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ORIGINAL ARTICLE

Phase II trial of sequential paclitaxel and 1 h infusion of bryostatin-1 in patients with advanced esophageal cancer

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Abstract
Background We sought to determine the response rate and toxicity profile of sequential paclitaxel and bryostatin-1, a novel, selective inhibitor of protein kinase C, in patients with advanced esophageal cancer.
Patients and methods Patients with advanced esophageal and gastroesophageal junction cancer were enrolled. All gave informed consent. They were initially treated with paclitaxel 90 mg/m^2 intravenously on Day 1 and bryostatin-1 50 μg/m^2 on Day 2 weekly for three consecutive weeks out of four. Because of severe myalgias, dosing was reduced to paclitaxel 80 mg/m^2 with bryostatin-1 40 μg/m^2 and then to paclitaxel 80 mg/m^2 with bryostatin-1 25 μg/m^2.
Results Twenty-four patients were enrolled, with 22 assessable for response. The partial response rate was 27%. 10 patients treated with bryostatin-1 40–50 μg/m^2 had a response rate of 40 versus 17% at bryostatin-1 25 μg/m^2 (p-value = 0.3). Median time-to-progression was 3.7 months and median survival was 8.3 months. Grade 3/4 myalgias were seen in 50% of patients. Myalgias appeared to be related to bryostatin-1 dose. Because of toxicity, the trial was closed prior to full accrual.
Conclusions Despite potential anti-tumor activity of this combination in patients with advanced esophageal cancer, further development is not warranted, given the severe toxicity, especially myalgias, that were seen.

Keywords Bryostatin-1 · Esophageal cancer · Gastroesophageal cancer · Paclitaxel · Protein kinase C

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Introduction

The anti-tumor activity of many cytotoxic chemotherapeutic agents is a consequence of their induction of apoptosis [4]. In turn, apoptosis is mediated by a balance between pro- and anti-apoptotic signaling [6, 11]. As activation of the phosphoinositide-protein kinase C (PKC) pathway is believed to effect the anti-apoptotic signals [10, 17], inhibition of the PKC pathway may represent a novel target for anti-cancer therapy in solid tumors.

Bryostatin-1 is a selective PKC inhibitor. It is a macrocyclic lactone isolated from the marine invertebrate *Bugula neritina* [18]. Although it is an activator of PKC with short-term exposure, more prolonged exposure leads to an overall decrease in PKC activity thought to be secondary to down-regulation of PKC [7]. In addition, bryostatin-1 is also an inhibitor of cyclin-dependent kinase 2 [2], whose derangement may contribute to the malignant phenotype [20].

Bryostatin-1 was found to enhance anti-tumor activity of chemotherapeutic drugs, including cytosine arabinoside [5], vincristine[15] and paclitaxel [13]. Koutcher et al. [13] concluded that the activity of bryostatin-1 is sequence-dependent and that it enhances the anti-tumor activity of paclitaxel only when paclitaxel precedes the bryostatin-1. Based on these data, we performed a phase I evaluation of

sequential paclitaxel followed 24 hours later by bryostatin-1 [12]. Objective responses were seen in patients with esophageal and pancreatic adenocarcinomas. This trial established the phase II dose of paclitaxel at 90 mg/m^2 and bryostatin-1 at 50 μg/m^2, with the most significant dose-limiting toxicity being myalgias. Ajani et al. then performed a phase II evaluation of sequential paclitaxel and bryostatin-1 in 37 patients with advanced gastric and gastroesophageal (GE) adenocarcinoma [1]. They reported a partial response rate of 29% and a median time-to-progression of 4.25 months. Significant toxicities included myalgia. Similarly, we conducted a phase II study of sequential paclitaxel and bryostatin-1 in patients with advanced esophageal cancer.

Patients and methods

Eligibility criteria

Eligibility criteria included unresectable or metastatic squamous cell carcinoma or adenocarcinoma of the esophagus or GE junction, with histology confirmed at Memorial Sloan-Kettering Cancer Center. All patients were ≥18 years old and had essentially normal organ function with an absolute neutrophil count (ANC) ≥1,500 μl^{-1}, platelets ≥150,000 μl^{-1}, serum creatinine ≤1.5 mg/dl and serum bilirubin ≤1.5 mg/dl. A Karnofsky performance status ≥70% was required. No more than one prior chemotherapy regimen was permitted and prior therapy with a taxane or bryostatin-1 was not permitted. Prior radiotherapy was allowed. Exclusion criteria included brain metastases, active angina/myocardial infarction within the previous six months or a pulmonary diffusing capacity (D_LCO) of < 60%.

The study was reviewed and approved by the Institutional Review Board. All patients gave written informed consent before initiation of therapy.

Treatment plan

Paclitaxel and bryostatin-1 were administered sequentially in repeated 4-week cycles that consisted of weekly treatment for 3 weeks, followed by a 1-week rest period. During each treatment week, paclitaxel was administered on day 1. Bryostatin-1 was then administered 24 h later on day 2. All therapy was administered in the outpatient setting.

All patients received premedication with dexamethasone 20 mg intravenously/orally, cimetidine 300 mg intravenously and diphenhydramine 50 mg intravenously 1 h prior to paclitaxel infusion.

Protocol amendment and dose modification for adverse events

Because of severe myalgias and pulmonary toxicity seen in the first four patients treated at the starting dose of paclitaxel 90 mg/m^2 with bryostatin-1 50 μg/m^2, the protocol was amended to reduce the starting doses to paclitaxel 80 mg/m^2 with bryostatin-1 40 μg/m^2. When persistent myalgias continued to be observed with the next seven patients, the starting doses were again modified to paclitaxel 80 mg/m^2 with bryostatin-1 25 μg/m^2. The schedule was also changed from 3 weeks out of 4 to 2 weeks out of 3.

During a treatment cycle, chemotherapy was held for 1 week for an ANC < 1,500 μl^{-1} or grade 3/4 myalgia. Treatment recommenced after a 1 week delay if these parameters had returned to acceptable levels. If they remained unacceptable, therapy was delayed for a second week. Patients who required more than a 2-week treatment delay could be removed from the study at the treating physician's discretion.

Myalgias were graded according to the National Cancer Institute (NCI) myalgia toxicity grading scale (see Table 1). Bryostatin-1 was reduced in 5 μg/m^2 stepwise increments for any occurrence of grade 3/4 myalgia uncontrolled by analgesics (after an 1-week treatment delay) or for grade 2 myalgia on the day of treatment. For every subsequent occurrence of grade 3/4 myalgia or for grade 2 myalgia on the day of treatment, the bryostatin-1 dose was further decreased by one more level.

A reduction in the dose of paclitaxel by 10 mg/m^2 was permitted for grade 4 hematologic toxicity and for grade 3/4 non-hematologic toxicities (except myalgia).

Treatment was continued indefinitely in those patients with responding or stable tumors unless recurrent grade 3/4 toxicity occurred despite dose reductions, in the event of death or serious illness or if patient consent was withdrawn.

Evaluation at baseline and during treatment

Pretreatment evaluations included a complete history and physical examination, complete blood count, biochemical screening profile, electrocardiogram and imaging of

Table 1 Myalgia toxicity grading scale

Grade 1	Mild, brief pain not needing analgesics; patient fully ambulatory
Grade 2	Moderate pain needing simple analgesics; settles in ≤7 days; patient remains ambulatory
Grade 3	Moderate to severe pain; regular analgesics required (not morphine); patient's mobility is severely restricted
Grade 4	Very severe, incapacitating pain; patient requires constant bedrest and regular morphine

measurable disease. When pulmonary toxicity was observed with the first 10 patients, subsequent patients also underwent a pulmonary function test (PFT) with measurement of D_LCO at baseline and prior to each additional cycle.

When myalgias were observed, selected patients underwent serum creatine kinase measurements. After the bryostatin dose was reduced to 25 μg/m^2, all patients underwent measurement of cytokine levels, including interleukin (IL)-2, IL-6 and tumor necrosis factor (TNF)-α, at baseline prior to paclitaxel infusion on Day 1 and prior to and 2 h after bryostatin-1 infusion on Day 2 during weeks 1 and 2 of the first two cycles.

Patients were evaluated for toxicity weekly while receiving treatment according to NCI Common Toxicity Criteria, version 2.0 [16]. Tumor imaging was repeated after the first two cycles of therapy and response was recorded according to World Health Organization (WHO) criteria [14].

Statistical plan

The study was a single institution, open-label, phase II evaluation. The primary endpoint was to determine the overall response rate (complete response or CR and partial response or PR) with sequential paclitaxel/bryostatin-1. A secondary endpoint was to assess the toxicity of therapy.

A Simon's two-stage optimal design was applied [22]. In the initial stage, 19 patients would be enrolled. If ≤3 major responses (PR and CR) were observed, the study would be closed. If ≥4 major responses were observed in this first stage, 14 additional patients would be enrolled. If there were ≥8 responses out of 33 patients, the combination would be considered to be worthy of further evaluation. If the true response rate of the therapy was ≤15%, the design had a 10% probability of recommending the therapy for further study. This probability increased to 90% if the actual anti-tumor activity was ≥35%.

The Kaplan–Meier method was used to estimate time-to-progression (TTP) and overall survival (OS) curves and the log-rank test was employed to compare survival curves of different groups. Associations between binary variables were tested using Fisher's exact test (two-sided test). $p < 0.05$ were considered statistically significant.

Results

Patient characteristics

24 patients were enrolled between March 2000 and November 2001. Because of prohibitive toxicity (primarily myalgias), the trial was closed prior to full accrual. Baseline characteristics are described in Table 2. The median age was 62. Most patients were male (83%), 22 of 24 (92%) had adenocarcinoma histology and most were untreated (92%). All patients were evaluable for toxicity while 22 were evaluable for response assessment. Two patients were taken off study prior to completing two cycles of therapy and undergoing disease assessment, for grade 4 pulmonary toxicity and for worsening dysphagia respectively.

Table 2 Patient characteristics

Characteristic	Patients ($N = 24$)	
	No.	%
Age, years		
Median	62	
Range	31–78	
Sex		
Male	20	83
Female	4	17
Karnofsky performance status		
Median	80	
Range	70–90	
Histology		
Adenocarcinoma	22	92
Squamous cell	2	8
Prior therapy		
Chemotherapy	1	4
Chemotherapy and radiotherapy	1	4
None	22	92

Response to treatment

Of the 22 evaluable patients, the overall response rate was 27%, with 6 PRs. Eight patients each (36%) had stable disease and progressive disease, respectively. Four out of ten evaluable patients who received bryostatin-1 at 40–50 μg/m^2 had a PR for a response rate of 40%. In comparison, 2 of 12 evaluable patients who received bryostatin-1 at 25 μg/m^2 had a PR for a response rate of 17%. This difference in response rates between the different bryostatin-1 doses was not statistically significant (p-value = 0.3).

Although not primary endpoints, the median TTP was 3.7 months (95% CI, 1.7–5 months) and the median OS was 8.3 months (95% CI, 3.9–12 months). The median TTP and OS were not statistically different between patients in the different bryostatin-1 dose groups (p-values = 0.3 and 0.7, respectively).

Toxicity profile

Two patients died on study. One patient (receiving therapy with paclitaxel 90 mg/m^2 with bryostatin-1 50 μg/m^2) was hospitalized for symptomatic bilateral pulmonary emboli,

developed cardiopulmonary arrest and died. The other patient (treated with paclitaxel 80 mg/m^2 with bryostatin-1 40 μg/m^2) developed respiratory failure thought to be bryostatin-related and died after a 4-week hospitalization despite intubation and aggressive medical care.

Hematologic toxicities were uncommon, with only one patient each (4%) developing grade 3 anemia and grade 3 leukopenia.

Among non-hematologic toxicities, myalgia was observed in 23 out of 24 patients. Six patients (25%) developed grade 1 myalgia, 5 patients (21%) developed grade 2 myalgia, 10 patients (42%) developed grade 3 myalgia and 2 patients (8%) developed grade 4 myalgia. The myalgias developed at a median of 8 days (1–41 days) and peaked at a median of 34 days (8–125 days). They were completely reversible but required a bryostatin-1-free interval of several weeks. The myalgias were managed symptomatically with such measures as narcotic and non-narcotic analgesics. Some patients required wheelchairs for ambulatory support.

Eleven out of 24 patients (46%) required at least one dose reduction for grade 2 or worse myalgia. Of these 11 patients, 7 were receiving bryostatin-1 at a starting dose of 40–50 μg/m^2. Ultimately, 6 of 24 patients (25%) were removed from study because of excessive toxicity due to myalgia. These data are summarized in Table 3.

Other significant non-hematologic toxicities included grade 5 pulmonary toxicity in one patient noted above and grade 4 pulmonary toxicity in another patient, who was hospitalized for presumed drug toxicity or exacerbation of underlying obstructive pulmonary disease and improved with intravenous steroids and antibiotics. Because of these toxicities, starting with the bryostatin-1 dose of 25 μg/m^2, PFTs and cytokine assessments were instituted. However, at this dose level, no changes in D_LCO or in IL-2, IL-6 or TNF-α within 2 h of bryostatin-1 administration were observed (data not shown).

All grade 3/4 non-hematologic toxicities, together with grade 1/2 non-hematologic toxicities seen in >20% of patients, are presented in Table 4.

Table 4 Toxicity profile (n = 24)

Toxicity	Grade (% of patients)				
	1	2	3	4	5
Anorexia	10 (42)	–	–	–	–
Diarrhea	9 (38)	6 (25)	1 (4)	–	–
Dyspnea	–	9 (38)	–	1 (4)	1 (4)
Fatigue	11 (46)	4 (17)	4 (17)	–	–
Myalgia	6 (25)	5 (21)	10 (42)	2 (8)	–
Nausea	8 (33)	4 (17)	–	–	–
Vomiting	10 (42)	–	–	–	–
Stomatitis	6 (25)	–	–	–	–
Thrombosis	–	–	1 (4)	–	1 (4)

NCI Common Toxicity Criteria, version 2.0

Discussion

Over 50% of patients with esophageal cancer present with metastatic disease, where chemotherapy is the mainstay of palliative therapy. The prognosis associated with metastatic or recurrent disease is dismal, with a median survival of only 8-10 months [3]. As such, more effective therapies are required.

One promising chemotherapeutic agent is paclitaxel. In a prior multicenter phase II evaluation, our group demonstrated that paclitaxel administered as a 1 h infusion weekly produces an objective response of 15% in chemotherapy-naïve patients with advanced esophageal cancer. The median TTP was 3.1 months and the median OS was 5.7 months [9].

In this study, we observed severe, frequently debilitating grade 3/4 myalgias. The etiology of the myalgias is unknown but they are not thought to be caused by myositis. The myalgias did appear to be a dose-related phenomenon of treatment with bryostatin-1. Unfortunately, at the time of this study, there was no pharmacological way to measure plasma levels of bryostatin-1.

One mechanism that has been postulated is impaired mitochondrial energy production as a result of reduced

Table 3 Description of myalgia, including incidence and severity by dose level and need for dose reduction and removal from study

Dose level			Myalgia (no. of pts/*N*)					
			Severity				Need to dose reduce	Need to take off study
Paclitaxel	Bryostatin-1	N	Gd 1	Gd 2	Gd 3	Gd 4		
90 mg/m^2	50 μg/m^2	4	–	–	2/4	2/4	3/4	2/4
80 mg/m^2	40 μg/m^2	7	1/7	1/7	5/7	–	4/7	2/7
80 mg/m^2	25 μg/m^2	13	5/13	4/13	3/13	–	4/13	2/13

N number of patients in each dose level, *pts* = patients; *gd* = grade (by NCI Common Toxicity Criteria, version 2.0)

blood flow caused by bryostatin-1-induced vasoconstriction [8, 13]. However, a small study in which patients receiving bryostatin-1 were also treated with nifedipine, a vasodilator, demonstrated an improvement in some parameters—oxygenation and proton efflux from cells—but evidence of impaired mitochondrial activity persisted [23]. The addition of nifedipine also did not improve the myalgias.

While a prior study noted elevations of IL-6 and TNF-α in a dose-dependent manner 2 and 24 h after bryostatin-1 administration[19], we did not note any increase in these cytokine levels over baseline 2 h after bryostatin-1 administration. However, these tests were only obtained starting at the bryostatin-1 dose of 25 μg/m^2, by which point the severity of the myalgias was reduced overall. Similarly, selected patients who experienced myalgias at all bryostatin-1 doses had creatine kinase measurements, all of which were normal.

The toxicities we observed on this trial are very similar to the toxicities observed by Ajani et al. [1] in their phase II evaluation of this combination in gastric cancer patients. They observed grade 3 myalgias in 55% of patients and 34% of patients discontinued therapy because of myalgias.

Despite the unexpected toxicity, there was a suggestion of anti-tumor activity from the combination. We observed a response rate of 27%, compared to the historical rate of 15% with paclitaxel alone. Clearly, interpretation across different clinical trials must be made with caution, particularly because of the small number of patients on this trial.

There did appear to be a potential dose-dependent relationship between bryostatin-1 dose and response rate. Although not statistically significant, patients treated with bryostatin-1 at 40–50 μg/m^2 had a response rate of 40% compared to 17% for patients treated with bryostatin-1 at 25 μg/m^2.

In addition, there were three other patients who had decreases in the sum of the cross-product of their measurable disease by 40–50% that did not meet the criteria for a partial response. The rate of these "near-PRs" plus PRs was 60% in the group of patients treated with bryostatin-1 at 40–50 μg/m^2 and was 25% in the patients who received bryostatin-1 at 25 μg/m^2 (p-value = 0.2).

Ultimately, however, the median TTP and OS were not statistically significant for patients receiving bryostatin-1 at 40–50 versus 25 μg/m^2. However, these were not primary endpoints that the trial was powered to evaluate. Furthermore, patients who discontinued therapy because of toxicity were censored from survival analyses, further limiting any informative comparison.

In this study, we adjudicated tumor response based on WHO criteria in order to permit a historical comparison with the single-agent paclitaxel study by Ilson et al., which employed WHO criteria. We have previously reported on the disparity in best tumor response for the first 19 assessable patients on this trial using WHO versus RECIST criteria [21]. The concordance between WHO and RECIST criteria was only 73.7%, with discrepancies occurring in patients with predominantly lymph node-only disease.

Although the objective responses seen in 5 of the first 19 patients were sufficient to permit full accrual to 33 patients, the trial was prematurely closed because of excessive toxicity. Further development of this combination would be indicated only if there is some mechanism to ameliorate the myalgias associated with bryostatin-1 at the higher doses of 40–50 μg/m^2.

Alternatively, several bryostatin-1 analogs that exhibit potent affinity to PKC have recently been synthesized [24–27]. Future characterization of their in vitro and in vivo anti-tumor activity and toxicities may lead to the clinical development of bryostatin-1 analogs that retain anti-tumor activity but have more acceptable toxicity profiles.

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