

## ONLINE FIRST

# Phase I Clinical Trial of O6-Benzylguanine and Topical Carmustine in the Treatment of Cutaneous T-Cell Lymphoma, Mycosis Fungoides Type

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**Objectives:** To evaluate the toxic effects and maximum tolerated dose of topical carmustine [1,3-bis (2-chloroethyl)-1-nitrosourea] following intravenous O6-benzylguanine in the treatment of cutaneous T-cell lymphoma (CTCL), and to determine pharmacodynamics of O6-alkylguanine DNA alkyltransferase activity in treated CTCL lesions.

**Design:** Open-label, dose-escalation, phase I trial.

**Setting:** Dermatology outpatient clinic and clinical research unit at a university teaching hospital.

**Patients:** A total of 21 adult patients (11 male, 10 female) with early-stage (IA-IIA) refractory CTCL, mycosis fungoides type, treated with topical carmustine following intravenous O6-benzylguanine.

**Intervention:** Treatment once every 2 weeks with 120 mg/m<sup>2</sup> intravenous O6-benzylguanine followed 1 hour later by whole-body, low-dose topical carmustine starting at 10 mg, with 10-mg incremental dose-escalation in 3 patient cohorts. Cutaneous T-cell lymphoma lesional skin biopsy specimens were taken at baseline and 6 hours, 24 hours, and 1 week after the first O6-benzylguanine infusion for analysis of O6-alkylguanine-DNA alkyltransferase activity.

**Main Outcome Measures:** Clinical response measured by physical examination and severity-weighted assessment tool measurements, safety data acquired by review of adverse events at study visits, and O6-alkylguanine-DNA alkyltransferase activity in treated lesion skin biopsy specimens.

**Results:** A minimal toxic effect was observed through the 40-mg carmustine dose level with 76% of adverse events being grade 1 based on the National Cancer Institute Common Terminology Criteria for Adverse Events. Mean baseline O6-alkylguanine-DNA alkyltransferase activity in CTCL lesions was 3 times greater than in normal controls and was diminished by a median of 100% at 6 and 24 hours following O6-benzylguanine with recovery at 1 week. Clinical disease reduction correlated positively with O6-alkylguanine-DNA alkyltransferase activity at 168 hours ( $P=.02$ ) and inversely with area under the curve of O6-alkylguanine-DNA alkyltransferase over 1 week ( $P=.01$ ). Twelve partial responses and 4 complete responses were observed (overall response, 76% [95% CI, 0.55-0.89]). Five patients discontinued therapy owing to adverse events with a possible, probable, or definite relationship to the study drug.

**Conclusion:** O6-benzylguanine significantly depletes O6-alkylguanine-DNA alkyltransferase in CTCL lesions and in combination with topical carmustine is well tolerated and shows meaningful clinical responses in CTCL at markedly reduced total carmustine treatment doses.

**Trial Registration:** clinicaltrials.gov Identifier: NCT00003613

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**E**ARLY-STAGE (IA-IIA) MYCOSIS fungoides (MF) tends to follow a chronic course. Treatment toxic effects are therefore of high consideration, and conservative skin-directed therapies and phototherapy are considered first-line therapies.<sup>1-3</sup> Disease relapses are common in MF,<sup>4,5</sup> and patients consequently exhaust skin-directed treatment options during their lifetime. Treatment-

resistant and refractory cases may then require more aggressive treatments, which incur greater risks of toxic effects.<sup>1,3,6,7</sup>

An alternative skin-directed therapy for early-stage MF is the topical chemotherapeutic agent carmustine [1,3-bis (2-chloroethyl)-1-nitrosourea] While producing high response rates, depending on the dosage, daily topical carmustine may incur systemic toxic effects not observed in most other skin-directed cutaneous

T-cell lymphoma (CTCL) therapies, including myelosuppression and hepatotoxicity.<sup>8</sup> Nearly all patients experience cutaneous reactions, including tender erythema, hyperpigmentation, and telangiectasias, which may be treatment limiting. Limiting these adverse effects could make carmustine a more useful therapeutic option for patients with MF.

Carmustine damages DNA through chloroethyl alkylation at the O6 site of guanine and subsequent cytotoxic interstrand crosslink formation.<sup>9-12</sup> Some malignant diseases have an escape mechanism for nitrosourea resistance via the DNA repair enzyme O6-alkylguanine DNA alkyltransferase (AGT), which covalently transfers the carmustine alkyl adduct to its cysteine residue.<sup>9-11,13,14</sup> The novel drug O6-benzylguanine may potentiate carmustine efficacy.<sup>15</sup> O6-benzylguanine is a guanine analog and AGT substrate, which transfers its benzyl group to the AGT cysteine residue, thereby irreversibly inactivating AGT and preventing DNA repair.<sup>16-20</sup> In tumor cell lines and xenograft models, O6-benzylguanine enhances the sensitivity of malignant diseases to nitrosoureas.<sup>21-25</sup>

Phase I and II trials have been published on O6-benzylguanine alone and in combination with systemic carmustine in advanced solid tumors, particularly malignant gliomas.<sup>26-37</sup> O6-benzylguanine and carmustine together (O6-benzylguanine–carmustine) have never been studied in the treatment of MF, and to our knowledge, topical application of carmustine has not previously been examined in combination with O6-benzylguanine. We conducted a phase I trial of a single topical carmustine administration following intravenous O6-benzylguanine administered once every 2 weeks in the treatment of MF to examine pharmacodynamics of AGT in MF lesions, to determine toxic effects and maximum tolerated dose (MTD), and to follow clinical efficacy measures. Hereinafter, “O6-benzylguanine–carmustine” will refer to the combination of O6-benzylguanine and topical carmustine, unless otherwise specified.

## METHODS

### ENROLLMENT AND ELIGIBILITY

Twenty-one patients with MF were enrolled in an institutional review board–approved, open-label, dose-escalation phase I trial of topical carmustine following intravenous O6-benzylguanine at our institution. All patients signed an institutional review board–approved informed consent. Eligibility criteria required histologically confirmed stage IA to IIA MF<sup>38</sup> refractory to at least 1 conventional CTCL treatment other than topical corticosteroids. Histologic diagnosis was made on hematoxylin-eosin–stained paraffin sections by a dermatopathologist experienced in lymphoproliferative cutaneous diseases (A.C.G. or G.S.W.). Minimal criteria used for histologic diagnosis of MF included tagging of basal keratinocytes by single or clustered haloed, small, atypical lymphocytes in the absence of significant epidermal spongiosis and papillary dermal fibroplasia with papillary dermal lymphocytic infiltrates.<sup>39</sup>

Patients were treatment free for at least 4 weeks prior to entry with no history of nitrosourea therapy. Patients were older than 18 years with Eastern Cooperative Oncology Group performance status grades of 0 to 2 and adequate organ function

(clinical trials.gov identifier NCT00961220). Exclusion criteria included women who were pregnant or lactating or those having known central nervous system involvement or any other malignant disease.

### PRETREATMENT EVALUATION

Screening and baseline complete blood cell count (CBC), chemical analyses, liver function tests, prothrombin time, creatine phosphokinase, electrocardiogram, chest radiography, urinalysis, pulse oximetry, and carbon monoxide diffusing capacity were obtained. For patients of childbearing potential, negative urine pregnancy tests were obtained at study entry, and contraceptive control was required.

At study entry and prior to each treatment cycle, a complete medical history and physical examination were performed. At baseline, up to 5 target index lesions were measured and the severity-weighted assessment tool (SWAT) score (Stevens et al<sup>40</sup>) was calculated.

### PROTOCOL

For each treatment cycle 120 mg/m<sup>2</sup> intravenous O6-benzylguanine (NCI Pharmaceutical Management Branch, Cancer Therapy Evaluation Program) was infused over 1 hour, followed 1 hour later by whole-body (excluding eyelids and ulcerated lesions but including normal-appearing skin) application of topical carmustine (BiCNU; Bristol-Myers Squibb) solution. The carmustine was supplied as 100 mg per vial and was dissolved with 3 mL of absolute alcohol, and then further diluted with 27 mL of sterile water, which provided 100 mg in 30 mL (3.33 mg/mL). Thus, a 20-mg dose is 6 mL of the solution (20 mg divided by 3.33 mg/mL=6 mL). The preparation was performed in a chemotherapy hood, with appropriate protections (gloves, mask, gown). A new vial was used for each dose preparation, owing to the potential for decomposition of the reconstituted carmustine (slowed with refrigeration). O6-benzylguanine dosing was fixed, whereas treatment with topical carmustine began at a 10-mg dose level, and the dose was escalated in 10-mg increments in cohorts of 3 patients without intraindividual escalation. Cycles were to be repeated every 2 weeks.

The MTD was defined as the dose level below which 2 patients experienced dose-limiting toxicities (DLTs). A DLT was defined according to National Cancer Institute common toxicity criteria (NCI-CTC) adverse event of grade 2 or higher toxic effect that was probably or definitely due to the treatment regimen and which occurred within the first 6 weeks of therapy or a 25% reduction in carbon monoxide diffusing capacity occurring within the first 6 weeks of therapy. Following a DLT at any dose level, 3 additional patients were treated before the next dose escalation or until a second DLT occurred. Treatment cycles were held up to 2 weeks to allow DLT resolution, and treatments were restarted at the previous dose level. Persistent DLTs resulted in withdrawal from the study.

### INTERIM AND POSTTHERAPY EVALUATION

Patients were interviewed and examined once every 2 weeks for drug toxic effects and disease response. Disease response was assessed using SWAT scores and was defined according to the following guidelines: complete response was defined as clinical resolution of all active disease lasting at least 4 weeks; partial response was at least a 50% decrease in SWAT lasting at least 4 weeks; marginal response was at least a 25% but less than 50% decrease in SWAT; stable disease was a response of less than a 25% increase in SWAT for at least 8 weeks; progressive disease was an increase of at least 25% in SWAT.

Screening laboratory tests and pulse oximetry were repeated once every 2 weeks prior to each treatment cycle. Interim laboratory evaluations were also performed on the weeks between treatment cycles and included a CBC, blood urea nitrogen level, creatinine level, liver function test, and creatine phosphokinase. Following demonstrated safety midway through the trial, laboratory monitoring was reduced from weekly to once every other week if there were no abnormalities at 2 months and then to monthly after 6 months. Measurement of carbon monoxide diffusing capacity was repeated every 8 weeks.

### AGT ACTIVITY ANALYSIS

During the first O6-benzylguanine infusion cycle, a 4-mm lesional punch biopsy specimen was taken prior to infusion and then 6 hours, 24 hours, and 1 week after infusion for analysis of AGT activity. Six skin specimens from normal volunteers were obtained as comparative controls. Biopsy tissue protein was extracted, and AGT was measured as removal of the [<sup>3</sup>H]methyl adduct from O6-guanine in DNA by incubating extracts with DNA substrate as previously described.<sup>27</sup> The AGT activity was expressed as femtomoles of O6-methylguanine removed per microgram of DNA.

### STATISTICAL ANALYSIS

The AGT activity over time was summarized by the area under the curve (AUC) of AGT. Sample correlations between two measurements were estimated using Spearman rank correlation. Linear regression was used to evaluate the predictive value of AUC of AGT activity on SWAT reduction. Factors predicting clinical response were identified by logistic regression. All tests were 2 sided and conducted at  $P = .05$ . Analyses were performed with SAS software (SAS Institute Inc, Cary, North Carolina).

We obtained SWAT measurements for most patients to quantify disease burden. SWAT assessments were not performed for patient 1, so body surface area (BSA) was used to determine response. In addition, when SWAT measurements were not available, morphologic characteristics of the lesions and BSA measurements as recorded in the physician notes were used to retrospectively estimate SWAT and measure response (patients 2, 3, 4, and 8). Baseline SWAT values represent the SWAT measurements on the day prior to treatment or the first day of treatment for patients 6 to 14, 17, 18, 20, and 21. For patients 1, 2, and 5, the first available SWAT measurement was recorded or retrospectively calculated from data obtained within 3 weeks after initiating therapy. For patients 3, 15, 16, and 19, the baseline SWAT measurements were recorded or retrospectively calculated from values obtained within 10 days prior to starting therapy. For patient 12, response duration included follow-up after the patient was removed from the study because a complete response was noted on the last day the patient was in the trial; for all other patients, follow-up included only the time around their last study evaluation. Time to partial or complete response was calculated from the first day of treatment until a response was noted. If the response lasted at least 4 weeks, the first day the response was noted was used in the "time to response" and "response duration" calculations.

## RESULTS

### PATIENT DEMOGRAPHICS AND TREATMENT DATA

Eleven male and 10 female patients with stages IA-IIA MF were enrolled in the trial (**Table 1**). Three patients

were African American, 1 patient was Latino, and the remainder were of European descent. Patients received a median of 12 total cycles of O6-benzylguanine–carmustine (approximately 5.6 months of treatment). The addition of 3 patients to the 10-mg cohort was due to grade 3 elevation of aspartate aminotransferase level associated with concomitant alcohol intake (patient 2), which resolved with abstinence. Six patients were treated with a dose level of 30 mg owing to a grade 2 DLT related to contact dermatitis (patient 11). Another grade 2 contact dermatitis DLT occurred at a dose level of 40 mg (patient 16) and necessitated enrollment of 6 patients at that dose level. The trial was closed after completion of the 40-mg dose level without achieving the MTD, given the sufficient safety and response demonstrated, and to prepare for the initiation of a follow-up phase II trial.

### O6-BENZYLGUANINE–CARMUSTINE TOXIC EFFECTS

At the tested dose levels, O6-benzylguanine–carmustine resulted in mostly mild, nonhematologic toxic effects with little myelosuppression (Table 1). The most frequent adverse events with a possible, probable, or definite relationship to either BCNU, O6-benzylguanine, or the O6-benzylguanine–carmustine combination were headache (10 patients; 48%), fatigue (10 patients; 48%), contact dermatitis (9 patients; 43%), nausea (7 patients; 33%), and cutaneous hyperpigmentation (6 patients; 29%). Headache, fatigue, nausea, and dizziness appeared and resolved spontaneously within minutes to a few hours after treatment. Seventy-six percent of toxic effects were grade 1.

Hyperpigmentation occurred in areas of contact dermatitis or in areas of resolved MF lesions, likely representing postinflammatory hyperpigmentation. Telangiectasias occurred in 2 patients at the 30-mg dose level and resolved over time.

### ELEVATED AGT LEVELS IN SKIN OF PATIENTS WITH CTCL

The AGT data from the patients with CTCL and normal controls are shown in **Table 2**. The mean (SD) baseline pretreatment AGT activity in patients with CTCL was 5.14 (1.65) fmol/μg, compared with a mean of 1.65 (1.41) fmol/μg in normal controls ( $P = .005$ ). A trend in racial variation was evident, as the mean (SD) AGT level in normal controls was 0.35 (0.39) fmol/μg in white patients compared with 1.48 (1.67) fmol/μg in African Americans ( $P = .07$ ). Although there seemed to be a similar trend when comparing baseline CTCL AGT activity in white patients with baseline CTCL AGT activity in African American patients (white CTCL AGT level = 4.58 [1.37] fmol/μg vs African American CTCL AGT level = 8.31 [8.06] fmol/μg [patients 13, 14, and 17]), the differences were not statistically significant. Differences between AGT activity in patients with CTCL and race-matched controls were significant in whites ( $P < .001$ ) but not in African Americans ( $P = .34$ ).

**Table 1. Treatment Responses and Adverse Events (AEs)**

Patient (Carmustine Dose, mg)	Age at Entry, y	CTCL Stage	Prior Tx, No.	Total Carmustine	Tx Cycles, No.	Basal SWAT	End SWAT
1 (10)	80	IIA	7	110	11	70.0 <sup>a</sup>	80 <sup>a</sup>
2 (10)	73	IA	1	120	12	20 <sup>b</sup>	1.42
3 (10)	46	IA	2	310	31	9.5 <sup>b</sup>	5 <sup>b</sup>
4 (10)	80	IIA	8	70	7	11.25 <sup>b</sup>	55 <sup>b</sup>
5 (10)	47	IA	2	90	9	3.2	0.53
6 (10)	59	IB	5	350	35	11.9	12
7 (20)	71	IA	1	600	30	4.64	5.2
8 (20)	50	IA	6	260	13	2.86	10 <sup>b</sup>
9 (20)	53	IB	3	280	14	1.78	0.18
10 (30)	71	IA	2	190	7	4.81	0
11 (30)	47	IB	1	190	8	16.7	2.3
12 (30)	53	IB	2	560	19	30.0	0
13 (30)	56	IB	2	390	13	10.3	5.9
14 (30)	66	IA	4	360	13	12.2	3.4
15 (30)	78	IB	1	570	19	43.6	21.7
16 (40)	39	IA	1	150	4	3.3	2.7
17 (40)	80	IIA	5	160	4	46.7	51.3
18 (40)	66	IB	2	480	12	72.9	21.4
19 (40)	76	IA	2	200	5	5.8	0
20 (40)	50	IB	2	320	8	23.7	12.81
21 (40)	74	IA	3	740	23	2.3	0
Mean	62.6	NA	3.0	309.5	14.1	19.4	13.8
Median	66	NA	2	280	12	11.25	5

Patient (Carmustine Dose, mg)	Best SWAT	SWAT Reduction, % <sup>c</sup>	Response <sup>c</sup>	Time to PR, Mo	RD	Reason for Treatment Discontinuation	AEs (NCI Grade) <sup>d,e</sup>
1 (10)	50.0 <sup>a</sup>	29 <sup>a</sup>	MR <sup>a</sup>	...	...	Metastatic SCC	F (II)
2 (10)	1.25	94	PR	1.3	4.0	Grade 3 SGOT elevation	HA (I), N (I), F (II), TrE (III)
3 (10)	0.5	95	PR	2.8	11.4	Refused further therapy	H (I), F (I), N (I), N (I)
4 (10)	11.25 <sup>b</sup>	0	PD	...	...	Refused further therapy; CHF	N (I)
5 (10)	0.53	83	PR	3.3	2.3	Refused further therapy	H (I), F (I), N (I), An (I)
6 (10)	2.32	81	PR	4.0	11.7	Disease progression	LP (I), An (I), TCP (I)
7 (20)	1.07	77	PR	2.8	6.5	Disease progression	HA (I), N (I), LP (I), TrE (I)
8 (20)	2.25 <sup>b</sup>	21	SD	...	...	Disease progression	TrE (I)
9 (20)	0.18	90	PR	2.4	4.2	Refused further therapy	HyP (I), CDx (I), An (I)
10 (30)	0	100	CR	0.9 (2.3) <sup>f</sup>	2.3	Study completed	CDx (I), HA (II), D (II), F (II)
11 (30)	0.5	97	PR	0.9	4.2	Grade 3 AST elevation	HA (I), F (I), HyP (I), D (I), CDx (II), TrE (III)
12 (30)	0	100	CR	1.9 (8.9) <sup>f</sup>	11.9	Grade 3 AST elevation	LP (I), T (I), CDx (I), HyP (I), TrE (III)
13 (30)	1.1	89	PR	1.4	4.2	Disease progression	HA (I), F (I), HyP (I), CDx (I), N (I), An (I)
14 (30)	1.3	89	PR	2.3	3.7	Anemia	F (I), HyP (I), CDx (I), D (I), LP (I), N (II), An (II), HA (III)
15 (30)	14.3	67	PR	5.1	3.3	Disease progression	HA (I), F (I), D (I), T (I), TCP (I)
16 (40)	2.7	18	SD	...	...	Contact dermatitis	HA (I), CDx (II)
17 (40)	31.5	33	MR	...	...	Refused further participation	...
18 (40)	21.4	71	PR	2.8	3.0	Disease progression	CDx (II)
19 (40)	0	100	CR	(2.0) <sup>f</sup>	0.9	Refused further participation	...
20 (40)	10.6	55	PR	2.3	1.0	Disease progression	HyP (I)
21 (40)	0.0	100	CR	2.3 (3.2) <sup>f</sup>	8.4	Study closing	F (I), LP (I), CDx (II), TCP (II)
Mean	7.3	71	NA	2.4	5.2	NA	NA
Median	1.25	83	NA	2.3	4.1	NA	NA

Abbreviations: An, anemia; CDx, contact dermatitis; CR, complete response; D, dizziness; F, fatigue; HA, headache; HyP, cutaneous hyperpigmentation; LP, leukopenia; MR, marginal response; N, nausea; NA, not applicable; PR, partial response; RD, response duration; SCC, squamous cell carcinoma; SD, stable disease; SWAT, severity weighted assessment tool score (range, 0-300); T, telangiectasia; TCP, thrombocytopenia; TrE, transaminase elevation.

<sup>a</sup>SWAT score was not available; response was determined by total body surface area of involvement assessment.

<sup>b</sup>Response was retrospectively determined by total body surface area of involvement and lesional morphologic characteristics recorded on physician assessments.

<sup>c</sup>Basal SWAT-best SWAT.

<sup>d</sup>Reported National Cancer Institute Common Toxicity Criteria grades 1, 2, and 3 adverse events with a possible, probable, or definite relationship to the study drug.

<sup>e</sup>“Contact dermatitis” includes skin erythema and/or adverse events felt to represent a contact dermatitis, and is not a specific term listed in the National Cancer Institute Common Toxicity Criteria.

<sup>f</sup>Time to CR.

**Table 2. AGT Activity From Baseline to 1 Week Postinfusion Compared With SWAT Reduction**

Patient <sup>a</sup>	AGT Activity at Baseline <sup>b</sup>	AGT Activity at 6 hr <sup>b</sup>	Inactivation at 6 hr, %	AGT Activity at 24 hr <sup>b</sup>	Inactivation at 24 hr, %	AGT Activity at 1 wk <sup>b</sup>	Inactivation at 1 wk, %	Best SWAT Reduction, %
1	3.2	0.0	100	0	100	2.5	22	29
2	2.2	0.6	73	0.7	69	1.8	18	94
3	1.8	0.9	50	0	100	1.9	-6	95
4	2.2	0.2	91	0	100	3.4	-55	0
5 <sup>c</sup>	...	...	...	0.2	...	...	...	83
6	2.4	0.5	79	0.1	96	1.0	58	81
7	3.1	0	100	1.1	65	1.1	65	77
8	1.6	0.2	88	0.7	56	1.9	-19	21
9	4.8	0	100	0	100	3.6	25	90
10	4.1	0	100	0	100	3.0	25	100
11	2.9	0.4	86	0.5	84	1.9	33	97
12	4.5	0.3	93	0	100	5.0	-11	100
13	15.5	0	100	0.7	95	7.8	50	89
14	8.2	0.7	91	0.1	99	9.0	-10	89
15	3.4	0	100	0	100	2.61	23	67
16	5.17	0	100	0	100	6.63	-28	18
17	1.23	0	100	0	100	3.2	-60	33
18	6.6	0	100	1.7	74	6.0	9	71
19	10.6	0	100	0	100	4.4	58	100
20	10	0	100	0	100	7.5	25	55
21	9.3	0	100	0	100	4.2	55	100
Mean	5.14	0.19	93	0.28	92	3.92	14	71

Abbreviations: AGT, O6-alkylguanine DNA alkyltransferase; SWAT, severity weighted assessment tool.

<sup>a</sup>The AGT activity at baseline for three normal white controls was 0.1, 0.1, and 0.7; the AGT activity at baseline for three normal African American controls was 1.3, 3.7, and 4.0.

<sup>b</sup>AGT activity is expressed as femtomoles of O6-methylguanine removed per microgram of DNA.

<sup>c</sup>Protein from lesional samples for patient 5 was insufficient for the AGT assay.

### EFFECT OF O6-BENZYLGUANINE ON AGT ACTIVITY

Lesional specimens obtained at 6 hours after O6-benzylguanine infusion exhibited dramatically reduced AGT activity to a mean reduction of 93%. A positive correlation was found for baseline AGT activity vs AGT inactivation at 6 hours (Spearman correlation coefficient  $\rho=0.59$ ,  $P=.007$ ). At 24 hours after infusion, AGT activity was significantly regained in 4 patients (patients 2, 7, 8, and 18), while further and complete inactivation occurred in 3 patients (patients 3, 4, and 12). Overall, 15 of 21 patients experienced 100% AGT depletion within the first 24 hours, although only 12 remained 100% depleted at 24 hours.

One week after O6-benzylguanine infusion, AGT activity rebounded to a mean (SD) of 3.92 (1.03) fmol/ $\mu$ g or 14% inactivation, although 5 patients still maintained moderate inactivation at levels from 50% to 65%. AGT activity rebounded to a level greater than baseline in 7 patients. The AUC of AGT activity from 0 to 168 hours negatively correlated with baseline AGT ( $\rho=-0.62$ ,  $P=.004$ ).

### CLINICAL RESPONSE TO O6-BENZYLGUANINE/BCNU

O6-benzylguanine–carmustine produced clinically significant responses, resulting in 4 complete responses (CR), 12 partial responses (PR), 2 marginal response, 2 stable disease responses, and 1 progressive disease (PD), yielding an overall response rate (CR + PR) of 76% (95% CI,

0.55-0.89). Partial responses were substantial, with 82% mean SWAT reduction. Target lesions reflected similar responses. Of 59 total target lesions, 39% cleared completely, 34% decreased in size, 10% remained unchanged, and 17% increased in size.

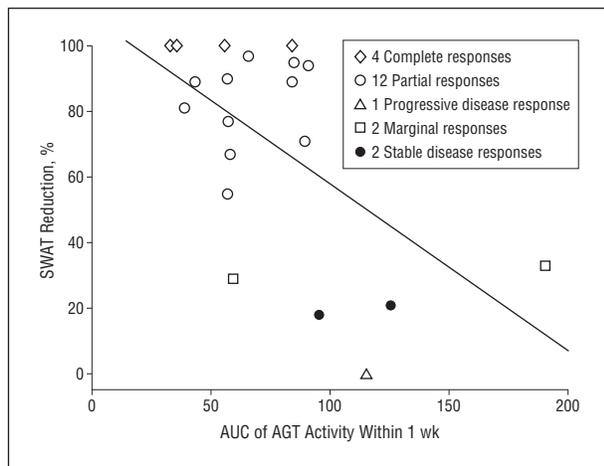
Among the 12 PRs, 6 discontinued therapy due to disease relapse. The other 6 patients discontinued therapy for various reasons while maintaining a PR (Table 1). Clinical responses correlated strongly with AGT inactivation at 168 hours ( $\rho=0.51$ ,  $P=.02$ ), and mean AGT inactivation within 168 hours ( $\rho=0.48$ ,  $P=.03$ ). The greatest and most statistically significant correlation with SWAT reduction was the AUC of AGT activity over the 0- to 168-hour period ( $\rho=-0.56$ ,  $P=.01$ ); the lower AUC of AGT activity correlated with greater SWAT reduction (**Figure**). Logistic regression analysis confirmed that the AUC of AGT activity was a significant predictor of clinical response; for every 10-fmol/ $\mu$ g DNA-day increase of AUC, the odds of achieving clinical response (PR + CR) was decreased by 2.1-fold ( $P < .05$ ). No significant correlations were found between absolute AGT activity values and SWAT reduction.

### COMMENT

Ideally, for most cases of early-stage disease (stage IA, or limited patches or plaques with <10% BSA involved), skin-directed therapies such as topical steroids, bexarotene, nitrogen mustard, and carmustine, as well as phototherapy, are generally used before proceeding to systemic therapies.<sup>3,41</sup> Since many patients ex-

haust the aforementioned skin-directed therapies during their lifetime, expanding and improving existing skin-directed therapies, such as BCNU, is desirable.

Most of the published data on topical carmustine for MF has come from Zackheim et al<sup>8,42</sup> and Zackheim<sup>43</sup>. Originally, Zackheim et al treated their patients with carmustine doses varying from 40 to 60 mg carmustine daily over 2 to 3 weeks, which was associated with myelosuppression, prompting a dose reduction to 10 mg daily over 6 to 12 weeks for most patients treated from 1980 to 1986; however, the lower dose produced inadequate responses in some patients. Subsequently, 20 mg of carmustine daily over 4 to 8 weeks provided an optimal balance between response and toxic effects. At 10 to 25 mg carmustine daily for 3 to 17 weeks (total dose, 200-1360 mg), a 7.4% rate of grade 1 or 2 leukopenia was reported. Mild increases in AST were also observed in 2 patients. Inflammatory cutaneous reactions manifesting as erythema or hyperpigmentation were seen in almost all patients, often accompanied by persistent tenderness and telangiectasia. Response rates were 66% CRs and 26% PRs among the 109 early-stage patients treated.<sup>8</sup> However, these results included the use of high-dose carmustine (40-60 mg) for the first 8 years of the study, which may have produced higher response rates. Data on toxic effects specific to these higher doses were not reported.



**Figure.** Scatterplot demonstrating negative correlation between clinical severity weighted assessment tool reduction and area under the curve of O6-alkylguanine DNA alkyltransferase activity.

O6-benzylguanine–carmustine toxic effects compare favorably against those of topical carmustine alone (**Table 3**). Telangiectasias and hyperpigmentation may occur less often with O6-benzylguanine–carmustine compared with a treatment course of topical carmustine alone. The lower frequency of cutaneous reactions is likely related to the lower frequency and less cumulative dosing of carmustine application. The mean carmustine doses used in this trial were approximately 30% of the doses used for carmustine alone. Observed adverse effects that are not seen with topical carmustine alone included mild transient postinfusion headaches, dizziness, and fatigue. Toxic effects of topical carmustine have not been attributed to O6-benzylguanine alone in other clinical trials.<sup>26-36</sup>

Taken together, O6-benzylguanine–carmustine may be advantageous over carmustine alone owing to less total drug exposure and less toxicity, allowing a longer duration of treatment. At the dose levels evaluated, however, response rates of O6-benzylguanine–carmustine seem to be lower but were still robust. A difference in patients' resistance to therapy might also account for the difference in response rates. While Zackheim et al<sup>8,42</sup> and Zackheim<sup>43</sup> reported a median of 4 months between MF diagnosis and therapy, the median duration in this study was 78 months, which may indicate a more refractory patient population. Indeed, 4 of the 5 nonresponders were the most refractory.

The AGT pharmacodynamics data reveal a prominent interrelation between AGT activity and SWAT reduction. The skin of patients with CTCL demonstrated elevated baseline AGT activity relative to controls, which may confer selective therapeutic targeting against malignant T-cells vs normal skin. These results mirror those of in vitro studies showing that tumor cells with high AGT activity demonstrate the greatest O6-benzylguanine enhancement of carmustine cytotoxicity.<sup>21</sup> The importance of prolonged AGT inactivation in determining response suggests that the ability of MF cells to regenerate AGT may play an important role in O6-benzylguanine–carmustine resistance. The consequences of long-term intermittent AGT inactivation in humans is unknown but is of potential concern for mutations if O6-methylguanine adducts are formed; however, knockout mice live normally.<sup>44</sup> Human studies of O6-benzylguanine infusions have not shown an adverse impact of repeated dosing.<sup>15</sup>

**Table 3. Comparison of Topical Carmustine With O6-Benzylguanine vs Topical Carmustine Alone**

Agent	Dose Frequency	Carmustine Dosing Regimen	Mean Total Dose of Treatment Course, mg	Overall Response, %	Cutaneous Reaction Incidence, %	Leukopenia
Carmustine alone <sup>a</sup>	Daily <sup>b</sup>	10 mg for 6-12 wk <sup>8</sup> 20 mg for 4-8 wk <sup>8</sup> 40-60 mg for 2-3 wk <sup>8</sup>	1100 <sup>9</sup>	55 CR, 31 PR <sup>8</sup>	About 100 <sup>8</sup>	Grade I-II <sup>8c</sup>
O6-Benzylguanine–carmustine	Once every 2 wk	10-40 mg for mean duration of about 28 wk	309.5	19 CR, 57 PR	About 52	Grade I

<sup>a</sup>For patients with stages IA to IVA disease; patients in stages IIB and IVA received radiation therapy to tumors (and carmustine to patches or plaques).

<sup>b</sup>Patients with less than 5% skin involvement treated twice daily.

<sup>c</sup>Data only for 95 patients treated with 10 to 25 mg carmustine daily for 3 to 17 weeks.

It is interesting to note that Dolan et al<sup>45</sup> reported low AGT protein levels in MF skin lesions measured by quantitative immunofluorescence microscopy using monoclonal AGT antibodies. The difference from the results in this trial may be attributed to the method used to detect AGT. Detection of AGT functional activity is likely a more sensitive method of detecting AGT and has greater clinical significance in reflecting carmustine resistance.

In conclusion, O6-benzylguanine-potentiated topical carmustine allows an alternative dosing schedule that may be more favorable for some patients with less toxic effects than topical carmustine alone and represents a skin-directed treatment with less significant toxic effects than currently available systemic modalities; as such, it may fill an important niche in the CTCL treatment paradigm. Given the safety and efficacy observed, the trial was closed without reaching the MTD to initiate a phase I and II trial, which is currently under way to evaluate the toxic effects and efficacy of *consecutive day treatments* of O6-benzylguanine to prolong AGT inactivation.

Assuming efficacy is maintained in larger studies, O6-benzylguanine–carmustine may be considered prior to systemic therapies in early-stage MF refractory to other skin-directed therapies. Treatment visits for O6-benzylguanine–carmustine can last several hours but occurred once every 2 weeks, which may represent an attractive option for patients uninterested in daily topical treatments and/or self-administered therapies.

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