Phase I Study of Bryostatin 1 and Gemcitabine

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Abstract

Purpose: Bryostatin 1 is a macrocyclic lactone with protein kinase C inhibitory activity. Gemcitabine is a nucleotide analogue with a broad spectrum of anticancer activity. Bryostatin 1 enhanced the activity of antitumor agents including gemcitabine in preclinical models. The primary objective of this phase I study was to determine the recommended doses for phase II trials of bryostatin 1 and gemcitabine.

Experimental Design: Eligible patients had histologic or cytologic diagnosis of nonhematologic cancer refractory to conventional treatment; life expectancy of >3 months; normal renal, hepatic, and bone marrow function; and a Southwest Oncology Group performance status of 0 to 2. Gemcitabine was administered i.v. over 30 minutes and was followed by bryostatin 1 by i.v. infusion over 24 hours on days 1, 8, and 15 of a 28-day cycle. Bryostatin 1 ($\mu g/m^2$) and gemcitabine (mg/m^2) doses were escalated as follows: 25/600, 25/800, 25/1,000, 30/1,000, 35/1,000, and 45/1,000, respectively.

Results: Thirty-six patients (mean age, 57 years; male/female 15:21) were treated. The median number of treatment cycles per patient was 3 (range, 0-24). Four patients developed dose limiting toxicities: myalgia, 2; myelosuppression, 1; and elevation of serum alanine aminotransferase levels, 1. Ten grade 3 toxicities were observed (anemia, 2; neutropenia, 5; thrombocytopenia, 3). No treatment-related death was seen. The recommended doses for phase II trials for bryostatin 1 and gemcitabine were 35 $\mu g/m^2$ and 1,000 mg/m^2, respectively. Two heavily pretreated patients with breast and colon cancer experienced partial responses lasting 22 and 8 months, respectively. Eight patients had stable disease.

Conclusion: The combination of bryostatin 1 and gemcitabine seemed to be well tolerated with limited grade 3 toxicity. The recommended dose of bryostatin 1 in combination with full doses of gemcitabine was 35 $\mu g/m^2$.

Protein kinase C (PKC) is a family of serine/threonine kinases composed of 12 isoforms subdivided into three major groups based on their cofactor requirement for activation (1). The PKC family has a central role in signal transduction and is linked to cell growth, differentiation, apoptosis, and angiogenesis (2, 3). Aberrant regulation of the PKC enzymes activity was shown in a number of malignancies including breast (4, 5), pancreatic (6), and non–small-cell lung cancers. Modulation of PKC activity *in vitro* promotes apoptosis and sensitizes cancer cells to the effects of cytotoxic agents (7). Consequently, PKC represents a rational target for drug development.

Bryostatin 1 is a macrocyclic lactone that has been shown to regulate PKC activity (8). In preclinical models, bryostatin 1 inhibited cell growth and angiogenesis, promoted apoptosis, and induced differentiation of cancer cells (9). In phase I trials, bryostatin 1 had minimal toxicity that included myalgia (10, 11). Myelosuppression was rarely observed with bryostatin 1. Single-agent activity of bryostatin 1 has been investigated in colorectal (12), renal (13), melanoma (14), and head and neck (15) cancers. Results indicated minimal or no appreciable clinical activity. The combinations of bryostatin 1 and cytotoxic agents were tested because PKC activation contributed to chemoresistance. Bryostatin 1 was shown to potentiate the proapoptotic effects of gemcitabine in human breast (16) and pancreatic cancer cell lines (6).

Gemcitabine (2,2′-difluorodeoxcytidine) is a potent and specific deoxycytidine analogue. In phase I trials, the maximal tolerated dose of gemcitabine administered weekly as an i.v. infusion over 30 minutes was between 790 and 1,370 mg/m^2 (17, 18). The dose limiting toxicity was myelosuppression. Gemcitabine showed a broad-spectrum of antitumor activity in patients with pancreas (19), breast (20), ovary (21), and lung (22) cancers.

Based on the preclinical data of potentiation of gemcitabine proapoptotic effects by bryostatin 1 and the nonoverlapping dose-limiting toxicities of the two agents, we conducted a phase I trial to define the recommended dose for phase II trials of the bryostatin 1 and gemcitabine combination.

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Materials and Methods

Patient eligibility. Patients were eligible for the study if they had a confirmed pathologic diagnosis of a nonhematologic malignancy for which no standard curative or palliative therapy was available. Patients were also required to have a Southwest Oncology Group performance status of ≤2, a life expectancy of at least 3 months, and adequate hematologic, renal, and hepatic function defined by the following variables: hemoglobin ≥8 g/dL, neutrophil count ≥1,500/μL, platelet count ≥100,000/μL, serum creatinine ≤1.5 mg/dL, total serum bilirubin within the institution's 1.5 times upper normal limit and serum aminotransferases <2.5 times upper normal limit. Patients were also required to have bidimensionally measurable disease. Irradiated tumors with no evidence of progression after radiation therapy were not considered measurable. Prior chemotherapy, surgery, or radiation therapy was permitted as long as the patient had recovered adequately from these procedures. Female patients of childbearing potential must have had a negative serum pregnancy test before enrollment, and all fertile patients must have agreed to use contraception during the study. The study was approved by the Wayne State University Human Investigations Committee and all subjects provided a signed informed consent in accordance with the Wayne State University Human Investigation Committee guidelines before participation on the study.

Patients were excluded from study participation if they had uncontrolled intercurrent illness such as active infection, symptomatic congestive heart failure, or unstable angina.

Drug administration. Cycles were repeated every 28 days. Gemcitabine (Gemzar, Eli-Lily Pharmaceuticals, Indianapolis, IN) was infused i.v. over 30 minutes on days 1, 8, and 15. Bryostatin 1 (provided by Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD) was administered by a 24-hour i.v. infusion on days 1, 8, and 15 via an indwelling central venous catheter. Bryostatin 1 infusion was started after the completion of gemcitabine administration.

Dose escalation. The starting doses for gemcitabine and bryostatin were 600 mg/m^2 and 25 μg/m^2, respectively. Table 1 summarizes the dose escalation schedule. No dose escalations were allowed within an individual patient. A minimum of three toxicity-evaluable patients was entered onto each dose level. Patients who received less than one cycle of therapy without toxicity were considered inevaluable for toxicity evaluation. If one dose limiting toxicity occurred, three additional patients were entered on that dose level. Dose escalation stopped when two or more dose limiting toxicities were observed. The recommended dose of bryostatin and gemcitabine for phase II trials was defined as the dose level at which one or less of six patients developed a dose limiting toxicity. At least 10 patients would be treated on the recommended dose for phase II trials to confirm its safety.

Table 1. Dose escalation schema of gemcitabine and bryostatin 1

Dose level	Gemcitabine dose (mg/m^2)	Bryostatin dose (μg/m^2)
1	600	25
2	800	25
3	1,000	25
4	1,000	30
5	1,000	35
6	1,000	45

NOTE: Dose level 1 was the starting dose level of the study. Both drugs were administered on days 1, 8, and 15 of a 28-day cycle. Gemcitabine was administered i.v. over 30 minutes, followed by bryostatin i.v. over 24 hours.

Table 2. Characteristics of the 36 patients treated with gemcitabine and bryostatin 1

	n
Age (y)	
Median	57 (18-79)
Race	
Caucasian	29
Other	7
Sex	
Male	15
Female	21
Performance status	
0	5
1	26
2	5
Primary tumor site	
Non–small-cell lung cancer	7
Breast	7
Colorectal	4
Pancreas	4
Sarcoma	3
Gastric	3
Esophagus	2
Small intestine	2
Cholangiocarcinoma	1
Renal	1
Merckel cell	1
Unknown primary	1
Prior cytotoxic therapy	
One regimen	6
Two regimens	13
Three regimens	6
Four to six regimens	11

Dose limiting toxicity was defined as the occurrence of (*a*) grade 3 or 4 nonhematologic toxicity excluding alopecia, nausea, and vomiting; (*b*) grade 4 thrombocytopenia; or (3) grade 4 neutropenia complicated by either fever or treatment delay of >1 week during any cycle of therapy.

On-study evaluation. Baseline evaluations included a complete history and physical examination, complete blood and differential counts, serum electrolytes, total serum bilirubin, serum alanine aminotransferase, serum aspartate aminotransferase, and urinalysis. Toxicity was graded by the National Cancer Institute Common Toxicity Criteria version 2. Evaluation for toxicity was undertaken after each treatment cycle and whenever clinically indicated. Radiological evaluation for response was done every two cycles and at the time of clinical disease progression. Objective response assessments were based on the WHO criteria.

Results

Patient characteristics. A total of 36 patients were enrolled on the study. Two patients were nonevaluable for toxicity: one patient on dose level 1 had treatment interruption due to infection and the second patient on dose level 4 had early disease progression. All patients had received prior cytotoxic therapy. Table 2 summarizes the patient characteristics. A total of 101 cycles of chemotherapy were administered with a median of three cycles per patient (range, 0-24).

Dose escalation and toxicity. Tables 3 and 4 summarize all the observed toxicities. No dose limiting toxicities were observed on dose level 1. Six patients were enrolled on dose level 2 because of one dose limiting toxicity. A patient developed grade 3 neutropenia requiring a 2-week delay in

treatment and dose reduction. No dose limiting toxicities were observed on dose levels 3 and 4. One dose limiting toxicity was observed on dose level 5. A patient developed hepatotoxicity consisting of grade 3 and grade 2 elevations of serum aspartate aminotransferase and alanine aminotransferase, respectively. Two of the six patients enrolled on dose level 6 experienced grade 3 myalgia. Subsequently, additional seven patients were enrolled on dose level 5 to verify the maximum tolerated dose of the combination. No additional dose limiting toxicities were observed. Overall, 10 episodes of grade 3 toxicities were observed (anemia, 2; neutropenia, 5; and thrombocytopenia, 3). No treatment-related deaths were seen.

Objective response. Two heavily pretreated patients had a partial response. The first patient had breast cancer and experienced a partial response lasting 22 months. She had previously been treated with doxorubicin, 5-fluorourcil, and paclitaxel, followed by high-dose chemotherapy with stem cell support. Subsequent to that, she had received trastuzumab and vinoralbine. The second patient had colon cancer and experienced a partial response lasting 8 months. This patient received two prior treatment regimens, oxaliplatin and capecitabine, and then irinotecan, 5-fluorouracil, and leucovorin with an investigational agent. Eight patients had stable disease. Three patients with non–small-cell lung cancer had disease stabilization lasting longer than 4 months.

Discussion

PKC promotes cellular proliferation and inhibits apoptosis through the activation of downstream signaling pathways that include Akt/nuclear factor κB, mitogen-activated protein kinase, and Ras. PKC also has a central role in angiogenesis (9). The binding of the vascular endothelial growth factor to its receptor leads to the activation of PKC resulting in proliferation and growth of endothelial cells. Inhibition of PKC can potentially sensitize cancer cells to the proapoptotic effects of cytotoxic agents and inhibit angiogenesis.

Table 3. The occurrence of treatment-related hematologic toxicity in 36 patients treated with gemcitabine and bryostatin 1

Toxicity	Dose level	Grade			
		1	2	3	4
Neutropenia	1	1	0	0	0
	2	0	0	1*	0
	3	0	0	0	0
	4	1	0	0	0
	5	0	1	4	0
	6	0	3	1	0
Anemia	3	1	0	0	0
	4	0	4	0	0
	5	3	4	1	0
	6	2	2	1	0
Thrombocytopenia	1	2	0	0	0
	2	3	0	0	0
	3	1	0	0	0
	4	1	1	0	0
	5	7	0	1	0
	6	1	1	2	0

*Associated with dose limiting toxicity.

Table 4. The occurrence of treatment-related nonhematologic toxicity in 36 patients treated with gemcitabine and bryostatin

Dose level	Myalgia			Hepatotoxicty			Fatigue		Infection		Edema	
	Grade			Grade			Grade		Grade		Grade	
	1	2	3	1	2	3	1	2	1	2	1	2
1	0	0	0	0	0	0	0	1	1	1	0	1
2	1	1	0	1	0	0	1	1	0	0	0	0
3	2	1	0	2	0	0	1	2	0	0	0	0
4	1	0	0	2	0	0	2	1	0	0	0	0
5	0	2	0	3	2	1*	2	2	1	1	0	1
6	0	0	2*	0	1	0	1	0	0	3	0	1

NOTE: Toxicity was assessed by the National Cancer Institute Common Toxicity Criteria version 2.
*Associated with dose limiting toxicity.

Bryostatin 1 is a modulator of PKC activation (8). Short-term exposure of tumor cells to bryostatin 1 results in PKC activation and translocation of the enzyme to the nuclear membrane. Prolonged exposure to bryostatin 1 leads to the depletion of PKC and inhibition of downstream signaling (23). Based on the mechanism of action of bryostatin 1, phase I trials of single agent bryostatin 1 were conducted using a prolonged continuous i.v. infusion schedule (11). The most frequent toxicity associated with bryostatin 1 was myalgia. The incidence and severity of myalgia was dose dependent and was cumulative. Therefore, the schedule of administration was changed to weekly infusions administered 3 out of 4 weeks. This decreased the incidence of myalgia and delays in treatment.

Phase II trials showed no significant clinical activity for single agent bryostatin 1 was observed in melanoma (14), colorectal (12), or renal (13) cancers by traditional tumor shrinkage criteria. In preclinical models, bryostatin 1 sensitized breast and pancreatic cancer cells to the effects of gemcitabine (6, 16). The potentiation of gemcitabine by bryostatin 1 was sequence dependent with maximal apoptosis observed when gemcitabine preceded bryostatin 1. A similar sequence-dependent potentiation was observed in gastric cancer cells when bryostatin 1 was used with mitomycin C and paclitaxel (9). The recognition of PKC overexpression and activation in most human cancers and the role of PKC modulation in potentiating conventional cytotoxic agents were the rationale to develop combinations of bryostatin 1 and broad-spectrum anticancer cytotoxic agents, such as gemcitabine.

In this phase I trial, the combination of gemcitabine followed by bryostatin 1 was well tolerated at doses of 1,000 mg/m^2 and 35 $\mu g/m^2$, respectively. This dose of gemcitabine was the recommended full dose in single-agent therapy. The dose limiting toxicities to gemcitabine and bryostatin 1 included myalgia and hepatotoxicity. The hematologic toxicity of the gemcitabine was not altered by the addition of bryostatin 1. Similarly, no potentiation of toxicity of either arabinofuranosylcytosine (24) or vincristine (25) by bryostatin 1 was observed in phase I trials. The lack of toxicity potentiation with bryostatin 1 may be explained by its mechanism of action that selectively targets activated PKC in the tumor cells (6, 16).

Clinical trials of bryostatin 1 have used different i.v. infusion schedules ranging from 1 to 72 hours. The optimal dose rate delivery based on pharmacokinetics of bryostatin 1 is unknown because of lack of reliable assays to quantify its serum levels. The choice of the 24-hour infusion was to produce a prolonged inhibition of PKC within tumor cells. Bryostatin 1 infusion was started after the completion of the gemcitabine delivery to mimic the preclinical findings with respect to the optimum sequence that resulted in maximal tumor cell kill (6, 16). Nevertheless, the optimal interval between gemcitabine and bryostatin 1 *in vivo* may be influenced by the half-life of gemcitabine. In this respect, gemcitabine has a relatively short plasma half-life ranging from 32 to 94 minutes (26). All patients in this study received prior cytotoxic therapies for their advanced cancers. Two patients with breast and colon cancer experienced partial responses lasting 22 and 8 months, respectively. The occurrences of those partial responses were of interest. Although responses to gemcitabine were expected in breast cancer, the duration of the response was longer than normally seen in this situation. Likewise, gemcitabine was considered an inactive drug in colorectal cancer. Eight additional patients had stable disease, with significant stability in patients with non–small-cell lung cancer, a disease that may respond to gemcitabine. In future studies, it will be necessary to conduct trials of bryostatin 1 or other PKC modulators in patients selected for their tumoral high expression of PKC isoenzymes. However, at this time, no information is available on the predictive value of individual PKC isoenzymes on bryostatin 1 antitumor effects.

In conclusion, the gemcitabine and bryostatin 1 combination was well tolerated and the preliminary results indicate promising activity. Based on the preclinical data and the tolerability of bryostatin 1 and gemcitabine, phase II trials in breast and pancreatic cancer represent a rational approach for further development of this regimen.

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