CASE WESTERN RESERVE UNIVERSITY/UNIVERSITY HOSPITALS
IRELAND CANCER CENTER

TITLE: **PHASE I/II MULTICENTER CLINICAL TRIAL OF O^6BENZYLGUANINE AND TOPICAL CARMUSTINE IN THE TREATMENT OF REFRACTORY EARLY-STAGE (IA-IIA) CUTANEOUS T-CELL LYMPHOMA.**

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TREATMENT SCHEMA: PHASE I/II TRIAL OF O^6BG AND BCNU IN CTCL
<u>Major Aim 1:</u> To determine the CTCL response rate and safety of O^6BG/BCNU when given as two biweekly consecutive daily doses.

120 mg/m^2 O^6BG IV over 1 hr + topical BCNU (see below) 1 hour after infusion on Day 1
120 mg/m^2 O^6BG IV over 1 hr
Repeated Q2 weeks.

Topical BCNU dose escalation in 1 patient cohorts:
Dose Level 1 - 20 mg after O^6BG IV (Day 1) + O^6BG IV (Day 2)
Dose Level 2 - 30 mg after O^6BG IV (Day 1) + O^6BG IV (Day 2)
Dose Level 3 - 40 mg after O^6BG IV (Day 1) + O^6BG IV (Day 2)

To serve as a control for apoptosis and proliferation studies, 1 lesion that is amenable to biopsy will be left untreated during the first cycle of therapy in all patients (see Table 1a and 1b). Treatments will be given q 2 weeks for 6 months or a total of 12 courses of therapy.

Table 1a. Treatment, Biopsy, and Blood Monitoring Schedule

	Week 1							**Wk 2**
Cycle Day	1	2	3	4	5	6	7	8
O^6BG	X	X						
BCNU	X	X						
Biopsy	X[1]	X[1.1]	X[1.2]					X[1]
Blood	X[2]							X[2]

1–At Cycle 1, BCNU-treated and BCNU-protected lesional biopsies will be performed according to the biopsy schedule detailed in Table 1b with patient enrollment alternating between Biopsy Schedule Arm 1 (1.1) and Biopsy Schedule Arm 2 (1.2)
– On week 13 of treatment (7th cycle), patients who have not achieved at least minor response will be requested for a 6 mm punch biopsy at baseline and a 4 mm punch biopsy at 24 hours for repeat AGT activity and mutational *MGMT* gene sequencing assays.
2–Lab monitoring will be performed once weekly for the first 4 wks, and then every 2 wks thereafter.

Table 1b. Detailed Cycle 1 Biopsy Schedule

Biopsy Time Post-O^6BG	**0 hrs**	**24 hrs**	**48 hrs**	**1 wk**
Arm 1 BCNU Treated Site	X[a]	X[a,b]		X[a]
Arm 1 BCNU Protected Site		X[b]		
Arm 2 BCNU Treated Site	X[a]		X[a,b]	X[a]
Arm 2 BCNU Protected Site			X[b]	

a– 4 mm punch biopsy (x1), fresh frozen, for AGT assay.

b– 6 mm punch biopsies (x1), fresh frozen (immunohistochemistry), apoptosis, cell cycle, and proliferation studies. Alternatively, a 1.0 cm length elliptical excision may be performed for the X[a,b] biopsies.

1.0 OBJECTIVES

This single-arm multicenter trial will consist of a fixed intravenous dose of O^6BG (120 mg/m^2) given as a bolus over one hour, followed by whole-body application of BCNU, with the regimen repeated 24 hours later on a consecutive day. Each treatment cycle will be repeated every two weeks for 6 months or a total of 12 treatment cycles. This Phase I/II trial has two major objectives:

1.1 Objective 1: To determine the CTCL response rate and safety of O^6BG/BCNU when given biweekly as two consecutive daily doses

1.2 Objective 2: To determine the laboratory correlates of clinical response and drug efficacy based upon the following surrogate marker studies:

a. AGT activity in CTCL lesions will be examined to determine the effects of consecutive day O^6BG administration on the extent and duration of AGT depletion.
b. Degree of induction of apoptosis and cell cycle arrest will be examined in the malignant T-cell population of lymphomatous tissue and in the constitutive cells of the skin to determine drug efficacy and toxicity through immunohistochemical techniques.
c. MGMT gene mutations and changes in AGT expression will be examined as potential mechanisms for O^6BG resistance in non-responding patients.

2.0 BACKGROUND

2.1 Clinicopathologic Features of Cutaneous T Cell Lymphoma (CTCL):

CTCL manifests most commonly as mycosis fungoides (MF), an epidermotropic skin infiltrate of atypical CD4+ helper T-cell clones, and its erythrodermic, leukemic variant Sézary syndrome.[1] The clinical presentation of MF is pleiomorphic, and includes patches, plaques, tumors, and erythroderma with other clinical variants such as hypopigmented and follicular MF being less common. MF has an incidence rate of 0.45 per 100,000 person-years based on the most recent epidemiological data from 1992.[2] An increase in the incidence rate occurred between 1973 and 1983, and may be due to improved and earlier diagnosis, though the rate has since stabilized.[3] While the mortality rate of MF improved by 22% between 1979 and 1991, diagnosis in the earlier stages of disease is a likely major contributing factor.[3] The black population is known to carry a higher incidence—1.7 times higher than whites—and has a worse prognosis. To date, the etiology of CTCL remains unknown, with no evident significant association with HTLV-I/II or occupational exposure, though superantigen stimulation is generally thought to play some role in disease stimulation.[1,4-6]

2.2 Prognosis and Therapy of CTCL

Prognosis and survival of CTCL is primarily influenced by stage at presentation.[1,7] Overall, survival of patients with Stage IA MF (less than 10% patch/plaque involvement) is similar to age-matched controls, whereas patients with more advanced stages have progressively lower median survivals: approximately 12 years for Stage IB (greater than 10% patch/plaque

involvement), 10 years for Stage IIA (patch/plaque disease with clinically palpable lymph nodes and negative nodal histology), 3 years for Stage IIB (tumor disease), 4.5 years for Stage III (erythroderma), and 2 years for extracutaneous Stage IV disease.[1,7-10]

No standard or consistently curative therapy exists for CTCL. Goals of therapy are to induce complete remission, reduce tumor burden, relieve symptoms, and to prevent disease progression. Since early-stage MF follows a more indolent and chronic course, treatment toxicity is of high consideration in the choice of therapy, and treatment objectives can be met with conservative skin-directed therapies. Progression to late-stage disease ultimately requires more aggressive therapies such as systemic chemotherapy, which induce greater toxicities like myelo- and immunosuppression. Common skin-directed initial therapies for early-stage MF may include topical mechlorethamine hydrochloride (nitrogen mustard), topical bexarotene, and PUVA/UVB phototherapy.[11-16] While such therapies are capable of inducing response rates ranging from 40% for topical bexarotene to 95% for PUVA, relapses are very common.[14,16] Given the generally good prognosis of early-stage disease, patients may consequently exhaust these conservative treatment options during their lifetime, and may then require more aggressive and toxic treatments such as interferon-α, systemic retinoids, and total-skin electron beam therapy.[11] Short and long-term use of conservative skin-directed therapies may also be associated with significant toxicities, including allergic contact dermatitis with nitrogen mustard, cutaneous irritation with bexarotene, and risk of skin cancer with nitrogen mustard and phototherapy.[11-17] Thus, in the treatment of early-stage MF, there remains a great need for additional modalities that produce durable responses while maintaining a good toxicity profile.

2.3 Treatment of CTCL with BCNU

The nitrosourea carmustine (BCNU) has been an available alternative treatment option for early-stage MF, although the modality has generally fallen out of favor due to its higher toxicity relative to topical nitrogen mustard and other skin-directed conservative therapies. Zackheim *et al* has performed the most extensive clinical investigation of topical BCNU therapy for MF, treating 143 MF patients over 15 years using dose schedules ranging from as high as 40 to 60 mg BCNU daily for 2 to 3 weeks to as low as 10 mg daily over 6 to 12 weeks.[18] Due to toxicity at the higher doses, and inadequate response at the lowest dose, a course of 20 mg BCNU daily over 4 to 8 weeks was found to provide an optimal balance between response and toxicity. Over the entire course of the study, topical BCNU achieved a 66% complete response (CR) and 26% partial response (PR) rate among the 109 early-stage (Stage IA-IIA) MF patients treated. Zackheim *et al* has suggested that topical BCNU may produce higher CR rates and more durable remissions than nitrogen mustard, and that BCNU may also have a shorter time to CR (11.5 weeks versus 7.9 months). However, topical BCNU incurs systemic toxicity not seen in the use of nitrogen mustard. In patients receiving 10 to 25 mg BCNU daily, a 7.4% rate of National Cancer Institute Common Toxicity Criteria (NCI-CTC) Grade 1 and 2 leukopenia was observed. Mild increases in AST were also observed in 2 of 143 patients. Inflammatory cutaneous reactions manifesting as erythema or hyperpigmentation were seen in almost all patients, often accompanied by persistent tenderness and telangiectasia. Given the responses demonstrated, if topical BCNU could be administered with fewer side effects, it could become a more accepted therapeutic option for CTCL.

2.4 BCNU and AGT Overview

As an alkylating agent, BCNU is known to initiate DNA damage through chloroethyl alkylation at the O^6 site of guanine to form O^6-chloroethylguanine.[19] Formation of the BCNU adduct is followed by intramolecular rearrangement to the cyclized intermediate O^6,N^1-ethanoguanine. An ethyl bridge subsequently forms between the N^1-guanine and the N^3-cysteine of the opposite complementary DNA strand to complete a cytotoxic 1-(N^3-deoxycytidyl)-2-(N^1-deoxyguanosinyl)-ethane interstrand crosslink, which is thought to initiate apoptosis in affected cells .[20] There are little data on BCNU pharmacodynamics, but a study of DNA interstrand cross-linking using 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea in human colon carcinoma xenografts in nude mice demonstrated that crosslinking begins and increases for approximately 8 hours with little change up to 19 hours.[21] In vitro studies support similar findings.[22,23] However, BCNU-induced interstrand cross-linking has been unmeasurable in humans, likely due to the relatively low number of adducts formed, and even less is known about cross-link formation induced by topical BCNU in the cutaneous setting.

A protective cellular defense exists, however, which provides an escape mechanism for nitrosourea resistance via an intranuclear DNA repair protein O^6-alkylguanine DNA alkyltransferase (AGT, also known as O^6-methylguanine-DNA methyltransferase, or MGMT), which covalently transfers the BCNU alkyl adduct to its own cysteine residue, thereby preventing crosslink formation.[24-27] Following repair, the alkylated AGT is irreversibly inactivated and is ubiquinated and degraded through the ubiquitin/proteosomal system. Thus, the ability for normal and tumor cells to repair O^6-alkylguanine is dependent on the amount of AGT synthesized. Evidence for the AGT mechanism for BCNU resistance has been observed in both in vitro cell line and in vivo human xenograft studies, which have demonstrated that AGT depletion increases the sensitivity of cells to nitrosourea cytotoxicity.[28-38] Elevated AGT levels have been observed in a number of malignancies, including colon cancer, glioma, lung cancer, breast cancer, rhabdomyosarcoma, acute myeloid leukemia, CTCL, and multiple myeloma.[39-42] This increased activity is thought to contribute to the primary and secondary resistance of these tumors to the nitrosoureas and related agents.

2.5 Modulation of Alkyltransferase with O^6 Benzylguanine

The drug O^6-benzylguanine (O^6BG) has been developed as an AGT substrate, which potently and irreversibly inactivates AGT by transferring its benzyl group to the alkyl-binding cysteine residue on AGT, thereby preventing DNA damage repair and allowing cancer cells to become vulnerable to nitrosoureas.[43,44] In both tumor cell lines and human tumor xenograft models, O^6BG has been found to enhance the sensitivity of malignancies to nitrosoureas.[45-50] The majority of experience with O^6BG in human subjects have focused on the pharmacokinetics, pharmacodynamics, and toxicity of the drug.[43,51-56] Results of a few Phase I and II O^6BG /BCNU trials have been published, and numerous other trials are ongoing.[39,40,51,52,54,57] Preliminary studies will be discussed in the following 3 sections: Preclinical studies of O^6BG/BCNU. Clinical studies of O^6BG/BCNU, and Phase I study of O^6BG/BCNU in CTCL treatment.

Preclinical Studies of O^6BG/BCNU

In vitro cytotoxic studies of O^6BG have been performed on a variety of human tumor cell lines, including colon, glioblastoma, and HeLa, and have demonstrated an enhancement of the cytotoxic effects of nitrosoureas with lower AGT levels, with the greatest enhancement of cytotoxicity occurring in cells with high basal AGT activity.[48,50] O^6BG has also been shown to increase the antitumor effects of BCNU in human tumor xenografts in mice, including medulloblastoma, glioma, and colon carcinoma models.[45-47,49] In some studies, nitrosourea-resistant tumors were observed to respond to BCNU given in combination with O^6BG , but not to BCNU alone.[45,47] In vivo studies also demonstrated correlation between augmentation of antitumor activity and mitigation of AGT activity. Two studies have attempted to address the question of the affect of O^6BG /BCNU on tumors with low AGT activity; however, contrasting observations were made with one demonstrating no enhancement of cytotoxicity in human tumor cell lines, and the other observing increased antitumor activity in human glioma xenografts.[48,58] It has also been discovered in tumor cell lines that treatment with O^6BG /BCNU may give rise to O^6BG-resistant forms of AGT through point mutations and in mismatch-repair deficient cells.[59,60]

The metabolism of O^6BG was first described in animal studies using radiolabelled O^6BG administered intraperitoneally in rats.[61] The major metabolite of O^6BG was found to be 8-oxo-benzylguanine, which is nearly as potent as the parent compound in inhibiting AGT. There is evidence that the cytochromal P450 system may play a major role in this process.[62] Studies of intravenous O^6BG in monkeys demonstrated that AGT in peripheral blood mononuclear cells (PBMCs) is reduced by 98% at 2 hours and 100% at 18 hours post-infusion.[63] A study of intraperitoneal O^6BG in mice has demonstrated quick recovery of AGT activity in the bone marrow, beginning at 12 hours, and reaching 91% of controls at 48 hours.[64] This study also demonstrated potentiated myelotoxicity in the mice receiving O^6BG /BCNU, although none was seen with O^6BG alone. However, CNS symptoms of lethargy, hypoactivity, prostration, and ataxia have been demonstrated in pre-clinical studies using single IV doses of 120 mg/m^2 to 315 mg/m^2 O^6BG on mice.[49]

Clinical studies of O^6BG/BCNU

The pharmacokinetics of O^6BG in humans is consistent to that observed in the preclinical studies. After infusion, O^6BG is rapidly converted to 8-oxo-benzylguanine and after 5 hours post-infusion, is undetectable in human plasma.[43] Thus, 8-oxo-benzylguanine is thought to exert most of the AGT inactivating effects. Analysis of AGT inactivation in PBMCs demonstrated depletion within 1 hour of O^6BG infusion with AGT activity recovering to approximately 50% at 72 hours post-infusion. To date, there have been two Phase I trials published on the use of O^6BG alone and two Phase I and one Phase II trial published on the combination of O^6BG and systemic BCNU in advanced solid tumors.[51,52-54,57] Treatment of malignant gliomas has been a primary focus of systemic BCNU because it is considered the gold standard of care for these highly chemotherapy-resistant cancers.

In a Phase I trial of O^6BG in malignant gliomas, 100 mg/m^2 O^6BG significantly inhibited tumor AGT activity to < 10 fmol/mg protein for at least 18 hours post-infusion.[53] No responses or toxicities were noted in the treated patients. Another Phase I trial by Spiro et al examined the

efficacy of various O^6BG dose levels (10-120 mg/m^2) in depleting AGT activity in 30 patients with resectable solid tumors, and established the 120 mg/m^2 over 1 hour dose as being the dose at which AGT activity is undetectable at 18 hours.[51] Subsequent cycles were given with low-dose systemic BCNU, which did not affect O^6BG kinetics or AGT depletion in PBMCs. Spiro et al also observed that AGT depletion in PBMCs does not reflect depletion in tumor tissues. No toxicity was observed in this trial with the exception of transient lymphocytopenia that was not dose-limiting. Dolan et al performed a Phase I trial directly comparing the AGT depletion capability of O^6BG at the 100 mg/m^2 and 120 mg/m^2 doses and confirmed 120 mg/m^2 as the dose that more consistently produced 100% depletion.[56] However, recent examination of O^6BG depletion of AGT in anaplastic gliomas determined that this dose achieved 100% depletion in only 45% patients at 18 hours, although the result was 94% at 6 hours.[65]

Among the Phase I O^6BG/BCNU maximal tolerated dose (MTD)-finding trials, Spiro et al conducted a dose escalation of BCNU and found the maximal tolerated dose (MTD) of O^6BG/BCNU to be 33 mg/m^2 BCNU with 120 mg/m^2 O^6BG.[51] Thrombocytopenia and neutropenia were identified as DLTs at high doses of BCNU. One patient with metastatic colon cancer achieved a partial response for 13 months and one other patient maintained stable disease for 20 months. The O^6BG/BCNU Phase I trial by Friedman et al on glioma patients also reported thrombocytopenia and neutropenia as toxicities, and found the MTD to be at 100 mg/m^2 O^6BG and 40 mg/m^2 BCNU given every 6 weeks. No objective responses were observed among 26 patients.[54] In another Phase I combination trial, Schilsky et al found no toxicity with O^6BG alone, but observed bone marrow suppression as the DLT with O^6BG/BCNU with the MTD being 120 mg/m^2 O^6BG and 40 mg/m^2 BCNU.[52] This study identified a possible threshold level of 40 mg/m^2 O^6BG, above which there is maximal AGT depletion of bone marrow progenitor cells. Other toxicities described included fatigue, anorexia, and increased bilirubin and transaminase. No responses were observed in this trial. Given these MTD data, a Phase II trial was conducted, treating 18 glioma patients with 120 mg/m^2 O^6BG and 40 mg/m^2 BCNU every 6 weeks. Three patients maintained stable disease on this protocol with NCI-CTC Grade 3 and 4 neutropenia, anemia, and thrombocytopenia being reported.

<u>Phase I trial of O^6BG/BCNU in the treatment of CTCL</u>

The Phase I trial (T97-0029) of O^6BG with topical BCNU in early-stage (Stage IA-IIA) refractory mycosis fungoides has been the first to utilize the topical formulation of BCNU with O^6BG and has been the first to examine the effects of the combined therapy on AGT levels in the skin.[39] This trial, which has completed enrollment, has treated 21 patients using a 120 mg/m^2 O^6BG IV bolus given over 1 hour followed by whole body low-dose topical BCNU at 1 hour post-infusion; cycles were repeated every 2 weeks. Topical BCNU was started at a 10 mg dose level and was dose-escalated in 10 mg increments in cohorts of 3 patients. The trial completed enrollment at the 40 mg BCNU dose level with one patient experiencing dose-limiting toxicity. Although a true maximal tolerated dose (MTD) was not achieved, this Phase I trial established 40 mg BCNU as a safe and tolerable dose. The clinical efficacy and safety data from this trial was sufficiently promising to initiate the current Phase I/II study.

Overall, minimal hematologic toxicity occurred with few transient sporadic cases of NCI-CTC Grade 1 myelotoxicity consisting of leukopenia (n=4), anemia (n=2), and thrombocytopenia (n=1). One case of Grade 2 anemia was treatment-limiting. The most frequent adverse events included transient post-therapy headaches (48%), contact dermatitis (43%), nausea (34%), and fatigue (38%). Nearly all cases were NCI-CTC Grade 1 toxicity with the exception of one case each of transient Grade 2 and Grade 3 toxicity headache, one case of Grade 2 nausea, and 3 cases of Grade 2 and 1 case of Grade 3 dose-limiting contact dermatitis. Two cases of Grade 3 transaminase elevation also occurred with the adverse event being treatment-limiting in both cases; hepatotoxicity resolved in both cases with treatment discontinuation.

AGT activity was examined from CTCL lesional biopsy specimens taken at baseline, and at 6, 24, and 168 hours (1 week) post-O^6BG infusion. Compared to baseline, AGT activity was diminished by a median of 100% at 6 hours, 100% at 24 hours, and 23% at 1 week post-O^6BG infusion. However, only 12 of 20 patients maintained 100% AGT depletion at 24 hours of infusion. Results from the AGT data demonstrate that the area-under-the-curve (AUC) of AGT activity had a statistically significant negative correlation with skin disease score reduction, indicating that lower AGT activity over the entire 1-week post-infusion period was associated with response. Baseline AGT levels were similarly negatively correlated with AUC of AGT activity. Higher baseline AGT levels predicted lower AGT activity during the entire 1-week period. Another interesting observation is that patients who did not clinically respond as well at the lower BCNU dose levels tended to regenerate AGT activity at the 1-week time point at a higher level than responding patients, and in some cases at a level higher than pre-therapy baseline AGT. Statistically, this reflected in a positive correlation between AGT inactivation at the 1-week time point and skin disease score reduction. This observation may indicate that, in poorly responding patients, the ability of CTCL cells that do not sustain lethal BCNU DNA damage may recover and upregulate AGT expression, leading to greater rates and levels of AGT regeneration. Thus, increased BCNU cytotoxicity through higher BCNU doses or prolonged therapy against CTCL cells might stem the ability to regenerate AGT in these poorly responding patients.

O^6BG and BCNU on this dosing schedule has produced 13 partial responses (8 with disease reduction greater than 75%), and 3 complete responses, yielding an objective response rate of 76% in refractory MF patients (median of 2 prior therapies). The mean disease improvement for all treated patients has been 68% based on SWAT skin scoring and partial responses have occurred at a median of 5 cycles of treatment or 2.6 months. When specifically evaluating the patients treated on the 30-40 mg BCNU dose levels the response rate is 83% with a 25% CR rate. Among all O^6BG/BCNU combination trials, this Phase I trial is one of two to demonstrate significant clinical response; the other trial achieving a 30% response rate in multiple myeloma patients.[19] Furthermore, these responses were achieved while exposing patients to a fraction (7%) of the daily BCNU dose used in conventional topical therapy.

2.6 Rationale

Despite the high overall response rates observed in the phase I trial, higher CR rates will be required to make the O^6BG/BCNU combination therapy a feasible therapeutic option when compared to the CR rates of conventional therapies, specifically topical BCNU alone. Since AGT

maintained total depletion in only 60% of patients through 24 hours, enhanced AGT depletion could translate into improved response; this could be achieved through an alternative O^6BG treatment schedule, such as reinfusion of a second dose of O^6BG 24 hours after the first. Consecutive daily dosing of O^6BG over 5 days is currently being utilized in one ongoing trial involving temozolomide in the treatment of childhood solid tumors. With the evidence that significant AGT depletion occurs between 6 hours and through 24 hours, there would appear to be a potential added benefit from exposing AGT depleted CTCL cells to longer durations of BCNU-induced DNA damage. This prolonged exposure could be gained through the administration of a second application of topical BCNU at 24 hours post-O^6BG infusion.

This Phase I/II clinical trial is being conducted to investigate the benefits and potential improvement in response rates that may be achieved with alternative O^6BG/BCNU treatment schedules utilizing two consecutive daily dosing. This Phase I/II clinical trial seeks to optimize the promising clinical efficacy and toxicity profile of O^6BG/BCNU that was observed in the phase I trial, and also serves to elucidate mechanisms of treatment and disease response. The results of this trial will support the ongoing search for novel therapeutic alternatives for CTCL patients.

Based on initial observations of 3 patients enrolled in this current study, we have noted dose limiting toxicities w/ respect to pruritis or pain in areas associated with lesions undergoing treatment when splitting the 40 mg BCNU over 2 days (20 mg BCNU day 1 and 20 mg BCNU day 2). The adverse effects appeared to resolve with a lower dose schedule of BCNU (20 mg BCNU day 1 and 10 mg BCNU day 2). Based on histology of a patient who developed an adverse event (AE), there was epidermal pallor and desquamatation and keratinocyte necrosis. These AEs occurred in areas of skin occlusion such as the axillae, inguinal regions, and thighs, also suggesting that the toxicity was related to higher local concentrations of topical BCNU. Because this did not occur w/ the single application of BCNU of up to 40 mg (as evidenced in the Phase I study), we feel that the second BCNU application induced keratinocyte damage, leading to the witnessed AEs. We are therefore modifying this protocol for patient safety. Specifically, we are removing the 2nd dose of BCNU but maintaining the second dose of O^6BG on day 2 in order to continue to test our initial hypothesis that enhanced AGT depletion (via a 2 day dosing schedule for O^6BG) can translate into improved response.

If we are able to achieve a 40 mg BCNU dose with 2 infusions of O^6BG, then we will be able directly compare the results, including safety, efficacy, and duration of MGMT inhibition, of this trial to the prior Phase I trial in which 6 patients were treated with 1 dose of 40 mg of BCNU and 1 infusion of O^6BG.

3.0 PATIENT SELECTION

3.1 Patient eligibility

3.1.1 Diagnosis of CTCL stages IA-IIA by histopathology and immunohistochemistry in screening biopsies confirmed at Case Western Reserve University within 6 months of enrollment. Biopsies may be performed at the site of collaborating institutions and shipped to UHC-CWRU.

3.1.2 Performance status ECOG grade 0, 1, or 2.

3.1.3 Patients must have recovered from toxicity of prior treatment and have received no CTCL therapy other than emolliation for at least 4 weeks, with the exception of topical corticosteroids, which may be used up to 2 weeks before the trial start date.

3.1.4 Patients must have signed a consent form indicating the investigational nature of the treatment and its potential side effects.

3.1.5 Adequate hepatic, renal, metabolic, pulmonary and bone marrow function as defined by the following parameters:

- Marrow - WBC at least 3.5 x10E9/L, ANC at least 1.6 x10E9/L , platelets > 100,000/µl
- Hepatic - bilirubin < 1.5 mg/dL; SGOT within normal range.
- Renal - creatinine $\leq$ 1.5 mg/dL
- Metabolic - electrolytes normal; glucose - controlled (diet and insulin) diabetes is permitted.
- Pulmonary - Demonstration of clinically normal lung function based on history and physical examination. Patients with clinical evidence of pulmonary disease as determined by the investigator should have baseline lung function tests performed with demonstration of DLCO $\geq$70%. A DLCO single breath, adjusted for hemoglobin, will be utilized. We will not use DLCO/VA for inclusion or exclusion in this study.

3.1.6 Age > 18 years

3.1.7 Patients must have cutaneous disease that is amenable to biopsy and must be willing to undergo several sequential biopsies.

3.1.8 Must have failed at least one conventional treatment for CTCL other than topical corticosteroids. This includes phototherapy, topical mechlorethamine, topical or oral bexarotene, radiation therapy, photopheresis, chemotherapy, and immunomodulatory agents such as interferon and other retinoids.

3.1.9 Inclusion of Women and Minorities

CTCL affects both genders and all racial/ethnic groups. The male/female ratio is about 2:1 and the black/white ratio is also about 2:1. The median age is 55-60, although adults of all ages can be affected. Our patient population reflects these statistics. Therefore, our study population will include both genders and a variety of racial/ethnic groups. Moreover, no patient will be excluded based on gender or racial/ethnic characteristics.

3.2 **Exclusion Criteria**

3.2.1 Patients who have received prior treatment with topical or systemic BCNU or other nitrosoureas.

3.2.2 Patients with known central nervous system involvement or primary CNS malignancies.

3.2.3 Patients with performance status ECOG grade 3 or 4.

3.2.4 Pregnant women, women who are breast feeding infants, or women with reproductive potential not practicing adequate contraception, because of potential toxicity to the fetus or infant.

3.2.5 Patients with an active infection which requires hospitalization, or which may affect the patient's safety if the patient was enrolled.

3.2.6 Patients with pulmonary disease as determined by history, physical examination, chest X-ray, or pulse oximetry with <70% predicted DLCO

3.2.7 CTCL patients with stage IIB-IVB disease.

3.3 REGISTRATION/CANCELLATION

3.3.1 Registration

All patients entering this Phase I/II trial will be registered with the UH-CWRU Clinical Trials Core facility by the principal investigator or his or her designee. Collaborating centers will contact the UH-CWRU Central Patient Registration Center by phone at (216) 844-7394, and/or fax at (216) 844-3092 for new patient registrations on this trial.

3.3.2 Cancellation Guideline

- Only untreated patients will be canceled.
- A letter of explanation will be written to the Director of the Clinical Trials Core Facility documenting the reason for cancellation.
- An on-study form will be submitted to the Clinical Trials Core Facility and the patient will continue to be followed for survival.

4.0 TREATMENT PLAN

A single-arm Phase I/II multi-center trial is planned with enrollment coming from a total of 6 university-based academic institutions that are centers for CTCL referrals, including the coordinating center at University Hospitals of Cleveland, Case Western Reserve University (UHC-CWRU).

4.1 O^6BG and BCNU administration

All patients will receive 120 mg/m^2 O^6BG IV followed by topical BCNU as a total skin application 1 hr after completing the O^6BG infusion on Day 1. A second dose of O^6BG will be given on Day 2 (24 hrs after the first dose). This treatment cycle will be repeated every 2 weeks for 12 treatment cycles over a 6-month trial period, or until the patient is withdrawn from the trial.

4.1.1 O^6BG Administration

For each 2-week cycle, O^6BG is administered intravenously at a dose of 120 mg/m^2 over 1 hour on Day 1 and then 24 hours later on Day 2. Actual body weight and height will be used to calculate the body surface area.

4.1.2 BCNU Administration

BCNU will be applied topically to the total skin surface (excluding the entire scalp and/or face if uninvolved, and always excluding the lips, eyelids, and ulcerated lesions) one hour after completion of O^6BG infusion on Day 1. If a region of skin is hyperpigmented and thought to be due to BCNU, BCNU will not be applied to that region unless there are currently active lesions in that region, and BCNU will only be applied to the active lesions. BCNU will begin at a starting dose of 20 mg on Day 1. Beyond this first dose level, for each of the subsequent four patients enrolled, the BCNU dose will be escalated up to a limit of 40 mg total (given on day 1 only). There will be 3 BCNU dose levels, and BCNU dose levels will be escalated in patient cohorts of 1 each (unless there are dose limiting toxicities, in which case 3 patients will be in each cohort, as described in the next paragraph) as depicted in the following schematic:

Dose Level 1 - 20 mg after O^6BG IV (Day 1) + O^6BG IV (Day 2)
Dose Level 2 - 30 mg after O^6BG IV (Day 1) + O^6BG IV (Day 2)
Dose Level 3 - 40 mg after O^6BG IV (Day 1) + O^6BG IV (Day 2)

Thereafter, additional accrual will be performed at the third BCNU dose level (40 mg). Dose escalation to the next dose level will occur once the patient enrolled at the previous dose level has been observed for 4 weeks (completed 2 cycles) without evidence of a dose-limiting toxicity (DLT), which will be characterized as NCI common toxicity criteria Grade 2 or higher (NCI CTCAE Version 3). If a DLT occurs at any dose level, then a total of 3 patients will be required to be treated at that dose level before enrollment can occur at the next dose level. If 2 patients at any dose-level develop a DLT, then no further dose escalation will be performed and further enrollment will be performed at the prior dose level. If this scenario occurs at the first dose level, then patients will be enrolled at half the dose used in the prior cohort (i.e. if at least 2/3 patients in the a cohort enrolled using a BCNU dose of 20 mg on day 1 develop DLTs, then patients in the next cohort will be enrolled using a BCNU dose of 10 mg on day 1; likewise, if at least 2/3 patients in the a cohort enrolled using a BCNU dose of 10 mg on day 1 develop DLTs, then patients in the next cohort will be enrolled using a BCNU dose of 5 mg on day 1). The initial patients enrolled on the initial dose levels may be dose-escalated once safety at any of the higher dose-levels has been confirmed. Patients will continue to be treated for the entire 6-

month period of therapy unless the patient meets guidelines for withdrawal.

4.1.3 One CTCL lesion on each patient will be left untreated by BCNU during the first cycle for lab correlate studies. (Section 8.0)

4.1.4 Follow-up Procedures

After completion of the 12th cycle of therapy, patients will return for a follow-up and study discontinuation visit On Week 25 (Cycle 12, Day 15) of the trial. During this visit, patients will undergo final scheduled interview, physical, and laboratory evaluations (see Section 10, Table 2). Patients who withdraw or are removed from the study prior to completion of the 12th cycle will be required to attend the follow-up visit 2 weeks after initiation (Day 15) of the last completed cycle.

5.0 DOSE MODIFICATION

5.1 O^6BG Dose modifications: Based on past clinical studies, most adverse events experienced on O^6BG/BCNU are expected to be attributable to BCNU. Occasionally, however, it may be determined that an adverse event is definitely related to O^6BG infusion (e.g. symptoms that only occur during or after infusion, and prior to BCNU application), in which case O^6BG infusion may need to be held or reduced in dosage.

5.1.1 For unresolved Grade 2 or 3 adverse events thought to be related to O^6BG infusion, the next cycle will be held; once the Grade 2 or 3 adverse event has resolved, infusion will be restarted at a lowered 80 mg/m^2 dose. If the Grade 2 or 3 adverse event recurs and remains unresolved after dose reduction, then the patient will be withdrawn from the study. A lowered dose may be increased back to the starting dose if the associated adverse events have resolved at the discretion of the investigator. If more than 1 cycle in a row must be withheld to recover from any adverse event, then the patient will be withdrawn from the study.

5.1.2 For Grade 4 adverse events that are possibly related to the study drug, patients will be withdrawn from the study.

5.2 BCNU Dose modifications:

5.2.1 For unresolved Grade 2 or 3 adverse events thought to be related to BCNU, the next cycle will be held; once the Grade 2 or 3 adverse event has resolved, BCNU may be restarted at one lower dose level (see section 4.1.2). If the patient is already at dose level 1 (20 mg day 1), then the patient will be restarted at a dose that is half of their current dose which resulted in a Grade 2 or 3 adverse event (see section 4.1.2). If the Grade 2 or 3 adverse event recurs and remains unresolved after dose reduction resulting in an additional cycle in a row being held, then the patient will be withdrawn from the study. A lowered dose may be increased back to the starting dose if the associated adverse events have resolved at the discretion of the investigator. If a patient develops a Grade 2 rash thought to be due to the BCNU, we will only hold a cycle of dosing if the rash covers more than 10% of the body surface area. If the Grade 2 rash covers less than 10%

of the body surface area, then we will continue to apply BCNU to the body but avoid the affected areas until the Grade 2 rash resolves. If more than 1 cycle in a row must be withheld to recover from any adverse event, then the patient will be withdrawn from the study.

5.2.2 For Grade 4 adverse events that are possibly related to the study drug, patients will be withdrawn from the study.

6.0 SUPPORTIVE CARE

Tylenol, Benadryl, Compazine, Phenergan, or Ondansetron in the usual doses will be used to manage any constitutional symptoms of headache or nausea and vomiting which may be associated with O^6BG and BCNU. Triamcinolone 0.1% cream or equivalent potency topical corticosteroid may be used twice daily for symptomatic treatment of contact dermatitis, but can only be limited to the areas of the skin suffering the adverse event. Hematopoietic growth factors may be administered at the treating physicians discretion for management of myelosuppression; however, use of hematopoietic growth factors will necessitate the patient's removal from the study. Blood products will be administered at the treating physician's discretion.

7.0 DURATION OF TREATMENT

7.1 Criteria for Removal From the Study

7.1.1 Decision of the patient to withdraw from the study.

7.1.2 Patients who meet criteria for Progressive Disease as defined in Section 9.4, or who experience relapse of disease from the best response by 25% of the baseline SWAT score (Section 9.1).

7.1.3 Patients who require greater than a 2-week delay for recovery (missing more than 1 cycle of therapy) from a NCI common toxicity criteria Grade 2 or higher adverse event or toxicity (NCI CTCAE Version 3).

7.1.4 Patients who miss more than 2 consecutive treatment cycles for any reason, including adverse events.

7.1.5 Patients who require the administration of growth factors.

7.1.6 General or specific changes in a patient's condition which render the patient unacceptable for further treatment in the judgement of the investigator or attending physician.

7.1.7 Completion of the trial after 6 months of therapy.

8.0 CORRELATIVE SPECIAL STUDIES

For purposes of laboratory correlate studies, patients enrolled on the trial will undergo sequential skin biopsies during the first cycle of therapy (Table 1b). Patients will receive biopsies at baseline, 24 or 48 hours, and 1 week. Patients will alternately receive biopsies on schedules 1 or 2, which differ in a second set of biopsies at 24 or 48 hours. All biopsies must be obtained with prior consent.

Table 1b. Detailed Cycle 1 Biopsy Schedule

Biopsy Time Post-O^6BG	**0 hrs**	**24 hrs**	**48 hrs**	**1 wk**
Arm 1 BCNU Treated Site	X[a]	X[a,b]		X[a]
Arm 1 BCNU Protected Site		X[b]		
Arm 2 BCNU Treated Site	X[a]		X[a,b]	X[a]
Arm 2 BCNU Protected Site			X[b]	

a– 4 mm punch biopsy (x1), fresh frozen, for AGT assay.
b– 6 mm punch biopsies (x1), fresh frozen (immunohistochemistry), for apoptosis, cell cycle, and proliferation studiesAlternatively, a 1.0 cm length elliptical excision may be performed for the X[a,b] biopsies.

8.1 AGT Assay.

Since there is substantial evidence that lower AGT levels correlate with enhanced antitumor cytotoxicity by nitrosoureas, 100% depletion in all treated patients is desirable and may allow enhanced disease responses. A consecutive O^6BG/BCNU daily treatment schedule in this trial may allow for cumulative depletion following the second O^6BG dose, and could also delay AGT regeneration. Based on this rationale, we will examine AGT depletion on the consecutive daily O^6BG dosing schedule, comparing the 24 and 48 hour time points.

8.1.1 For AGT assays, three 4mm cutaneous punch biopsies of the most representative skin lesion in each patient will be obtained under local anesthesia with 1% lidocaine with epinephrine (1:100,000) at baseline, 24 hrs or 48 hrs (Biopsy Schedule 1 or 2), and 1 week after the first infusion.
8.1.2 In addition, patients who do not achieve a minor response after the 6th cycle will undergo 2 additional biopsies for AGT analysis at baseline and 24 hours after the 7th cycle of therapy. The baseline biopsy will be taken with a 6mm punch biopsy, which will be split in half with one half being used for the AGT assay, and the other half for MGMT gene sequencing for mutations.

8.1.3 Tissue procurement – the lesional biopsy will be frozen in liquid nitrogen and transported in liquid nitrogen or on dry ice. All biopsy specimens will be stored at -80º C prior to the assay. All specimens procured at enrollment sites outside of CWRU will be sent on dry ice via courier to CWRU for the assay.

8.1.4 AGT levels will be determined by biochemical activity assay. We have developed methods to measure activity of alkyl transferase in clinical samples. This HPLC assay determines

the ability of a whole tissue extract to remove O^6-methylguanine adducts from the substrate ^{3}H-methyl DNA by measurement of the loss of adduct.[66] The limit of detection for the enzyme is 0.4 fmol activity/sample, which corresponds to approximately 0.02 fmol/µg DNA.

8.2 Apoptosis, Cell Cyle/Proliferation, and DNA damageAssays

To gain increased understanding of BCNU alkylating effects in human lymphocytes, we will investigate BCNU-treated CTCL cells for induction of DNA damage and apoptosis induction, and changes in cell cycle and proliferation as an indirect measure of BCNU adduct and crosslink formation. Using this approach, we will be able to assess the cellular response to BCNU-induced damage, specifically in regards to BCNU adduct formation at the 24-hour and 48-hour time points, and thereby determine if there is enhanced BCNU cytotoxicity gained from the additional second BCNU dose administered at 24 hours. These findings will also be correlated with clinical efficacy.

8.2.1 A 6 mm punch biopsy will be taken at screening and split for paraformaldehyde and fresh frozen specimens. The specimens will be sent to CWRU for histology and immunohistochemical studies to assess for evidence of a malignant clone using CD3, CD7, and V_β antibodies. If a patient is determined to have a trackable malignant population with a moderately dense infiltrate, we will collect a lesional specimen for semi-quantitative analysis of cell cycle/proliferation and apoptosis markers. If there is no trackable clone identified (estimated 20%), we will collect lesional specimens for single-color immunohistochemistry for analysis of cell cycle/proliferation and apoptosis markers, and DNA strand break assays.

During the first cycle of therapy one 6 mm punch skin biopsy will be performed on lesional skin at 24 or 48 hours (See Table 1a-b, Schedule 1 or 2). BCNU-protected CTCL lesional specimens will also be taken using one 6 mm punch biopsy as a control for each of these specimens. These specimens will be sent fresh frozen in dry ice to CWRU. Specimens from these biopsies will be studied using immunohistochemical staining for Ki-67, PCNA, bcl-2, and caspase-3, as well as γ2HAX and TUNEL assays. The apoptotic index and proliferation rate will be calculated from these results. Immunohistochemistry will be used to assess expression of these proteins in keratinocytes, epidermal lymphocytes, and dermal lymphocytes, to determine the effects of BCNU cytotoxicity in each subpopulation of cells.

9.0 MEASUREMENT OF EFFECT

9.1 A modified version of the Severity Weighted Assessment Tool (SWAT) will be used to measure cutaneous response.[67] SWAT provides an accurate and reproducible assessment of cutaneous disease involvement based on body surface area of involvement and lesional thickness. In the modified SWAT, the percentage total body surface area (%TBSA) involvement will be measured separately for patches, plaques, and tumors using the patient's palm as a measure of 1% TBSA and the skin score will be calculated using the following formula:

SWAT = (%TBSA involved by patch x 1) + (%TBSA involved by plaque x 2) + (%TBSA involved by tumor or ulceration x 4)

Based on changes in modified SWAT assessment, patient responses will be classified as complete clinical response (CCR), partial response (PR), stable disease (SD), or progressive disease (PD). Baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 2 weeks before the beginning of the treatment. Cutaneous toxicity (erythema, tenderness, hyper-pigmentation and telangiectasia) will be assessed using the same methods and severity will be graded on a scale of 1 to 4 based on NCI common toxicity criteria.

9.2 Target lesions

Up to 5 target lesions will be identified at baseline and their length and width will be measured in metric notation using a ruler or calipers. Target lesions should be selected on the basis of their suitability for accurate repeated measurements and should be well demarcated and as isolated as possible from other lesions to decrease the chance of confluence of lesions during the study.

9.3 Photographs

Performing digital or kodachrome photography is highly recommended on a monthly basis to assist in the documentation of response.

9.4 Response Criteria

Complete clinical response (CCR): No evidence of disease, 100% improvement for a duration of at least 4 weeks.

Partial response (PR): Greater than or equal to 50% decrease in SWAT score compared to baseline and improvement is maintained for at least 4 weeks.

Stable disease (SD): Less than 50% decrease in SWAT score compared to baseline.

Progressive disease (PD): Increase of greater or equal to 25% of the SWAT score compared to baseline while the patient is actively taking the study drug

10.0 STUDY PARAMETERS

10.1 Pretreatment Evaluation

10.1.1 A complete history and physical examination including documentation of all measurable disease, as well as signs and symptoms, shall be done prior to entry. Time since onset of lesions and time since diagnosis will be noted. Patients will be staged according to standard TNM criteria.

10.1.2 Baseline laboratory studies are indicated in Table 2 and shall be completed within 2 weeks of initiating therapy.

10.2.1 Evaluation during study – For the first 4 weeks of therapy, Patients will be followed with weekly CBC and differential, BUN and creatinine, alkaline phosphatase, total bilirubin, and SGOT. Every 2 weeks, during treatment visits, pulse oximetry, urinalysis, calcium, phosphorous, and prothrombin time will also be performed. Following the first 4 weeks, the previously mentioned laboratory studies will only be performed during treatment visits every 2 weeks. Physical exam and vital signs will be performed before treatment every 2 weeks. Target lesions and SWAT will be assessed and documentation on a monthly basis with the last assessment occurring 2 weeks after the last treatment on week 25.

10.2.2 Patients should come to regularly scheduled Cycle Day 1 visits, although they may not receive treatment at a particular visit if they are currently experiencing an adverse event and will undergo the study evaluations scheduled for that visit.

Table 2 Study Calendar

	Pre-Study	Wk 1	Wk 1	Wk 2	Wk 3	Wk 3	Wk 4	Wk 5	Wk 5	Wk 6	Wk 13	Wk 13	Wk 23	Wk 23	Wk 25
Cycle# / Day#		C1 D1	C1 D2	C1 D8	C2 D1	C2 D2	C2 D8	C3 D1	C3 D2	C3 D8	C7 D1	C7 D2	C12 D1	C12 D2	C12 D15
O6BG		X	X		X	X		X	X		X	X	X	X	
BCNU		X	X		X	X		X	X		X	X	X	X	
Physical Exam and Vital Signs	X	X			X			X			X		X		X
Performance Status	X														
Measurable Lesions		X						X			X				X
CBC + Dif, Plt	X	X		X[1]	X		X[1]	X			X		X		X
BUN, Creatinine	X	X		X[1]	X		X[1]	X			X		X		X
Prothrombin Time	X	X			X			X			X		X		X
CA++, PO^4, Uric Acid	X	X			X			X			X		X		X
Alk phos, SGOT, total bilirubin	X	X		X[1]	X		X[1]	X			X		X		X
Serum Electrolytes	X	X			X			X			X		X		X
Photographs		X						X			X				X
ECG	X														
Chest x-ray	X														
Urinalysis	X	X			X			X			X		X		X
Pulse oximetry	X	X			X			X			X		X		
Negative Pregnancy Test	X														
Skin Biopsies (Table 1a,b)	X[2]	X[3]	X[3]	X[3]							X[4]	X[4]			

1 – interim labs during the first 4 weeks of therapy only.
2 – skin biopsy at screening for immunohistochemical detection of malignant clone (6 mm punch biopsy split for paraformaldehyde and fresh frozen specimens).
3 – skin biopsies at baseline, 24 hours, and 48 hours post-treatment as detailed in Table 1b.
4 – skin biopsies at cycle 7 of therapy will be taken in patients who demonstrate less than partial response.

11.0 DRUG FORMULATION AND PROCUREMENT

11.1 O^6Benzylguanine [O^6BG]

Study drug will be provided by the Pharmaceutical Management Branch of CTEP.

Chemical Name - 6-(Phenylmethoxy)-1H-purin-2-amine

Other Names - O^6-Benzylguanine (O^6BG) (NSC 637037)

Molecular Formula - $C_{12}H_{11}N_5O$

How Supplied - In dual pack with diluent (NSC 659805) for 0^6-Benzylguanine.

Active Drug - For injection, 100 mg vial. White lyophilized powder with 670 mg mannitol, USP and sodium hydroxide to adjust pH to 7-8.5

Diluent - 30 ml vial. Sterile solution of 40% polyethylene glycol 400 in pH 8 phosphate buffer (106 mg dibasic sodium phosphate, 102 mg monobasic potassium phosphate in sterile water for injection USP).

Solution Preparation - Withdraw 30 mL from the diluent vial; add to the vial of 0^6-Benzylguanine. Shake until complete solution is observed. Each milliliter of the resulting solution will contain 3.3 mg 0^6-Benzylguanine, 22 mg of mannitol, USP, 0.4 mL of polyethylene glycol 400 and approximately 0.6 mL pH 7 phosphate buffer.

Storage - Refrigerate dual pack or intact vials (2-8^{o}C).

Stability - Shelf-life surveillance of intact vials is ongoing. Samples from a pilot lot of freeze dried vials showed significant loss of potency after one month's storage at 50^{o}C.

Solutions of drug reconstituted as above were stable for at least 24 hours when stored at room temperature. The drug, further diluted to 0.04 mg/mL with 0.9% saline or dextrose 5% in water, was also stable for at least 24 hours at room temperature. However, at many concentrations between those two extremes an inconsistent precipitation problem has been documented. Therefore, based on current doses, the NCI recommends that the 3.3 mg/mL concentration be used for patient administration.

CAUTION: This single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded 8 hours after initial entry.

Route of Administration: Intravenous

Reported Adverse Events and Potential Risks:
Adverse events with possible relationship to O-6-Benzylguanine: nausea; vomiting

Also reported on O-6-Benzylguanine trials but with the relationship to O-6-Benzylguanine still undetermined: fatigue; fever; hyperpigmentation; rash; anorexia; pharyngeal mucositis/stomatitis; CNS hemorrhage; GI hemorrhage; hemorrhage with thrombocytopenia; infection; ALT; AST; hyperbilirubinemia; hyperglycemia; hyponatremia; confusion; neuropathy; bladder pain; headache; DL(co); pleural effusion; urinary frequency

Animal Data: The following toxicities have been observed in animal studies with O-6-Benzylguanine: leukopenia; rapid heart rate; lethargy; elevated hepatic enzymes; agitation; convulsion

Note: O-6-Benzylguanine in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.
O-6-Benzylguanine can exacerbate BCNU-induced myelosuppression.

11.2 BCNU

General - BCNU (Carmustine^{R}, 1,3-bis-(2-chloroethyl)-1-nitrosourea) was the first of the nitrosourea series to receive extensive clinical evaluation. Significant response has been observed in Hodgkin's disease and CTCL, and to a lesser extent in other lymphomas and myelomas. Because of its ability to cross the blood brain barrier it has been used in meningeal leukemia and in primary and metastatic tumors of the brain, with some activity. Modest response rates have been reported for melanoma, gastrointestinal cancers, breast and bronchogenic carcinomas.

Mechanism of action - BCNU will be used as the effector drug in this combination. It is thought that BCNU exerts its cytotoxic effects by cross-linking opposite strands of DNA. DNA crosslinks are formed after BCNU undergoes a spontaneous, nonenzymatic degradation to a carbonium ion which can alkylate a variety of substances including the nucleobases of DNA. In addition to the generation of carbonium ions, the spontaneous degradation of BCNU results in an isocyanate that carbamoylates lysine residues of proteins. This reaction is thought to be related to the myelosuppression associated with the nitrosoureas.

Clinical Pharmacology - BCNU is rapidly absorbed by the oral route but is usually administered by the intravenous route because tissue uptake and metabolism occur quickly. Pharmacokinetic studies of BCNU applied topically to the skin have not been performed; however, some systemic absorption is evident because dose-dependent myelosuppression does occur (see Section 2.0). The drug is highly lipid soluble and readily crosses the blood brain barrier. Cerebral spinal fluid concentrations occur rapidly and represent 15 to 70% of plasma values. Urinary excretion accounts for 70% of elimination by 96 h in the form of metabolites. Plasma protein binding and

an enterohepatic circulation are reported to account for the slow excretion of metabolites. A small fraction of metabolites are excreted in the respiration of $C0_2$ and in the feces.

Clinical Toxicity - BCNU is usually administered at doses of 100 to 200 mg/sqM as an 1 h intravenous infusion every 6 weeks. Myelosuppression is the most frequent toxicity associated with BCNU. Leukopenia and thrombocytopenia may be delayed with nadirs as late as 6 weeks; however, with the doses and schedules proposed in this study, the expected nadirs are at 8-12 days. Bone marrow toxicity is cumulative. Other toxicity includes nausea and vomiting, stomatitis and a metallic taste. The drug is not a vesicant but a local burning pain is associated with intravenous administration. A reversible hepatic toxicity with bilirubin, alkaline phosphatase and transaminase elevations has been reported. An interstitial pulmonary fibrosis has been associated with high doses or cumulative doses of > 1200 mg/M^2. Topical BCNU toxicities include bone marrow suppression, contact dermatitis, telangiectasia and hyperpigmentation.

Pharmaceutical Data - Formulation: BCNU is supplied as a 100 mg vial. First, dissolve BCNU with 3 mL of the supplied sterile diluent (Dehydrated Alcohol Injection, USP). Second, aseptically add 27 mL Sterile Water for Injection, USP. Each mL of resulting solution contains 3.3 mg of BCNU in 10% ethanol. The topical formulation is prepared by diluting to a final concentration in 60 ml of a sterile 30% ethanol/water solution. All preparations will be made in glass containers.

BCNU storage and stability - BCNU solution will be stable for 8 hours in glass containers at room temperature. BCNU vials must be stored at refrigerated temperatures (2-8^{o}C), and temperatures above 30^{o}C should be avoided, due to BCNU's low melting point. BCNU solutions should be protected from light exposure.

BCNU administration - The manufacturer recommends only glass containers be used for administration. The topical formulation will be applied evenly to the total skin surface, except that eyelids and ulcerated lesions will be avoided. Care will be taken to keep the solution from getting into the eyes. The patient will apply the BCNU solution with the help of nursing staff using a paint brush or gauze pad.

Pharmacy personnel involved in the preparation of the BCNU topical solution will use safety equipment appropriate for the preparation of cytoxic materials, including but not limited to a biological safety cabinet (BSC), chemotherapy gloves and gowns, and will be trained in the preparation, handling and disposal of cytotoxic chemotherapy drugs, according to institutional policy.

Nursing personnel involved in the application of the topical BCNU to the subject will use safety equipment appropriate for the handling of cytotoxic materials, including but not limited to chemotherapy gloves and gowns, and will be trained in the proper administration, handling and disposal of chemotherapy drugs, according to institutional policy.

Supplier - BCNU is commercially available and should be purchased by a third party. This drug will not be provided by the NCI.

Note - Please refer to the BCNU package insert for comprehensive information

12.0 REGULATORY AND REPORTING REQUIREMENTS

12.1 This study will be monitored by the NCI CTEP Clinical Data Update System (CDUS). All data will be reported to UHC-CWRU as the coordinating center, which will be responsible for compilation and submission of data to CTEP through CDUS. During the phase 1 dose-escalation portion of the study, data will be reported on a monthly basis. During the phase 2 portion of the study, data will be reported quarterly.

12.2 All reportable toxicities shall be reported immediately to the Principal Investigator and reported in writing to the Cancer Center Clinical Trials Development and Review Committee and University Hospitals of Cleveland Institutional Review Board.

12.3 The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for adverse event reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 11 (Pharmaceutical Information). A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

12.4 The Adverse Event Expedited Reporting System (AdEERS) will be retired in May of 2014. Expedited AE reporting for this study must use the CTEP Adverse Event Reporting System (CTEP-AERS). CTEP-AERS is live and open for use in place of AdEERS. Information regarding CTEP-AERS and instructions for training is available on the CTEP website: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined in the table below.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made to NCI by telephone at: 301-897-7497, or 301-897-7402 for CIP studies. An electronic report MUST be submitted immediately upon re-establishment of internet connection", for the reason that all paper CTEP-AERS forms have been removed from the CTEP website and will no longer be accepted.

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

<u>Expedited Reporting Guidelines</u> – CTEP Adverse Event Reporting System(CTEP-AERS) Reporting Requirements for Adverse Events that occur within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 2 and 3 Trials

Phase 2 and 3 Trials									
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**

CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within <u>24 hours</u> of learning of the event followed by a complete CTEP-AERS report within <u>5 calendar days</u> of the initial 24-hour report.
  - "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within <u>10 calendar days</u> of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Revised O-6-Benzylguanine CAEPR – Version 2.1, August 12, 2013
Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
O-6-Benzylguanine (NSC 637037)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 265 patients.* Below is the CAEPR for O-6-Benzylguanine.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.1, August 12, 2013[1]

Adverse Events with Possible Relationship to O-6-Benzylguanine (CTCAE 4.0 Term) [n= 265]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		
GASTROINTESTINAL DISORDERS			
	Constipation		
	Diarrhea		
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Blood bilirubin increased		
	Carbon monoxide diffusing		

	capacity decreased		
Lymphocyte count decreased			***Lymphocyte count decreased (Gr 2)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
White blood cell decreased			***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Acidosis		
	Anorexia		
	Hyperglycemia		
	Hypoalbuminemia		
	Hypocalcemia		
	Hypokalemia		
	Hyponatremia		
	Hypophosphatemia		
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on O-6-Benzylguanine trials but with the relationship to O-6-Benzylguanine still undetermined:

CARDIAC DISORDERS - Palpitations; Supraventricular tachycardia
EAR AND LABYRINTH DISORDERS - Ear pain
EYE DISORDERS - Flashing lights
GASTROINTESTINAL DISORDERS - Abdominal pain; Dyspepsia; Gastrointestinal hemorrhage[2]; Mucositis oral
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Injection site reaction

INFECTIONS AND INFESTATIONS - Infection[3]
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising
INVESTIGATIONS - Creatinine increased; GGT increased; Weight gain; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypoglycemia; Hypomagnesemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness; Myalgia
NERVOUS SYSTEM DISORDERS - Depressed level of consciousness; Dysphasia; Intracranial hemorrhage; Peripheral motor neuropathy; Peripheral sensory neuropathy
PSYCHIATRIC DISORDERS - Anxiety; Confusion
RENAL AND URINARY DISORDERS - Urinary frequency; Urinary retention; Urinary tract pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Epistaxis; Hypoxia; Pharyngeal mucositis; Pleural effusion; Pneumonitis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Pruritus; Purpura; Rash maculo-papular; Skin hyperpigmentation
VASCULAR DISORDERS - Flushing; Hypotension; Phlebitis; Vascular disorders - Other (hemorrhage with thrombocytopenia)

Note: O-6-Benzylguanine in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

12.5 Data Safety and Monitoring Plan

This protocol will adhere to the policies of the Ireland Cancer Center Data and Safety Monitoring Plan guidelines in accordance with NCI regulations. The Data and Safety Toxicity Committee will review all serious adverse events and toxicity reports as well as annual reviews.

12.6 CTEP Multicenter Guidelines

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

12.7 Cooperative Research and Development Agreement (CRADA)/ Clinical Trials Agreement (CTA)

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Keryx Pharmaceuticals, Inc.") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http:// ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies

utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):

   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

   b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

   c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected , used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for

review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
Executive Plaza North, Suite 7111
Bethesda, Maryland 20892
FAX 301-402-1584
Email: anshers@ctep.nci.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator(s) confidential/ proprietary information.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The overall response rates for CTCL patients are 55-80% when treated with conventional therapy.[11] In this phase I/II trial, an expected overall response rate of 80% for O^6BG/BCNU (P_1 = 0.80), would be sufficiently promising to justify further testing of the regimen and schedule. In contrast, a response rate of no greater than 55% (P_0 = 0.55) would be sufficiently discouraging to justify no further testing of the regimen and schedules.

R. Simon's optimal two-stage phase II design was used on clinical response.[68] The design characteristics are as follows: if the overall response rate of O^6BG/BCNU is less than P_0, then there is a probability of 0.1 or less of accepting the drug; and if the alternative hypothesis is true—the response rate is greater than P_1—the probability of rejecting the drug is less than 0.1. Simon's optimal designs minimize the average sample size.

Eight patients will be enrolled in Stage one of the trial. If there are 4 or less partial or complete clinical responses observed, enrollment of patients in this phase II trial will end, and the treatment will be considered less than 80% effective for this patient population. If there are 5 or more partial or complete responses observed, 18 additional patients will be enrolled. If 17 or fewer responses are observed in the total 26 patients, the treatment and schedule will be considered to be less than 80% effective. On the other hand, if there are more than 17 responses, then the treatment will be considered to be > 55 % effective. With 10% attrition, the sample size for this trial is 28 patients.

Of note, we changed/modified the parameters of Simon's two-stage design during the course of the trial because of very positive responses in 7 out of 8 patients enrolled in Stage one of the trial with 5 complete responses, 2 partial responses, and 1 stable disease. Our 89% response

rate was higher than expected when we originally did the power analysis. Therefore, we did an interim analysis when we reached 10 patients in order to determine if the full complement of 26 patients is needed in order to perform a comparison to single dose of O^6BG. For these 10 patients, 5 had complete responses (1 unconfirmed), 4 had partial responses, and 1 had stable disease. Since there were more than 7 patients with partial or complete responses observed out of the 10 patients, the number required in stage two by the design was reduced from 18 to 5 patients. Furthermore, assuming that 10 % failed to complete the study, a total of 17 patients (to achieve 15 evaluable patients) were enrolled.

13.2 Sample Size/Accrual Rate

As discussed in the section above, we will enroll a total of 26 to 28 patients, depending on attrition. Accrual rate to reach this goal over 2 years will be approximately 1 to 2 patients per month.

13.3 Analysis of Secondary Endpoints

13.3.1 Analysis of clinical response

Response rate and its confidence interval will be estimated based on binomial distribution theory. We will perform statistical pair-wise comparisons of response rates between this Phase I/II trial and the 30 and 40 mg (MTD) dose level cohorts from the Phase I trial using chi-square or Fisher's exact test.

13.3.2 Analysis of AGT data.

The clinical response rate and its 95% confidence interval will be estimated based on binomial theory. The correlation between AGT activity and clinical response will be determined using logistic regression. Because of repeated measurements of AGT activity at three different time points, the activity will be summarized by area under the curve (AUC). AGT results will be compared to the phase I trial AGT data.

13.3.3 Analysis of Apoptosis, Cell Cycle/Proliferation, DNA Damage data

Statistical analysis will be performed to compare differences in the apoptosis, cell cycle/proliferation and DNA damage assays at the 24 hour and 48 hour time points using paired T-test, and between BCNU-treated and BCNU-protected lesions using T-test or Wilcoxon rank sum test after examining normality. Spearman's correlation coefficient will be used to determine if the degree of apoptosis and cell cycle arrest in the CTCL malignant T-cell population and in the constitutive skin cells is associated with clinical response and local toxicity, respectively.

13.3.4 Analysis of AGT inactivation in non-responding patients

Statistical analysis will be performed to assess differences between AGT inactivation at the first and seventh cycles of treatment using paired the T-test. The predictive values of AGT activity

and the development of MGMT gene mutations on clinical response will be determined using logistic regression. Because of repeated measurements of AGT activity at three different time points, the activity will be summarized by area under the curve (AUC).

13.4 Reporting and Exclusions

13.4.1 Evaluation of toxicity

All Patients Receiving Drug Will Be Considered Evaluable For Toxicity

13.4.2 Evaluation of response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) minor response, 4) stable disease, 5) progressive disease, 6) early death from malignant disease, 7) early death from toxicity, 8) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the patients who meet the eligibility criteria and receive any treatment on the trial will be included in an intent-to-treat analysis of the response rate. Patients in response categories 4-9 will be considered as failing to respond to treatment. Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

14.0 REFERENCES

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Appendix A

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.