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Multicenter Phase II Trial of Temozolomide in Mycosis Fungoides/Sézary Syndrome: Correlation with O⁶-Methylguanine-DNA Methyltransferase and Mismatch Repair Proteins

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Abstract

Purpose—Temozolomide (TMZ) is an oral derivative of dacarbazine that induces DNA damage by methylating nucleotide bases. Resistance has been associated with high levels of O⁶-methylguanine-DNA methyltransferase (MGMT). Malignant CD4⁺ T cells of patients with mycosis fungoides/Sézary syndrome (MF/SS) have been shown to have low levels of MGMT and may be particularly sensitive to this methylator.

Experimental Design—The efficacy of TMZ was evaluated in a multicenter phase II trial of patients with advanced stages of MF/SS. TMZ was given orally at daily doses of 200 mg/m² for 5 days every 28 days. MGMT and mismatch repair protein expression was assessed by quantitative immunofluorescence and immunohistochemistry in skin and blood samples.

Results—Twenty-six patients (stages IB–IVB) were evaluable for response. Patients had a median of four prior treatments. Median follow-up time was 19 months (range, 1–95). The overall response was 27% with two complete remissions (8%) and five partial remissions (19%). Median disease-free survival was 4 months. The median overall survival was 24 months. The most frequent toxicities included constitutional symptoms, gastrointestinal symptoms, and hematologic toxicities. Treatment was discontinued in three patients following grade 3 thrombocytopenia,

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Disclosure of Potential Conflicts of Interest

M.E. Dolan is coinventor of O⁶-benzylguanine. All other authors declared no potential conflicts of interest.

lymphopenia, and skin reaction. The relationship between pretreatment MGMT and mutL homolog 1 (MLH1)/mutS homolog 2 (MSH2) mismatch repair protein expression levels in skin biopsies of cutaneous lesions and clinical response to TMZ were evaluated.

Conclusions—Pretreatment levels of MGMT and MLH1/MSH2 protein levels are not predictive of response to TMZ in MF/SS, suggesting that other resistance mechanisms are important.

Introduction

The pathogenesis of mycosis fungoides/Sézary syndrome (MF/SS) is characterized by an altered immune biology and the accumulation of cytogenetic abnormalities during disease progression (1). Evidence of epigenetic silencing of hypermethylation of individual genes or gene-specific promoters associated with tumor progression has been observed in advanced stages of cutaneous T-cell lymphoma (CTCL; refs. 2, 3). Microsatellite instability (MSI), consistent with deficits in DNA repair, was found in a large number of microdissected tissue samples from patients with MF, with a higher prevalence in tumor stages suggesting a multilineage progression.

Chromosomal and somatic mutations have been frequently shown in hereditary nonpolyposis colon carcinoma and correlated with mismatch repair gene defects and MSI (4–6). In addition, several reports have identified the loss of mismatch repair function leading to MSI in a number of leukemias and lymphomas (7).

Temozolomide (TMZ) is an oral imidazotetrazine derivative of dacarbazine that has shown clinical activity in various tumor types such as non-Hodgkin's lymphoma, brain tumors, and melanoma (8–10). It is generally well tolerated, with an excellent oral bioavailability. TMZ causes methylation damage on DNA including the O^6 -position of guanine, with subsequent base pair mismatch resulting in cytotoxic and mutagenic DNA adducts.

Resistance has been associated with high levels of the DNA repair protein O^6 -methylguanine-DNA methyltransferase (MGMT), also referred to as O^6 -alkylguanine-DNA alkyltransferase, and defects of DNA mismatch repair (11). Previous studies have shown that increased levels of MGMT in tumor samples of patients with brain tumors receiving carmustine (bischloroethylnitrosourea, BCNU) or TMZ therapy are associated with resistance to chloroethylating and methylating agents (12, 13). Moreover, MGMT levels in tumor tissue specimen may be a predictive marker of survival in this patient population (14). The loss of mismatch repair function is associated with tumor progression during TMZ treatment (15). Interestingly, MGMT expression varies considerably in normal skin tissue shown by previous cell-type-specific *in situ* mapping for MGMT (16).

Topical BCNU is an effective treatment of patch- and plaque-stage MF (17, 18). On the basis of these unique findings, MGMT protein levels were studied by quantitative immunofluorescence in MF patch, plaque, and tumor tissue samples as well as in blood and lymph node samples. Overall, low levels of MGMT protein were detected but virtually undetectable in malignant CD4⁺ T cells in samples from patch/plaque disease with higher levels in tumor lesions, circulating Sézary cells, and lymph nodes (19). Healthy donor CD4 T cells and skin samples of benign reactive dermatitides had also low or nondetectable levels of MGMT expression.

No treatment is considered to be curative and patients with advanced MF/SS are often refractory to therapy and have a poor prognosis with a 5-year survival rate of less than 40% (20, 21). Low levels of MGMT in malignant CD4⁺ T cells suggest that treatment with TMZ may be an effective strategy to improve clinical outcomes. Given these data, a prospective

phase II trial of TMZ was initiated in relapsed patients with advanced stages of MF/SS, correlating the clinical response to levels of MGMT and DNA mismatch repair proteins mutL homolog 1 (MLH1) and mutS homolog 2 (MSH2).

Materials and Methods

Patient selection

The current study (trial registration ID: NCT00004106) was approved by the local Institutional Review Board of each participating center, and all patients gave informed consent prior to enrollment. Twenty-nine patients were enrolled into this open-label phase II trial, conducted in 3 academic centers, between 1998 and 2004. Patients 18 years or older with stage IB to IVA disease of histologically proven MF/SS were considered eligible for this protocol. Patients must have failed at least one previously received systemic therapy and have recovered from toxic effects of prior therapy, with a minimum of 4 weeks from receiving biologic therapy, chemotherapy, and topical agents. Radiation for local control had to be completed at least 2 weeks before study entry. Other eligibility criteria included Eastern Cooperative Oncology Group (ECOG)/WHO performance status of 2 or less, preserved renal (creatinine ≤ 2 mg/dL), and hepatic function (bilirubin ≤ 2 mg/dL). Pregnant or lactating women, women at childbearing age who were not using a reliable method of contraception, and patients with active infections or known to be HIV positive, severe heart disease (NYHA classes III–IV), poorly controlled diabetes mellitus, clinically significant peripheral venous insufficiency, and/or hematologic abnormalities with a decreased leukocyte count ($<3.0 \times 10^3/\text{mm}^3$) and decreased platelet count ($<1.0 \times 10^5/\text{mm}^3$) were ineligible.

Treatment plan

TMZ (Temodar; Schering-Plough) was given after an overnight fast at daily doses of 200 mg/m² for 5 days, for a total dose of 1,000 mg/m² every 28 days (1 cycle). Response was assessed after 2 cycles. Patients with disease progression were discontinued from study treatment. Complete responders received an additional 2 cycles and were permitted to continue for a maximum of 1 year. Treatment in patients with partial response and stable disease was continued up to 1 year or as tolerated, until disease progression or development of intolerable toxicities occurred. Adverse events were categorized as grades 1 to 4, using the NCI Common Toxicity Criteria Version 2.0. Twenty-five percent dose reductions were required in patients with any grade 3 toxicity and 50% dose reduction with any grade 4 toxicity that delayed treatment. Reescalation was not allowed. All grade 2 to 4 toxicities must have resolved to at least grade 1 before repeat dosing. If grade 3 hematologic toxicities persisted for more than 3 weeks, then the patient was discontinued from the study. Growth factors were not allowed. Therapy was permanently discontinued if grade 3 to 4 toxicity reoccurred.

Patient monitoring and response classification

The disease status of all patients was monitored at least once every 2 weeks during the first 2 cycles and thereafter on day 1 of each subsequent cycle with appropriate physical examination, complete blood cell count, Sézary cell count, and biochemical profile to assess the effect of TMZ. Imaging studies at 2 months were carried out to follow lymphadenopathy and systemic disease if present. The Severity-Weighted Assessment Tool (SWAT) was used to quantify skin disease burden. Criteria for response were defined as followed: complete response (CR), complete resolution of cutaneous and/or extracutaneous disease, and Sézary cell count for at least 4 weeks; partial response (PR), at least 50% reduction of index lesions or Sézary cell count with no evidence of new lesions, for at least 4 weeks; stable disease (SD), less than 50% reduction of index lesions or Sézary cell count; progressive disease

(PD), a greater than 25% increase in cutaneous or extracutaneous disease, or Sézary cell count. A Sézary cell count of less than 5% was considered not significant.

Analysis of MGMT and MLH1 and MSH2 mismatch repair proteins

Skin biopsies and blood samples were taken from patients prior to study entry. Isolation of malignant CD4⁺ T cells from peripheral blood samples and subsequent determination of MGMT protein levels by quantitative immunofluorescence were carried out as previously published (19, 22). Paraffin-embedded skin biopsies from 24 patients were analyzed for MGMT and for mismatch repair proteins MLH1 and MSH2 by quantitative immunofluorescence according to previously described methods (19) and/or by semiquantitative immunohistochemistry. For all 3 markers, only nuclear staining was taken into account for the respective scoring system. A semiquantitative scoring on a 4-point scale based on the percentage of positively staining tumor cells was employed according to the indicated references (<10%: 0, 10%–30%: 1, 30%–50%: 2, >50%: 3). For statistical analysis, groups of negative and weak staining (scores 0 and 1) or moderate and strong staining (scores 2 and 3) were combined.

Monoclonal antibodies against MGMT (MT3.1; Chemicon), hMLH1, and hMSH2 (clone 14 and clone FE11; Oncogene) were used for immunohistochemical staining in conjunction with the Vectastain ABC kit (Vector Laboratories) according to the manufacturer's protocol. Positive control tissue consisted of nuclear staining in colon cancer.

Statistical methods

All statistical analysis was based on 29 enrolled patients. Baseline variables for each treatment group were summarized as determined statistically by Fisher's exact test. Overall survival and disease-free survival was analyzed using the Kaplan–Meier method. The χ^2 test was used to test differences in response rates between disease with a high or low expression of MGMT, MLH1, and MSH2. $P < 0.05$ was considered significant.

Results

Patient characteristics

Most patients were Caucasian and presented with a median age of 61 years (range from 33 to 87 years; Table 1). Most patients had advanced disease: 2 (7%) stage IIB; 10 (34%) stage III; 13 (45%) stage IVA. Half of the cohort has received more than 3 prior treatments and approximately one-third was female.

Response

Twenty-six patients were evaluable for response excluding 3 patients who discontinued therapy within the first cycle, due to toxicity (2 patients) or unknown reasons (1 patient). Median follow-up time was 19 months (range, 1–95). The overall response rate (ORR) was 27%, with 2 CR (8%) and 5 PR (19%). One patient with stage IB, IIB, and IVA each and 4 patients with stage III responded to therapy. Four patients had SD (15%). Median progression-free survival was 4 months (range, 2–17 months). The median overall survival of all patients was 24 months. Kaplan–Meier survival estimates were 86% at 1 year, 50% at 2 years, and 25% at 5 years. Twenty-one patients (72%) had died, 19 (66%) attributable to CTCL. Other causes of death included complication after allogeneic bone marrow transplant and unknown condition. Among the 7 responders, 4 patients eventually died of progressive disease. One patient was lost to follow-up, and 7 patients (24%) were alive at last follow-up.

MGMT and MLH1 and MSH2 mismatch repair protein expression

Results of MGMT protein expression were available in circulating malignant CD4⁺ T cells on 13 patients and in skin specimens on 23 patients. Of circulating malignant CD4⁺ T cells, 9 patients were deficient of MGMT whereas the remaining 4 had MGMT expression ranging from 1,973 to 3,366 molecules/nucleus by immunofluorescence assay. Two of 9 MGMT-deficient patients responded to therapy (1 CR and 1 PR), but this was not statistically significant compared with patients with high MGMT activity ($P = 0.21$). Of the 23 skin samples, 14 (61%) were negative and the remaining 9 (39%) were positive for MGMT. The correlation between MGMT expression in cutaneous lesions and clinical responses to TMZ therapy is shown in Table 2. MGMT expression tended to be low or negative in patients with CR, PR, and SD but was not found to be significantly correlated with response (CR and PR) or overall disease control (CR, PR, and SD; $P = 0.72$). Low levels of the mismatch repair proteins MLH1 and MSH2 have been associated with resistance to TMZ, in contrast to tumors with high mismatch repair protein expression that have been reported to be more sensitive to TMZ (11). Both DNA repair proteins were investigated in 24 skin specimens (Fig. 1). Our results suggest that approximately 50% of the tumor specimens express these proteins, but none of the proteins significantly correlated with response rates (CR and PR) or overall disease control (CR, PR, and SD; data not shown). The correlation between both MGMT and mismatch repair protein expression and clinical responses to TMZ therapy is shown in Table 3. The frequency of negative MGMT score with positive MLH1 and/or MSH2 score was higher in patients who responded to TMZ, but this was not found to be significantly correlated with response (CR and PR) or overall disease control (CR, PR, and SD; $P = 0.99$). Our data suggest that resistance to TMZ therapy is independent of cellular levels of MGMT and DNA repair proteins in CTCL.

There was no significant correlation between MGMT levels in circulating malignant T cells and progression-free ($P = 0.27$) or overall ($P = 0.72$) survival. There was also no significant correlation between MGMT levels in cutaneous lesions and progression-free ($P = 0.36$) or overall ($P = 0.50$) survival (Fig. 2). Furthermore, there was no significant difference between overall survival time and MGMT expression with or without MLH1/MSH2 expression ($P = 0.47$).

Toxicity

In general, TMZ treatment was well tolerated, with the majority of toxicities being grade 1 or 2 in severity and transient (Table 4). The most frequent toxicities included constitutional symptoms, gastrointestinal symptoms, and hematologic toxicities. One patient developed sepsis. Treatment was discontinued in 3 patients following grade 3 thrombocytopenia, lymphopenia, and skin reaction. One patient experienced a transient ischemic attack during cycle 1, but this was not related to the study drug.

Discussion

The present study investigated the efficacy of TMZ in patients with advanced and refractory MF/SS and correlated pretreatment levels of MGMT and DNA mismatch repair protein with clinical response. Our results show that TMZ with an ORR of 27% has moderate clinical activity as a single agent in this disease.

TMZ is believed to exert its cytotoxic effects primarily by generating *O*⁶-methylguanine in DNA. One identified mechanism of TMZ resistance is mediated by the expression of the DNA repair protein MGMT. Epigenetic silencing of the *MGMT* gene by promoter methylation prevents repair of *O*⁶-methylguanine lesions and has been shown to predict survival benefit from TMZ therapy in glioblastoma (13). One question remaining is whether

the sensitivity of MF/SS to TMZ may include the mismatch repair function and MGMT expression. The current study suggests that MGMT expression varies considerably in skin specimens among patients with advanced MF/SS. MGMT expression tended to be low or negative in patients who responded to or remained stable on TMZ therapy and further supports the concept that low MGMT-expressing cells are preferentially killed by TMZ, but these findings did not reach statistical significance. This is likely due to a small number of patients studied. In addition, all patients had advanced disease and were heavily pretreated, leading to further accumulation of mutations that may influence other critical pathways in controlling cellular response to TMZ.

MSI has been recently shown to be associated with disease progression in MF (3, 23). Such progressive genetic defects have been extensively shown in hereditary colon carcinoma and correlated with DNA mismatch repair defects (4). Defects in mismatch repair may result in cellular resistance to TMZ, independent of MGMT activity. In a recent analysis, a panel of mismatch repair proteins appeared normal in skin biopsies of patients with MF/SS (24). Our results suggest that a detectable positive MLH1 and/or MSH2 score was higher in patients who responded to TMZ, but this was not found to be significantly correlated with response rate or overall disease control. It seems that TMZ resistance is not related to a dysfunctional MLH1/MSH2; however, we were not able to study the mismatch repair protein expression during or after completion of treatment and therefore do not know whether their expression has been altered by TMZ. It may also be plausible that dysfunctional MLH1/MSH2 does not play a causal role in tumor progression in our patient population, but studies that rely on mutation frequency and functional data have not been done. In addition, the methodologies applied in the current study may not be sensitive enough to detect subtle differences in MGMT and mismatch repair to better define resistance to TMZ therapy. Eight patients were found to be previously treated with topical or systemic alkylating agents either alone or in combination. We therefore cannot exclude any effects of these agents on the current regimen as well.

The toxicity profile using the standard 5-day dosing regimen of TMZ (200 mg/m²) was safe and feasible with the majority of toxicities being grade 1 or 2 in severity and transient. Most patients received more than 2 cycles of TMZ. The major reason for the discontinuation of treatment was tumor progression. The treatment was discontinued in 3 patients following grade 3 thrombocytopenia, lymphopenia, and skin reaction.

In conclusion, the current trial confirms that TMZ has moderate activity in patients with advanced CTCL. Furthermore, our analysis of the pretreatment levels of MGMT and MLH1/MSH2 in skin biopsies failed to predict response to TMZ. However, most of our patients had advanced disease, which might have contributed genetic alterations involving other repair pathways. One strategy to reverse the limited therapeutic efficacy to TMZ is inactivation of MGMT with protracted dosing regimen, but this may be of limited efficacy due to increased hematologic toxicity as previously reported (25). Clinical trials depleting MGMT activity with *O*⁶-benzylguanine have been shown to potentiate the cytotoxic activity of BCNU in patients with multiple myeloma and advanced melanoma, but at the expense of significantly increased hematologic toxicity (26, 27). In contrast, susceptibility of neoplastic cells to TMZ can be increased through pharmacologic modulation of the expression level and functional activity of DNA repair proteins. Correspondingly, PARP inhibitors have been shown to overcome TMZ resistance in clinical trials in patients with TMZ-resistant leukemias, melanomas, and other solid tumors and may be a promising strategy in advanced CTCL (28).

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Translational Relevance

The efficacy of temozolomide (TMZ) treatment depends on low *O*-6-methylguanine-DNA methyltransferase (MGMT) activity and on high mismatch repair. Treatment of advanced cutaneous T-cell lymphoma (CTCL) remains difficult as virtually all patients become refractory to most standard therapies. We have previously shown low levels of MGMT in malignant T lymphocytes from patients with CTCL, suggesting that treatment with TMZ may improve clinical outcomes. In this study, we showed that TMZ has moderate activity in patients with advanced CTCL with a low toxicity profile and compares favorably with novel agents when used as a single agent in this disease, but it did not correlate with mismatch repair function and MGMT expression, despite the trend of low or negative levels in patients who responded to or remained stable on TMZ therapy. The relevance and clinical utility of MGMT methylation status in CTCL remain to be determined.

Figure 1. Skin biopsies of 2 responding patients showed high MLH-1 and low MSH-2 expression (A and B) and low MLH-1 and high MSH-2 expression, respectively (C and D). Skin biopsy of another patient showed low expression for both MLH-1 and MSH-2 and was associated with disease progression (E and F; original magnification: $\times 400$).

Figure 2. Overall survival of patients with MF/SS stratified by MGMT expression in skin. The median overall survival is 24 months.

Table 1

Characteristics of patients ($N=29$)

Characteristic	<i>n</i> (%)
Age at study entry, median (range), y	61 (33–87)
Gender	
Female	11 (38)
Male	18 (62)
Race	
White	20 (69)
Black	8 (28)
Asian	1 (3)
TNMB stage	
IB	3 (10)
IIA	1 (3)
IIB	2 (7)
III	10 (34)
IVA	13 (45)
Prior treatments	
3	13 (45)
>3	14 (48)
Unknown	2 (7)

Abbreviation: TNMB, tumor, node, metastasis, blood.

Table 2

MGMT expression in skin biopsies of patients with MF/SS versus response to TMZ therapy

Response	MGMT expression		<i>P</i>
	Negative	Positive	
CR	1	1	0.72
PR	3	1	
SD	3	1	
PD	7	5	
NE	0	1	
Total	14	9	

NOTE: *P* value compares CR/PR versus SD/PD between groups.

Abbreviation: NE, not evaluable.

Table 3

Expression of MGMT and two mismatch repair proteins, MLH1 and MSH2, in skin biopsies of patients with MF/SS versus response to TMZ therapy

Response	MGMT expression		<i>P</i>
	Negative MLH1/MSH2 ^a positive	Positive MLH1/MSH2 negative	
CR	1	1	0.99
PR	3	1	
SD	2	0	
PD	6	7	
NE	0	0	
Total	12	9	

Abbreviation: NE, not evaluable.

^a Expression of either MLH1 and MSH2 proteins or both; *P* value compares CR/PR versus SD/PD between groups.

Table 4

Side effects associated with TMZ

Side effect	Toxicity grade (NCI Common Toxicity Criteria Version 2.0)			
	I	II	III	IV
<i>Hematologic</i>				
Anemia	4			
Leukopenia		2		
Neutropenia		3	1	
Lymphopenia		2		
Thrombocytopenia	6	3	1	
<i>Constitutional</i>				
Fatigue	10	5		
Fever/chills	10			
Pain/pruritus	7	13		
<i>Mucocutaneous</i>				
Stomatitis	3			
Rash, erythema	7	5	2	
Alopecia	4			
<i>Infection</i>	2	5	1	1
<i>Gastrointestinal</i>				
Nausea/vomiting/GERD	23	22	1	
Anorexia/weight loss	2			
Diarrhea/constipation	3	4		
<i>Hepatic</i>	5	2		
<i>Renal</i>		1	1	
<i>Edema</i>	5	4		
<i>Cardiac</i>	2			
<i>Pulmonary</i>	2			
<i>Neurologic</i>				
Headache	3			
Insomnia	1			
Lightheadedness/transient ischemic attack	1		1	
Mood disorder	2			
<i>Hyperglycemia</i>	3			
<i>Allergic reaction/vasculitis</i>	2			
<i>Hemorrhage</i>	1			

Abbreviation: GERD, gastroesophageal reflux disease.