

A Phase II Trial of Bryostatin 1 in the Treatment of Metastatic Colorectal Cancer¹

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ABSTRACT

Current chemotherapy for patients with advanced colorectal cancer is relatively ineffective and may be associated with significant toxicity. Bryostatin 1 (bryo 1) influences cell proliferation, intracellular metabolism and signaling, differentiation, and apoptosis in human cancer cell lines via modulation of protein kinase C (PKC) activity. This trial investigates the efficacy and toxicity of bryo 1 as a novel therapeutic agent for patients with advanced colorectal cancer who have had previous 5-fluorouracil therapy. The primary end point was tumor response to bryo 1. Toxicity was also assessed. Twenty-eight patients with advanced colorectal cancer were enrolled. The mean age was 59 years (range, 38–76), with 16 men and 12 women, and good minority representation (11 African-Americans). The first 10 patients initially received 25 $\mu\text{g}/\text{m}^2$ of bryo 1 weekly as a 24-h infusion for 3 weeks of every 4-week cycle, with dose escalation to 35 $\mu\text{g}/\text{m}^2$ starting with the second cycle. The remaining patients were started at 35 $\mu\text{g}/\text{m}^2$ and escalated to 40 $\mu\text{g}/\text{m}^2$, if toxicity was minimal. Twenty-five patients were evaluable for objective tumor response, and complete data on toxicity were collected on 26 patients. No partial or complete tumor responses were observed. All 25 patients had disease progression within four cycles. Myalgia was the most common toxicity. Myelosuppression was not seen. bryo 1 as a weekly 24-h continuous infusion lacks single-agent antitumor activity in advanced colorectal cancer. Toxicity differs from that of traditional chemotherapeutic drugs.

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INTRODUCTION

Approximately 15% of patients with colorectal cancer have metastatic disease at presentation. Additionally, nearly two-thirds of patients with resected disease involving locoregional lymph nodes will recur within 5 years of initial treatment (1). Median survival for patients with untreated metastatic colorectal cancer is 6–8 months.

The most extensively studied drug in the treatment of metastatic colorectal cancer is 5-FU.³ Median survival for patients treated with 5-FU is ~11 months, with a 23% response rate (2). Recently, additional drugs for colorectal cancer have been introduced. Irinotecan has been approved for the treatment of patients who have failed 5-FU therapy. This is largely on the basis of two studies, one evaluating irinotecan *versus* best supportive care in 5-FU-refractory metastatic colorectal cancer (3) and another comparing irinotecan to continuous infusion 5-FU (4). One-year survival was in the 40% range in the irinotecan arms of these trials. Grade 3–4 diarrhea and neutropenia was relatively frequent in both studies. Oxaliplatin shows promise (5, 6), particularly when combined with 5-FU (7) or irinotecan (8), but at the time of this writing, oxaliplatin is not licensed for use in the United States. Taken together, the current data indicate that more effective, less toxic therapy for metastatic colorectal cancer is needed.

PKC is a family of closely related, lipid-dependent and diacylglycerol-activated isoenzymes with an important role in intracellular signaling pathways (9–12). PKC may have a role in normal colonic cell homeostasis and neoplastic transformation (13). Modulation of PKC affects *in vitro* differentiation of colon cancer cells (14, 15). 12-*O*-Tetradecanoylphorbol 13-*O*-acetate, a tumor promoter that modulates PKC activity, induces colon cancer cell terminal differentiation in several cell lines (15–17). PKC activation is implicated in tumor promotion in colonic epithelial cells by endogenous and dietary factors such as bile acids, free fatty acids, and diacylglycerol (18).

bryo 1, a macrocyclic lactone derived from the marine invertebrate *Bugula neritina* (19, 20), has a panoply of PKC-mediated biological effects. bryo 1 influences hematopoiesis (21, 22), lymphocyte function (23), platelet activation (24), and cell differentiation (15, 25–27). bryo 1 also has direct cytotoxic effects in some human cancer cell lines (28) and modulates the *in vitro* cytotoxic effects of several chemotherapeutic agents, both in hematological (29) and nonhematological (30–34) tumors. Phase I studies demonstrate that bryo 1 has minimal toxicity; myalgia is usually dose-limiting, and myelosuppression is observed rarely (35–39).

This Phase II study was designed to evaluate the efficacy of bryo 1 as a novel therapeutic agent in the treatment of metastatic

³ The abbreviations used are: 5-FU, 5-fluorouracil; PKC, protein kinase C; bryo 1, bryostatin 1.

Table 1 Treatment scheme for 28 patients with advanced colorectal cancer treated with bryostatatin 1

	<i>n</i>	Starting dose	Dose escalation	Median no. of cycles given
First cohort	10	25 $\mu\text{g}/\text{m}^2$	35 $\mu\text{g}/\text{m}^2$	2
Second cohort	18	35 $\mu\text{g}/\text{m}^2$	40 $\mu\text{g}/\text{m}^2$	2

colorectal cancer when administered as a weekly 24-h continuous infusion i.v. Additionally, information regarding toxicity of this drug was obtained.

PATIENTS AND METHODS

Study Design. This was a single-institution, Phase II clinical trial. All patients were treated with bryo 1, with initial dosing and planned dose escalation as described below. A complete response was defined as the disappearance of all measurable and evaluable tumor without the appearance of any new lesions on two successive measurements separated by 8 weeks. Partial response was defined as reduction of the sum of the cross-sectional areas of measurable lesions by $>50\%$ on two successive measurements. Stable disease was characterized by shrinkage of $<50\%$ or growth of $<25\%$, whereas progressive disease was defined as a $>25\%$ increase in the sum of the cross-sectional areas of measurable lesions. The study had a 0.79 power to distinguish regions of (true, unknown) response rates of 5% or less *versus* 20% or more.

Patient Eligibility. Patients with measurable, histologically proven, metastatic colorectal cancer were eligible for this trial. Prior treatment with up to one 5-FU-based regimen was acceptable, as was prior radiation therapy. Patients treated previously with other investigational agents within 1 month of study entry were excluded. All patients had to have a Southwest Oncology Group scale performance status of 0–2. Adequate bone marrow function (absolute neutrophil count, $\geq 1500/\text{cmm}$; platelet count, $\geq 100,000/\text{cmm}$), liver function (total bilirubin, $\leq 1.5 \times$ institutional upper limit of normal, aspartate aminotransferase $\leq 2.5 \times$ institutional upper limit of normal) and renal function (serum creatinine ≤ 1.5 mg/dl) were required. Patients with active infections, other serious systemic conditions, or an active second primary malignancy (other than *in situ* carcinoma of the cervix or adequately treated basal cell carcinoma of the skin) were excluded, as were patients with uncontrolled brain metastases. Patients who were lactating or pregnant were not eligible. Entrants with reproductive potential were required to use effective contraception. The study was approved by the Human Investigation Committee at Wayne State University. All patients provided signed informed consent.

Treatment Scheme. Patients were treated with bryo 1 via central venous access in 4-week cycles (three consecutive weekly 24-h infusions followed by a week break). bryo 1 was kindly supplied by the National Cancer Institute, Cancer Therapy Evaluation Program. Initially, the starting dose of bryo 1 was 25 $\mu\text{g}/\text{m}^2$ with planned dose escalation to 35 $\mu\text{g}/\text{m}^2$ if there was minimal toxicity. Because each of the first 10 consecutive patients enrolled met criteria for dose escalation, the trial was amended, and all remaining patients were started at 35 $\mu\text{g}/\text{m}^2$, with dose escalation to 40 $\mu\text{g}/\text{m}^2$ if no significant toxicity was

Table 2 Characteristics of 28 patients treated with bryostatatin 1 for metastatic colorectal cancer

Mean age	59 years (range, 38–76)
Sex	Male: 16 (57%) Female: 12 (43%)
Race	Caucasian: 17 (61%) African-American: 11 (39%)
SWOG performance scale status	
0	8 (29%)
1	18 (64%)
2	2 (7%)
Primary tumor site	Colon: 18 (64%) Rectum: 10 (36%)
Prior 5-FU regimen	Mayo Clinic regimen: 12 (43%) Weekly: 8 (29%) Continuous infusion: 6 (21%) Unknown: 2 (7%)
Mean time from diagnosis	20 months (range 6–55)
Sites of disease	Liver: 16 (57%) Lungs: 11 (39%) Lymph nodes: 2 (7%) Peritoneum/mesentery: 5 (18%) Colon/rectum: 2 (7%) Other: 3 (11%)
Median no. of disease sites per patient	2

observed in the initial cycle. This scheme is summarized in Table 1. Treatment cycles were repeated until disease progression or unacceptable toxicity occurred. Treatment was delayed for any grade 2 or higher hematological or nonhematological toxicity until recovery. Any grade 3 or 4 toxicity (except alopecia, nausea, or vomiting) prompted a dosage reduction of 5 $\mu\text{g}/\text{m}^2$ for subsequent cycles.

Trial Procedures. Initial evaluation of patients included a complete history and physical examination, performance status assessment, a complete blood count, and a multiphasic chemistry panel. Additionally, a computed tomographic scan of the abdomen and a computed tomographic scan or X-ray of the chest within 4 weeks prior to starting therapy was required.

Before each cycle, the complete blood count and multiphasic chemistry panel, performance status, and a toxicity profile were evaluated. Tumor measurements, if possible, were documented; if the tumor was only assessable radiographically, this was done after cycles 2 and 4. Toxicity assessment used the National Cancer Institute common toxicity criteria (<http://ctep.info.nih.gov>).

RESULTS

Patients. A total of 28 patients were enrolled. Table 2 provides a summary of patient characteristics. The mean age was 59 years (range, 38–76). There were 16 men and 12 women. Eleven of the patients were African-American, with the other 17 being Caucasian. Eighteen of the patients had colon primaries, and the other 10 had rectal tumors. The mean time from initial diagnosis to treatment with bryo 1 was 20 months (range, 6–55). 5-FU had been received as adjuvant therapy for 14 of the patients and for metastatic disease in the other 14. There was a median of two sites of metastases/patient, the most frequent being liver and lung.

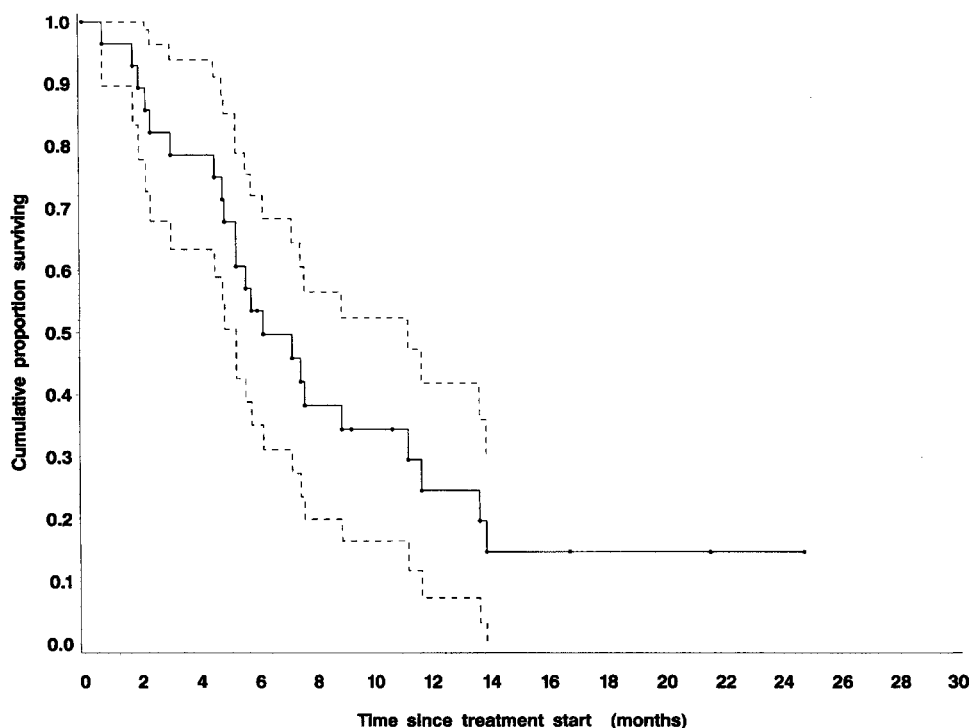


Fig. 1 Survival curve for 28 patients with advanced colorectal cancer treated with bryostatin 1. Dashed lines are the 95% confidence limits for the survival rate at a specific time point.

Efficacy. Twenty-five of 28 enrolled patients were evaluable for objective tumor response. Three patients were not assessable for tumor response, because they voluntarily withdrew from the study after fewer than two cycles of bryostatin 1. Of the remaining twenty-five patients, 21 had progressive disease after two cycles of bryostatin 1, and the other four patients had disease progression by completion of four cycles. There were no complete or partial responses observed (response rate, 0 of 25, 0%; 95% confidence interval, 0.00–0.14). The median overall survival of the 28 patients entered into this study as determined by the Kaplan-Meier method was 6.1 months (95% confidence interval, 5.2–11.2 months; Fig. 1). Of the 28 enrolled patients, 22 have expired, yielding a censoring rate of only 6 of 28 (21%).

Toxicity. Twenty-six patients were evaluable for toxicity. Two patients, one of whom received less than one complete cycle of bryostatin 1 and another who withdrew prior to receiving any bryostatin 1, were not considered eligible for toxicity evaluation. Table 3 shows the frequency and severity of side effects observed. Two patients required hospitalization, one for grade III nausea, vomiting, and dehydration likely related to a bowel obstruction, and a second for Gram-negative sepsis and pneumonia (without neutropenia). No myelosuppression was noted. Myalgia was the most frequently observed side effect (13 of 26, 50% incidence rate; 95% confidence interval, 0.30–0.70), although in 11 of 13 patients, it was only grade I or II. The grade III/IV myalgia incidence rate was 2 of 26 (8%; 95% confidence interval, 0.02–0.26) at a dose level of 35 $\mu\text{g}/\text{m}^2$ in each case. Fatigue, nausea, and vomiting were the next most common side effects of therapy, but these symptoms were generally mild. Other toxicities included elevated liver transaminases, diarrhea, sensory neuropathy, joint pain, mucositis, and depression. Two patients with preexisting diabetes experienced grade III hyper-

Table 3 Worst grade of toxicity per patient among 26 colorectal cancer patients treated with bryostatin 1

Toxicity	NCI-CTC grade				
	0	1	2	3	4
Myalgia (no. of patients)	13	8	3	1	1
Fatigue	19	2	5	0	0
Nausea/Vomiting	20	2	3	1 ^a	0
Diarrhea	24	2	0	0	0
Neuropathy	25	1	0	0	0
Fever	24	0	2	0	0
Arthralgia	25	1	0	0	0
Mucositis	25	1	0	0	0
Depression	24	0	2	0	0
Dyspnea	25	1	0	0	0
Hyperglycemia	24	0	0	2	0
Hepatotoxicity	24	1	1	0	0
Rash	25	1	0	0	0

^a Probably disease-related.

glycemic episodes during the study. In one case, the patient had an acute central venous catheter infection that was likely causative, and the other patient had multiple instances of hyperglycemia documented before and after the period he was being treated with bryostatin 1. Thus, it is unclear whether bryostatin 1 contributed to hyperglycemia in either case. Seven patients experienced no toxicity; 3 of 10 patients started at 25 $\mu\text{g}/\text{m}^2$, and 4 of the remaining 18 started at 35 $\mu\text{g}/\text{m}^2$.

DISCUSSION

In human colon cancer cell lines, PKC is involved in cell proliferation (16, 17, 40, 41), intracellular metabolism (42) and

signaling (43, 44), differentiation (15–17, 45), and apoptosis (46). Using bryo 1 to modulate PKC activity or expression represents a novel treatment approach for colorectal malignancies. However, this Phase II study failed to demonstrate any clinically meaningful activity against advanced colorectal cancer by bryo 1 when used as a single agent. If bryo 1 has a true response rate of 20% (which we had hypothesized), then the probability of observing no responses among 25 patients is extremely low ($P = 0.0038$). Similarly disappointing results have been reported in Phase II trials of single-agent bryo 1 for melanoma (47–49) and in Phase I trials for bryo 1 in certain hematological malignancies (38). The treatment schedule herein was based on a previous Phase I study (35), but it is distinctly possible that a different treatment schedule might result in enhanced antitumor activity. Because no reliable assay to measure bryo 1 levels in biological fluids exists (50, 51), investigators have explored varying administration schedules of bryo 1 on the basis of theoretical, but unproven, grounds. The present study was limited to evaluation of tumor size. The answers to fundamental questions, such as whether bryo 1 influenced the PKC levels in the tumor cells *in vivo* or whether it affected cell differentiation or apoptosis, were not determined. Logistically, this type of data are difficult to collect, but future trials, if undertaken, might attempt to incorporate such an analysis.

The toxicity profile of bryo 1 at doses of 25–40 $\mu\text{g}/\text{m}^2$ was quite favorable. Unlike previous clinical trials of bryo 1 (35–38), myalgia was rarely dose limiting. The reason for this difference is not entirely clear but may be related to dosing schedule. This study confirmed previous findings that myelosuppression is not a dose-limiting toxicity of bryo 1 in doses up to 40 $\mu\text{g}/\text{m}^2$.

There is an emerging body of data showing that bryo 1 probably has little or no activity against nonhematological tumors when used as a single agent (36, 47–49). However, there is *in vitro* and animal xenograft data suggesting a potential role for bryo 1 as a response modifier to traditional cytotoxic agents (29, 30, 32, 52–54). Recent Phase I trials have demonstrated the feasibility of combining bryo 1 and these drugs (55, 56). With our growing understanding of the role of PKC in cellular growth and function, the *in vitro* data on synergy between bryo 1 and traditional chemotherapeutic drugs and the lack of myelosuppression or gastrointestinal toxicity observed in clinical trials of bryo 1, investigation of bryo 1 as a potential tumor response modifier to other drugs is clearly warranted.

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