bone marrow and biochemical function (hemoglobin $>$ 10 g/dL, WBCs $> 3 \times 10^9$/L, absolute neutrophil count $> 1.5 \times 10^9$/L, and platelets $>$ 100,000/μL), creatinine $\leq$ 1.25 upper limit of normal (ULN), bilirubin $\leq$ 1.25 ULN, and AST $\leq$ 5 (metastases to liver) or 2 $\times$ ULN.

Patients were excluded if within 4 weeks of radiotherapy or immunotherapy; pregnant or nursing; still recovering from surgery; suffering from significant comorbidity; and had a known CNS metastases, a history of seizures, or were on antiepileptic medication.

The study was conducted in accordance with the principles of the International Conference on Harmonisation of Good Clinical Practice guidelines and the Declaration of Helsinki. The trial was approved by an independent ethics committee according to national and local requirements at each trial center. All patients gave informed, written consent.

Study Design and Statistical Considerations

The trial was a multicenter, open study in which 80 patients were to be randomly assigned (1:1) into one of two treatment arms: LM/TMZ or TMZ alone. Patients experiencing disease progression in the TMZ alone arm were permitted to continue study treatment by changing to the LM/TMZ combination. The sample size was selected on the basis that 40 patients would permit a satisfactory estimate of response rate for each trial arm. In addition, we anticipated that approximately half of progressors on TMZ alone would go on to receive combination therapy, allowing assessment of LM's ability to reverse resistance to TMZ.

The aim of the study was not to compare performance between the trial arms, and descriptive statistics were generated for efficacy, toxicity, pharmacokinetic, and pharmacodynamic end points. Median time to progression and median survival time were estimated using Kaplan-Meier survival curves.

In the course of the trial, it became apparent that MGMT persisted in tumor biopsy samples taken 24 to 72 hours after the end of cycle 1 LM/TMZ. Therefore, the trial was extended by 20 patients, with the LM dose in those assigned combination treatment being increased to 60 mg/d, then to 80 mg/d.

Treatment

LM enteric–coated 10-mg capsules were obtained from Kudos Pharmaceuticals (Kenilworth, NJ), and TMZ purchased from Schering-Plough (Welwyn Garden City, United Kingdom), as 5-, 20-, 100-, and 250-mg capsules.

Patients randomly assigned to LM/TMZ received LM 40 mg/d orally for 5 consecutive days every 4 weeks for up to six cycles. Those among the last 20 patients randomly assigned to LM/TMZ received LM 60 mg/d or 80 mg/d. TMZ was administered at 125 mg/m^2/d orally 2 hours after LM. Patients fasted for 1 and 2 hours before and after TMZ and LM, respectively. On the TMZ alone arm, patients received a starting dose of 200 mg/m^2/d, administered days 1 through 5 of a 28-day cycle. Patients receiving LM/TMZ after progressing on TMZ alone were treated as above, although the TMZ dose was adjusted to take into account any prior dose reductions on TMZ.

A treatment delay of up to 2 weeks was allowed for resolution of drug-related toxicity. Dose reductions in TMZ were mandated in the event of grade 4 hematologic toxicity, grade 3 toxicity lasting 7 or more days, or any grade 3 or 4 nonhematologic toxicity. These were in increments of 25 or 50 mg/m^2/d according to the treatment arm and type of toxicity encountered. A need to reduce doses of TMZ to less than 75 mg/m^2/d (LM/TMZ) or 100 mg/m^2/d (TMZ alone) required the patient to be removed from the study. Patients also could be withdrawn from the study for progressive disease, serious violation of the study drug protocol, or withdrawal of consent.

Evaluation of Response and Toxicity

All eligible patients who received any part of the treatment were considered assessable for response and toxicity. Patients were assessed for adverse events at each attendance. Physical examination, performance status, and vital signs were recorded at the beginning of each treatment cycle. CBC was checked before treatment and on days 14, 21, and 28, with blood chemistry tested on days 1, 14, and 28. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2. Tumor response was assessed every second cycle based on clinical and radiological findings in accordance with Response Evaluation Criteria in Solid Tumors Group.

Pharmacodynamics

In a subset of patients samples for PBMC MGMT activity and/or DNA methylation were obtained before treatment on day 1 of cycle 1 at 6 and 24 hours after dosing and once during days 5 to 8 (ie, after the end of dosing). From 5 to 10 mL of venous blood was collected for isolation of PBMC and analysis of MGMT.[13] Where available, tumor biopsies were obtained by excision biopsy before, and up to 72 hours after completion of, the first cycle of treatment. The biopsies were snap frozen on dry ice and stored at -80°C before determination of MGMT activity.[13] Additional pharmacodynamic samples were drawn from patients being evaluated for pharmacokinetics at 2, 4, and 8 hours after LM administration on day 1 of cycle 1.

Pharmacokinetics

This was to be undertaken in up to eight patients receiving the LM/TMZ combination only. Two 5 mL venous blood samples were drawn predose and at 0.5, 1, 2, 3, 4, 6, and 8 hours after dosing on days 1 and 5 of cycle 1 to determine the LM and TMZ concentrations according to previously published methods.[12,14]

RESULTS

A total of 104 patients were recruited into the study, split equally into two treatment arms (Table 1). Of the 52 patients initially treated with TMZ alone, 27 transferred to LM/TMZ at progression. All of the patients were assessable for safety, and all but four (three on TMZ alone and one on LM/TMZ) for efficacy.

Four patients received drug doses significantly different from those required by the study protocol. In all cases (one on LM/TMZ, two on temozolomide, and one on LM/TMZ after progressing through TMZ), doses of medication were omitted in error during one cycle of treatment. Additionally, one patient receiving TMZ was

Table 1. Patient Characteristics at Random Assignment

Characteristic	LM/TMZ (n = 52)	TMZ (n = 52)
Age, years		
Median	56	57
Range	32 to 82	21 to 82
Sex		
Male	33	36
Female	19	16
Disease status		
Stage 3	2	1
M1a	4	3
M1b	16	15
M1c	30	33
ECOG PS		
0	32	27
1	19	24
Missing	1	1
Prior therapy		
Immunologic/biologic	15	14
Radiation	22	15

Abbreviations: LM, lomeguatrib; TMZ, temozolomide; ECOG PS, Eastern Cooperative Oncology Group performance status.

re-treated on day 22, rather than day 29, of cycle 1. Sixteen patients completed six cycles of treatment with LM/TMZ and nine with TMZ alone. Most patients were withdrawn from treatment because of disease progression.

Treatment Efficacy

Responses to treatment are summarized in Table 2. Two patients treated with TMZ alone and one with LM/TMZ achieved complete responses. Rates of overall response and disease stabilization were similar for the two treatments. Stable disease was observed in 14 patients on the combination arm and in 13 patients on the TMZ alone arm. No patient responded to LM/TMZ having progressed through TMZ: four patients had stable disease after two cycles, but all four progressed before cycle 4.

Median time to disease progression was similar for the two arms at 65.5 days for LM/TMZ (95% CI, 55 to 116 days) and 68 days for TMZ (95% CI, 53 to 106 days). Median overall survival was 7.6 months (95% CI, 6.9 to 10.3 months) and 7.7 months (95% CI, 6.3 to 10.7 months), respectively.

Adverse Events

Four patients died while undergoing treatment: one on the LM/TMZ combination and three on TMZ alone. The cause of death was attributed to melanoma in two cases, pulmonary embolism in one patient on TMZ, and a cerebrovascular event in an additional patient treated with TMZ. No deaths were related to treatment.

All of the patients on the study experienced adverse events. The pattern of these was similar for the two treatments, though an increased incidence of thrombocytopenia and neutropenia was seen in patients receiving LM/TMZ, and more vomiting was recorded in those on TMZ alone. The most frequent treatment-related adverse events are listed in Table 3. Patients on the LM/TMZ combination had an increased incidence of grade 3 and 4 adverse events compared with those on TMZ alone, but they had a lower rate of withdrawal due to toxicity, indicating that these events were manageable. Hematologic toxicity was more frequent when LM was administered at 60 or 80 mg/d or when LM/TMZ was administered after progression on TMZ (Table 4).

Forty-eight patients had treatment delays: 25 patients on LM/TMZ had a total of 45 dose delays, compared with 19 patients on TMZ who experienced 25 delays. Four more patients had delays on LM/TMZ after progressing on TMZ. Twenty-four of the treatment delays on LM/TMZ were caused by grade 3 or 4 hematologic toxicity, as were the majority of the delays in patients on the combination having progressed through TMZ. Ten out of 25 delays on TMZ were caused by hematologic toxicity. Most other delays were for logistic reasons, such as awaiting scan results. Twenty patients on LM/TMZ required dose reductions in TMZ, compared with seven on TMZ alone. In all but two cases, reductions were made for hematologic toxicity.

Pharmacodynamics

MGMT activity in PBMCs fell more rapidly and completely with combination therapy than with TMZ alone. At 4 and 22 hours after TMZ dosing patients on LM/TMZ had no detectable MGMT activity, whereas all but one TMZ-treated patient had residual activity (Table 5). Tumor biopsies were obtained on days 6 through 8 of cycle 1 in nine patients on LM/TMZ at 40 mg/d LM and in five on TMZ alone. Unexpectedly, given the more than 92% depletion of MGMT observed after a single LM dose in the phase I trial, residual MGMT activity was detected in five of the nine LM/TMZ patients' samples (0.7, 0.8, 1.5, 1.6, and 4.5 fmol/μg DNA). Two of the five samples taken after TMZ treatment had detectable MGMT (2.3 and 2.8 fmol/μg DNA). Four patients who received LM dose at 60 or 80 mg/d underwent tumor biopsies on day 6, and residual MGMT activity was detectable in three of these cases. In four further patients, tumor biopsies were obtained on day 5, a few hours after the final dose of TMZ, and no residual MGMT activity could be detected.

Pharmacokinetics

Samples were obtained from seven patients, but in two cases, the profiles were incomplete. Both drugs had similar pharmacokinetic parameters on days 1 and 5 of administration (Table 6).

Table 2. Best Response by Treatment Arm

Response	LM/TMZ (n = 52)	TMZ (n = 52)	LM/TMZ on Progression (n = 27)
Complete response	1	2	0
Partial response	6	7	0
Stable disease	14	13	4
Progressive disease	30	28	22
Not evaluated	1	2	1
Response rate, %	13.5	17.3	0

Abbreviations: LM, lomeguatrib; TMZ, temozolomide.

DISCUSSION

The results of this study do not support the hypothesis that clinical inactivation of MGMT enhances the activity of TMZ in melanoma. We observed similar antitumor activity in the two treatment arms and no reversal of resistance to TMZ with the addition of LM. The response rate of 13.5% with combination therapy is disappointing, as it represents no improvement on that seen in the phase II

Table 3. Most Common Treatment-Related Toxicities

Event	LM/TMZ (n = 52)		TMZ (n = 52)		LM/TMZ on Progression (n = 27)	
	No.	%	No.	%	No.	%
All adverse events						
Thrombocytopenia	24	46	14	27	18	67
Neutropenia	27	52	5	10	17	63
Febrile neutropenia	5	10	2	4	4	15
Anemia	8	15	5	10	5	19
Nausea	37	71	36	69	11	41
Vomiting	11	21	17	33	4	15
Constipation	8	15	10	19	6	22
Diarrhea	3	6	5	10	1	4
Dyspepsia	4	8	2	4	0	0
Fatigue	18	35	22	42	8	30
Pyrexia	3	6	4	8	0	0
Anorexia	11	21	15	29	5	19
Headache	5	10	9	17	5	19
Alopecia	3	6	0	0	1	4
Serious adverse events						
Thrombocytopenia	5	10	2	4	5	19
Neutropenia	13	25	1	2	12	44
Febrile neutropenia	4	8	2	4	4	15
Anemia	2	4	5	10	0	0
Nausea	3	6	1	2	1	4
Vomiting	3	6	2	4	0	0

NOTE. Adverse events considered possibly, probably, or highly probably related to treatment occurring in 5% or more patients in any study arm.
Abbreviations: LM, lomeguatrib; TMZ, temozolomide.

comparison of TMZ and dacarbazine, a trial with similar entry criteria to the current study.[13]

The patients treated with TMZ alone had response rates, median survival, and toxicity similar to those reported in previous large studies.[1,13] The combination of LM and TMZ used in this study also was well tolerated. Hematologic toxicity was more frequent, but readily managed, and nonhematologic adverse effects were very similar to those seen with the methylating agent alone. Higher doses of LM appeared to result in greater myelotoxicity, an interesting finding given the total depletion of MGMT in PBMCs during the first day of treatment at the 40-mg dose level. PBMCs may not be as good a surrogate for bone marrow progenitor cells as previously thought.[14] Alternatively, the dose of LM may have influenced the duration, rather than the extent, of depletion, and this may have influenced the degree of myelosuppression observed. No new LM-specific toxicity was encountered.

The TMZ-treated group was included in the trial design for two reasons. We wished to check that our patient population behaved similarly to those treated with the drug in previous studies, and this proved to be the case. We also wished to test the hypothesis that the

Table 4. Treatment-Related Hematologic Toxicity on LM/TMZ

Event	40 mg/d First Line (n = 42)		60 to 80 mg/d First Line (n = 10)		40 mg/d Progression (n = 22)		60 to 80 mg/d Progression (n = 5)	
	No.	%	No.	%	No.	%	No.	%
All adverse events								
Thrombocytopenia	17	41	7	70	13	59	5	100
Neutropenia	19	45	8	80	12	55	5	100
Febrile neutropenia	2	5	3	30	3	14	1	20
Anemia	6	14	2	20	4	18	1	20
Serious adverse events								
Thrombocytopenia	5	12	2	20	4	18	1	20
Neutropenia	9	21	4	40	9	41	3	60
Febrile neutropenia	2	5	2	20	3	14	1	20
Anemia	1	2	1	10	0	0	0	0

Abbreviations: LM, lomeguatrib; TMZ, temozolomide.

Table 5. MGMT Concentration in PBMCs on the First Day of Treatment

Mean MGMT (fmol/mg DNA ± SEM)	LM/TMZ	TMZ	LM/TMZ Progression
Pretreatment	16.5 ± 1.68	16.8 ± 1.69	9.5 ± 1.49
No.	28	28	7
6 hours	0	14.8 ± 2.37	0
No.	27	25	6
24 hours	0	12.5 ± 1.41	0
No.	26	26	6
Day 6 or 7	0.62 ± 0.28	0.83 ± 0.83	0.5 ± 0.5
No.	5	3	3

NOTE. Only data from patients treated with lomeguatrib 40 mg/day are presented.
Abbreviations: MGMT, O^6-methylguanine DNA-methyltransferase; PBMC, peripheral blood mononuclear cells; LM, lomeguatrib; TMZ, temozolomide.

inactivation of MGMT could reverse melanoma resistance to TMZ treatment. Just more than half of the patients from the TMZ arm received combination treatment on progression, but none achieved an objective response. In two instances, patients exhibited shrinkage in individual tumor deposits that had previously been growing, but in neither case was there a significant decrease in the overall tumor burden. If reversal of resistance can occur, it does not appear to be a frequent occurrence, on the basis of our results.

The pharmacokinetics of LM and TMZ were assessed in a small number of patients. It is not possible to compare the results directly for LM with those from the phase I trial, as the formulation used here was different and there are only very limited data from the earlier study for the oral administration of the dose we used. We found no evidence that coadministration of LM affected TMZ pharmacokinetics, in that the parameters were comparable to those previously published, taking into account the lower dose used in this study.[13]

There are several possible reasons why MGMT inhibition failed to enhance the efficacy of TMZ in our trial. Inactivation of the protein in normal as well as tumor tissue necessitates a reduction in the dose of the accompanying chemotherapy. The relative overexpression of MGMT in tumor tissue should mean that a gain in therapeutic index, as seen in animal models,[11] can be achieved. In practice, this did not occur, perhaps because of greater accessibility of the drug to bone marrow. Some targeting of drug to tumor may be required to realize the benefits of DNA repair inhibition. Likewise, the combination of O^6-benzylguanine with carmustine requires that the dose of the latter agent is reduced and that enhanced activity has not been observed.[15]

MGMT may not be critical to cell survival after O^6-alkylating agent therapy, at least in certain clinical situations, because other factors ensure that damage is tolerated rather than resulting in cell death. Even though there is extensive preclinical evidence that MGMT is important in determining cellular sensitivity to methylating agents, in the clinic, a clear relationship between protein level and response to treatment has only been shown for primary CNS tumors. Here, low MGMT expression (or hypermethylation of the gene promoter) has been linked with improved survival or an increased chance of response to O^6-alkylating agent treatment in a number of trials .[16-18] In melanoma, we failed to demonstrate a relationship between pretreatment tumor MGMT and clinical outcome with TMZ,[19] though others have suggested that the probability of a response to DTIC increases with reducing amounts of tumor MGMT.[20] It is interesting to note that where responses to DNA damage are intact, such as in bone marrow progenitor cells, pretreatment MGMT concentration is a good predictor of cell survival.[21]

It may be that we have not adequately tested the premise that MGMT inactivation enhances TMZ activity. We found residual protein activity in tumor biopsies taken 24 to 72 hours after the end of the first treatment cycle. This was not expected, for we observed complete inactivation of MGMT after a single dose of LM in three of five patients in the phase I trial of LM/TMZ and more than 96% in the other two cases.[12] Pharmacodynamic measures from the current study show that MGMT recovers rapidly after treatment with LM/TMZ. This finding is significant in that O^6-methylguanine is toxic only through replication and may be repaired effectively at any point beforehand.[3] The presence of MGMT soon after dosing suggests that repair of potentially cytotoxic lesions is taking place and, hence, that a longer period of LM administration is needed.

Coadministration of LM and TMZ for 5 days is well tolerated, but it has efficacy similar to TMZ alone. LM dosing should be

Table 6. Pharmacokinetics of Lomeguatrib and Temozolomide

Drug	Day 1 Mean	Day 1 SD	Day 5 Mean	Day 5 SD
Lomeguatrib, n = 5				
V_z/F, L	1,025.0	775.3	1,319.0	771.3
CL/F, L/h	555.3	469.6	582.1	362.0
AUC_{last}, h × ng/mL	83.34	61.73	85.03	52.81
$T_{1/2}$	1.35	0.277	1.81	0.941
Temozolomide, n = 5				
V_z/F, L	37.70	16.53	34.01	10.64
CL/F, L/h	13.74	7.156	12.91	3.550
AUC_{last}, h × μg/mL	15.76	4.128	16.45	2.348
$T_{1/2}$	1.96	0.230	1.81	0.123

Abbreviations: SD, standard deviation; V_z, volume of distribution, terminal phase; F, bioavailability; CL, clearance; AUC_{last}, area under the serum concentration-time curve up to the last sampling; $T_{1/2}$, terminal half-life.

continued beyond that of TMZ to maintain MGMT inactivation. Such a combination will test whether repair protein depletion can improve the outcome for patients, and a trial with this design in melanoma is under way.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Middleton MR, Grob JJ, Aaronson N, et al: A randomized, phase III study of temozolomide (TMZ) versus dacarbazine (DTIC) in the treatment of patients with advanced, metastatic malignant melanoma. J Clin Oncol 18:158-166, 2000
2. Eggermont AM, Kirkwood JM: Re-evaluating the role of dacarbazine in metastatic melanoma: What have we learned in 30 years? Eur J Cancer, 40:1825-1836, 2004
3. Margison GP, O'Connor PJ: Biological consequences of reactions with DNA: Role of specific lesions, in Cooper CS, Grover P (eds): Handbook of Experimental Pharmacology. Berlin, Germany, Springer-Verlag, 1990, pp 547-571
4. Newlands ES, Stevens MFG, Wedge SR, et al: Temozolomide: A review of its discovery, chemical properties, pre-clinical development and clinical trials. Cancer Treat Rev 23:35-61, 1997
5. Middleton MR, Margison GP: Improvement of chemotherapy efficacy by inactivation of a DNA-repair pathway. Lancet Oncol 4:37-44, 2003
6. Yarosh DB, Hurst-Calderone S, Babich M, et al: Inactivation of O^6-methylguanine-DNA methyltransferase and sensitization of human tumor cells to killing by chloroethylnitrosourea by O^6-methylguanine as a free base. Cancer Res 46:1663-1668, 1986
7. Jelinek J, Fairbairn LJ, Dexter TM, et al: Long-term protection of hematopoiesis against the cytotoxic effects of multiple doses of nitrosourea by retrovirus-mediated expression of human O^6-alkylguanine-DNA alkyltransferase. Blood 87:1957-1961, 1996
8. Pegg AE, Dolan ME, Moschel RC: Structure, function, and inhibition of O^6-alkylguanine-DNA alkyltransferase. Prog Nucleic Acid Res Mol Biol 51:167-223, 1995
9. Durkacz B, Omidiji O, Gray D, et al: $(ADP\text{-}ribose)_n$ participates in DNA excision repair. Nature 283:593-596, 1980
10. Dolan ME, Pegg AE, Moschel RC, et al: Effect of O^6-benzylguanine on the sensitivity of human colon tumor xenografts to 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU). Biochem Pharamacol 46:285-290, 1993
11. Middleton MR, Kelly J, Thatcher N, et al: O^6-(4-bromothenyl)guanine improves the therapeutic index of temozolomide against A375M melanoma xenografts. Int J Cancer 85:248-252, 2000
12. Ranson M, Middleton MR, Bridgewater J, et al: Lomeguatrib, a potent inhibitor of O^6-alkylguanine DNA alkyltransferase: Phase I safety, pharmacodynamic and phamacokinetic trial and evaluation in combination with temozolomide in patients with advanced solid tumors. Clin Cancer Res 12:1577-1584, 2006
13. Danson S, Lorigan P, Arance A, et al: A randomized phase II study of temozolomide given 8-hourly, or daily with either interferon alfa-2b or thalidomide in metastatic malignant melanoma. J Clin Oncol 21:2551-2557, 2003
14. Gerson SL, Philips W, Kastan M, et al: Human $CD34^+$ hematopoietic progenitors have low, cytokine-unresponsive O^6-alkylguanine-DNA alkyltransferase and are sensitive to O^6-benzylguanine plus BCNU. Blood 88:1649-1655, 1996
15. Quinn JA, Pluda J, Dolan ME, et al: Phase II trial of carmustine plus O(6)-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma. J Clin Oncol 20: 2277-2283, 2002
16. Hegi ME, Diserens AC, Gorlia T, et al: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997-1003, 2005
17. Friedman HS, McLendon RE, Kerby T, et al: DNA mismatch repair and O^6-alkylguanine-DNA alkyltransferase analysis and response to temodal in newly diagnosed malignant glioma. J Clin Oncol 16:3851-3857, 1998
18. Esteller M, Garcia-Foncillas J, Andion E, et al: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 343:1350-1354, 2000
19. Middleton MR, Lunn JM, Morris C, et al: O^6-methylguanine-DNA methyltransferase in pretreatment tumour biopsies as a predictor of response to temozolomide in melanoma. Br J Cancer 78:1199-1202, 1998
20. Ma S, Egyhazi S, Ueno T, et al: O^6-methylguanine-DNA-methyltransferase expression and gene polymorphisms in relation to chemotherapeutic response in metastatic melanoma. Br J Cancer 89:1517-1523, 2003
21. Middleton MR, Walker E, Danson S, et al: Predicting the myelotoxicity of O^6-alkylating agent chemotherapy: The utility of pre-treatment O^6-methylguanine-DNA methyltransferase determination in peripheral blood lymphocytes. Proc Amer Assoc Cancer Res 43:1000, 2002 (abstr 4958)