Phase Ib Trial of Bryostatin 1 in Patients with Refractory Malignancies

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ABSTRACT

A Phase Ib trial of bryostatin 1, a macrocyclic lactone and protein kinase C (PKC) activator, was conducted in patients with refractory nonhematological malignancies with the primary goal of determining whether down-regulation of peripheral blood mononuclear cell (PBMCN) PKC activity could be achieved in vivo in humans. Patients (four patients/cohort) received bryostatin 1 (25 μg/m²) as a 1-h infusion weekly three times every 4 weeks, but to study the schedule dependence of pharmacokinetics and pharmacodynamics, the first dose was administered according to one of three schedules: (a) a 1-h infusion; (b) a 24-h infusion; or (c) a split course (12.5 μg/m² as a 10-min infusion) on days 1 and 4. Conventional toxicities (grades I-III) included myalgias, fever, anemia, fatigue, phlebitis, and headache; in addition, two patients in cohort 3 experienced transient elevations in liver function tests, although these patients had preexisting liver metastases. No objective clinical responses were encountered. Effects on PBMCN PKC activity were heterogeneous. Several patients in cohorts 1 and 2 experienced significant declines in activity (~50%) that were sustained in some cases for periods of ≥72 h. Comparison of 72-h with baseline values for all three patient cohorts combined revealed a trend toward PKC down-regulation (P = 0.06; signed rank test). For each schedule, plasma bryostatin 1 levels were below the level of detection of a platelet aggregation-based bioassay (3–4 nm). Bryostatin 1 administration failed to produce consistent alterations in lymphocyte immunophenotypic profiles, interleukin 2-induced proliferation, or cytocytotoxicity, although two of three samples from patients in cohort 3 did show significant posttreatment increase in proliferation. Moreover, in some patients, bryostatin 1 treatment increased lymphokine-activated killer cell activity. These findings indicate that bryostatin 1 doses of 25 μg/m² can induce in vivo PBMCN PKC down-regulation in at least a subset of patients and raise the possibility that higher bryostatin 1 doses may be more effective in achieving this effect.

INTRODUCTION

Bryostatin 1 is a macrocyclic lactone derived from the marine bryozoan, Bugula neritina (1). In addition to immunomodulatory actions (2), bryostatin 1 has been shown to exert in vitro and in vivo activity against a variety of experimental tumor types, including carcinomas of the breast (3) and lung (4), melanoma (5), lymphoma (6), and leukemia (7), among others. In contrast to its inhibitory effects toward neoplastic cells, bryostatin 1 stimulates the in vitro growth of normal hematopoietic progenitors (8), possibly through an indirect mechanism (9). Based on these and other properties, bryostatin 1 has been targeted for clinical development by the National Cancer Institute, and several Phase I trials have now been completed. When administered as a weekly 1-h bolus or 24-h infusion, the maximum tolerated dose has been reported as 25–50 μg/m² (10–13). Myalgias have been the major dose-limiting toxicity; other side effects have included fevers, anemia, leukopenia, fatigue, phlebitis, headaches, dyspnea, hypotension, and bradycardia (10–13). Limited responses have been observed in patients with ovarian carcinoma and lymphoma (12). Bryostatin 1 also exerts in vivo immunomodulatory actions, enhancing expression of lymphocyte IL-2 receptors and selectively stimulating CD8+ cells (13).

Although the mechanism by which bryostatin 1 exerts its antineoplastic actions remains unknown, considerable evidence suggests the involvement of the Ca²⁺- and lipid-dependent serine-threonine kinase PKC. In in vitro studies using nanomolar concentrations, bryostatin 1 has been shown to bind to and activate PKC (14). This results in translocation of the enzyme to the membrane, accompanied by depletion of cytosolic activity (15). In this regard, bryostatin 1 mimics to a certain extent, the actions of tumor-promoting phorboids such as phorbol 12-myristate 13-acetate, although its spectrum of activity is distinctly different from such compounds. For example, bryostatin 1 blocks those phorboid-associated actions that it does not possess, including tumor promotion in mouse skin (16) and induction of differentiation in some leukemic cell sublines (15, 17).

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3 The abbreviations used are: IL, interleukin; PKC, protein kinase C; PBMCN, peripheral blood mononuclear cell; ara-C, 1-β-D-arabinofuranosylcytosine; LAK, lymphokine-activated killer cell; Ab, antibody.
The basis for the unique actions of bryostatin 1 is obscure but may stem from specific patterns of PKC isoform activation (18) or nuclear translocation (19). In addition, prolonged exposure of cells to bryostatin 1 (e.g., for 24 h) leads to profound reduction in total cellular PKC activity (20), possibly as a consequence of proteosomal degradation after ubiquitination (21). This phenomenon, referred to as down-regulation, is distinct from the early loss of cytosolic activity accompanying PKC activation, which results from enzyme translocation to the cell membrane (22).

Bryostatin 1 has been shown to modulate the apoptotic response of human leukemia cells to the antimetabolite ara-C (23). For example, pretreatment of human promyelocytic leukemia cells (HL-60) with bryostatin 1 substantially increases their susceptibility to ara-C-induced apoptosis, an event temporally associated with PKC down-regulation (24). This finding is consistent with reports that PKC activation opposes apoptosis (25, 26), whereas PKC inhibition promotes cell death (27, 28). In light of evidence that various chemotherapeutic drugs, including ara-C, induce leukemic cell apoptosis in vitro and in vivo (29, 30), it seems plausible that prior administration of bryostatin 1 might increase the efficacy of certain antileukemic agents.

To be effective, this strategy would require that bryostatin 1 exert its in vitro actions, i.e., PKC down-regulation, in vivo. Currently, little is known about the in vivo effects of bryostatin 1 on PKC activity. Recently, we reported that C57BL/6 mice receiving a single i.v. bolus injection of bryostatin 1 (1 μg) experienced significant and sustained PKC down-regulation in normal splenocytes, an effect accompanied by splenic enlargement (31). We have observed that in vitro exposure of normal PBMCs to bryostatin 1 results in PKC down-regulation virtually identical to that displayed by HL-60 cells. Thus, PBMCs may serve as a surrogate hematopoietic target tissue capable of mimicking the response of leukemic blasts to this agent. To assess the potential clinical relevance of these preclinical findings, a Phase Ib trial of bryostatin 1 has been conducted in patients with advanced malignancies. The primary goal of this trial was to determine whether and to what extent in vivo administration of bryostatin 1 modulates PKC activity in human hematopoietic cells.

MATERIALS AND METHODS

Study Design. A fixed dose of bryostatin 1 (25 μg/m²) was studied on the basis of experience from previous Phase Ia trials (10–13). Patients received bryostatin 1 (25 μg/m²) as a 1-h infusion weekly × 3 every 4 weeks; however, the first dose was administered by random assignment to one of three schedules: (a) 1-h i.v. infusion; (b) 24-h i.v. infusion; or (c) 12.5 μg/m² as a 0.5-h i.v. infusion on days 1 and 4.

Bryostatin 1 Source and Administration. Bryostatin 1 (NSC 339555) was supplied by the Cancer Treatment and Evaluation Program, Division of Cancer Treatment, National Cancer Institute. It was stored at 4°C in flint vials containing 0.1 mg of bryostatin 1 and 5 mg of povidone USP lyophilized from 40% t-butanol. Material was reconstituted in 1 ml of sterile PET (60% polyethylene glycol 400, 30% ethanol, and 10% Tween 80) diluent. The resulting solution was diluted further with 9 ml of 0.9% sodium chloride. Tubing for the drug infusion was primed with bryostatin 1 in the PET formulation diluted with sodium chloride to minimize adsorption of drug. For infusions of ≤1 h, bryostatin 1 was administered by syringe pump via a peripheral venous line. For 24-h infusions, bryostatin 1 was administered by syringe pump via either a peripheral venous or central line with coinfusion of 2 liters of normal saline.

Patient Eligibility. All patients were ≥18 years of age and had histologically confirmed lymphoma or solid tumor malignancy refractory to standard treatment. Other eligibility criteria included a life expectancy ≥12 weeks, Zubrod performance status ≤2, the presence of measurable disease, the absence of primary or metastatic malignant central nervous system disease, and no chemotherapy or radiotherapy within 4 weeks of planned therapy. Laboratory criteria included a WBC in the range 3.7–15.0 × 10⁹/μl, platelets in the range 130–500 × 10⁹/μl, hemoglobin ≥10 g/dl, normal protime, bilirubin ≤1.5, normal aspartate aminotransferase and alanine aminotransferase (≤2.5 × the upper limit of normal for metastatic disease), and creatinine ≤1.5 or creatinine clearance >70. Pregnant or nursing patients were excluded from the study.

Toxicity Grading. All patients received an initial evaluation that included a complete physical examination, serum chemistries, complete blood count, protime, and urinalysis. Toxicities were graded on a scale of 0–4 according to Cancer and Acute Leukemia Group B common toxicity criteria, with the exception of myalgias, which were evaluated based on criteria previously described by Philip et al. (10).

Response Criteria. The following criteria were used to assess the responses of patients to bryostatin 1: (a) complete response, absence of all measurable disease, signs, symptoms, and biochemical changes related to the tumor, including calcification of all lytic bone lesions, for >4 weeks and no new lesions; (b) partial response, ≥50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions compared to baseline measurements persisting for ≥4 weeks, no enlargement of an existing lesion, and no new lesions; (c) stable disease, neither response nor progression for ≥8 weeks; and (d) progression of disease, an increase in the product of the perpendicular diameters of any measured lesion by ≥25% compared to baseline measurement or any new lesion.

PBMC PKC Activity. At designated intervals (preinfusion and 1, 8, 24, 48, 72, 96, and 120 h postinfusion), 20 ml of blood were collected in a syringe containing EDTA (Sigma Chemical Co., St. Louis, MO) and stored at 4°C for not more than 20 min before processing. Samples were then diluted 1:2 with sterile RPMI 1640 (Life Technologies, Inc., Grand Island, NY) and layered over a 10-ml cushion of Ficoll-Hypaque (specific gravity, 1.077–1.081) in 50-ml sterile polypropylene conical centrifuge tubes. The tubes were centrifuged at 400 × g at room temperature according to the method of Boyum (32). At the end of this period, the interface layer containing mononuclear cells was extracted with a sterile pipette, transferred to 15-ml plastic centrifuge tubes, and washed twice with fresh medium.

S. Grant and H. D. Bear, unpublished observations.
Total PKC activity in PBMNC was determined as described previously (33) using materials supplied by Life Technologies, Inc. Briefly, pelleted cells were homogenized in 2 × 10⁻³ M Tris, 5 × 10⁻⁴ M EDTA, and 5 × 10⁻⁴ M EGTA (pH 7.5) containing 25 μg/ml protease inhibitors (aprotinin and leupeptin), with thirty-five 1-s strokes of a motorized micropipette homogenizer (Glas-Col) at setting 35. The homogenate was incubated on ice for 30 min, after which normalized quantities of protein (15 μg) were added to an assay mixture containing mixed micelles of phosphotidylserine and phorbol 12-myristate 13-acetate in suspension. The reaction was initiated by addition of 2.5 × 10⁻³ Ci/ml [γ-³²P]ATP, 2 × 10⁻⁵ M nonisotopic ATP, and 5 × 10⁻⁵ M synthetic peptide substrate (acetylated myelin basic protein amino-terminal peptide AcMB₁₋₁₄) in a buffer containing 1 mM CaCl₂ as per the manufacturer’s instructions. After a 5-min incubation at 30°C, aliquots of the reaction mixture were transferred to scintillation filters, and the reaction was terminated by immersion of discs in cold 1% (v/v) phosphoric acid. Preliminary studies established that this reaction was linear over at least a 30-min interval. The discs were washed thoroughly, and radioactivity was quantified by conventional liquid scintillation. With this method, replicate determinations generally did not vary by more than 15%. Baseline pretreatment PKC values for cells obtained from the 12 patients studied ranged from 1705-4136 pmol myelin basic protein phosphorylated/mg protein/5 min.

**Bryostatin 1 Plasma Assay.** Samples were collected in tubes containing EDTA and centrifuged at 400 × g for 10 min at 4°C, generally within 5 min of collection, after which the plasma was extracted with a sterile Pasteur pipette and transferred to sterile polypropylene centrifuge tubes. All assays were performed within 30 min of blood collection. Bryostatin 1 plasma levels were measured by addition of aliquots of patient plasma samples to donor whole blood and monitoring ATP release as described previously (67). Plasma samples (450 μl) were mixed with 450 μl of whole blood, and after 5 min at room temperature, 450 μl were added to 450 μl of 0.15 M NaCl in an aggregometer cuvette. Platelet ATP release was measured using Chrono-Lume reagent and a Chrono-log whole blood lumi-aggregometer. Standard curves using known quantities of authentic bryostatin 1 were assayed in parallel and used to determine concentrations of bryostatin 1 present in plasma samples. Alternatively, parallel studies were performed assaying the lag phase of platelet aggregation as described previously (34). These methods permit detection of plasma bryostatin 1 levels at concentrations as low as 5 nM (34).

**IL-2 Responsiveness and Lymphocyte Markers.** Samples of whole blood were obtained from each patient before treatment with bryostatin 1 and at intervals of 24, 72, and 144 h after initiation of treatment. In cohort 3 (days 1 and 4), day 4 samples were obtained after the second infusion of bryostatin 1. After density gradient centrifugation as described above, mononuclear cells were harvested, washed, and frozen in FCS + 10% DMSO (Sigma). Subsequently, samples were thawed, washed, and resuspended in complete medium consisting of RPMI 1640, 10% FCS (Hyclone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 10 mM HEPES buffer, and 5 × 10⁻⁵ M β-mercaptoethanol (Sigma). For proliferation assay, cells were plated in triplicate at 10⁴ cells/well in a total volume of 0.2 ml in 96-well plates (Costar, Cambridge, MA) with or without 40 IU/ml recombinant IL-2 (Chiron, Emeryville, CA). After 3 days of culture at 37°C in a 5% CO₂, fully humidified atmosphere, 0.05 mlCi of [³¹H]thymidine (Amersham, Arlington Heights, IL) was added to each well. After an 8-h incubation, cells were harvested onto glass fiber filters using a PHD Harvester (Cambridge Biotechnology, Inc., Cambridge, MA), and radioactivity was quantified by liquid scintillation.

Cell samples were also plated at 10⁶ cells/well in 24-well plates (Costar) under the following conditions: (a) 40 IU/ml IL-2; (b) wells precoated with anti-CD3 monoclonal Ab (OKT3; Ortho, Raritan, NJ) at 1 μg/ml, after which the cells were washed and cultured in the presence of 40 IU/ml IL-2 as described above; and (c) 500 IU/ml IL-2 alone to stimulate LAKs. After a 3-day incubation at 37°C, the cells were harvested, washed, and assayed for cytotoxicity against target cells using a 4-h [⁵¹Cr] release assay as described previously (35). Target cells tested included the P815 murine mastocytoma, the murine lymphocytic lymphoma EL-4, and human Daudi cells. Assays involving P815 cells, which have cell surface Fc receptors, were performed with and without addition of anti-CD3 monoclonal Ab (1 μg/ml); in the presence of anti-CD3, CTLs mediate Ab redirected cytotoxicity against Fc receptor + targets (36).

For analysis of cell surface markers, PBMNCs were stained with fluorescein-labeled antibodies directed against CD3, CD4, CD8, CD25, CD56, and DR (MHC class II) and analyzed using a FACScan analyzer (Becton Dickinson, Sunnyvale, CA) as described previously (37).

**Informed Consent.** The study was conducted in accordance with local and federal regulations for the protection of human research subjects, and all patients offered an informed consent before enrolment.

**Statistical Analysis.** PBMNC PKC data for cohorts as a whole were analyzed by the signed rank test. The significance of differences between PKC values at individual time points (relative to baseline control values) was determined using Student’s t test for unpaired observations.

**RESULTS**

**Patient Characteristics.** The characteristics of the patients participating in this trial are summarized in Table 1. The 12 patients included 7 males and 5 females. The median age was 55 years, with a range of 39–76 years. Eastern Cooperative Oncology Group performance status was 0 in 2 patients and 1 in 10 patients. Disease types and previous therapy were as shown in Table 1.

**Toxicities.** All patients entered on study were available for evaluation of toxicity. Table 2 lists the mean number of cycles administered and the associated side effects. In no patient was treatment discontinued due to bryostatin 1-related toxicity.

Patients treated initially according to schedule 1 (1-h bolus infusion) experienced myalgias (three of four patients), anemia (two of four patients), fever (two of four patients), and pruritus (one of four patients). In all cases, toxicity was grade II or lower. Myalgias occurred during both early and late treatment cycles and were responsive to standard analgesic therapy.
Phase Ib Trial of Bryostatin 1

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Tumor type</th>
<th>Prior treatment</th>
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<td>49</td>
<td>M</td>
<td>Pancreatic cancer</td>
<td>Surgery, chemotherapy</td>
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<tr>
<td>2</td>
<td>62</td>
<td>M</td>
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<td>4</td>
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<td>F</td>
<td>Non-small cell lung cancer</td>
<td>Chemotherapy, radiotherapy</td>
</tr>
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<td>5</td>
<td>62</td>
<td>F</td>
<td>Non-small cell lung cancer</td>
<td>Chemotherapy, radiotherapy</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>F</td>
<td>Breast cancer</td>
<td>Surgery, chemotherapy, radiotherapy</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>M</td>
<td>Pancreatic cancer</td>
<td>Surgery, chemotherapy, hormonal therapy</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>F</td>
<td>Breast cancer</td>
<td>Surgery, chemotherapy, radiotherapy, hormonal therapy, immunotherapy</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>M</td>
<td>Kidney cancer</td>
<td>Surgery, chemotherapy</td>
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<tr>
<td>10</td>
<td>44</td>
<td>F</td>
<td>Colon cancer</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>11</td>
<td>49</td>
<td>M</td>
<td>Melanoma</td>
<td>Surgery, chemotherapy, immunotherapy</td>
</tr>
<tr>
<td>12</td>
<td>43</td>
<td>M</td>
<td>Soft tissue sarcoma</td>
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Table 2 Toxities observed

<table>
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<tr>
<th>Cohort</th>
<th>Type of toxicity</th>
<th>Side effect</th>
<th>Grade</th>
<th>No. of subjects</th>
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<td>Anemia</td>
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<td></td>
<td></td>
<td>Fever</td>
<td>≤2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myalgia</td>
<td>≤2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pruritus</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>General</td>
<td>Leukopenia</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thrombocytopenia</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myalgias</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phlebitis</td>
<td>2</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Fullness (head)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Performance status</td>
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<td>2</td>
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<td>Cohort 2</td>
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<td>Anemia</td>
<td>≤2</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
<td>Leukopenia</td>
<td></td>
<td>1</td>
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<tr>
<td></td>
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<td>Thrombocytopenia</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>General</td>
<td>Fever</td>
<td>≤2</td>
<td>3</td>
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<td></td>
<td>Myalgias</td>
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<td>Phlebitis</td>
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<tr>
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<td>Fullness (head)</td>
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<td>1</td>
</tr>
<tr>
<td></td>
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<td>Performance status</td>
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<td>Fever</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myalgias</td>
<td>≤2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Bilirubin</td>
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<tr>
<td></td>
<td></td>
<td>Transaminase</td>
<td>≤2</td>
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<tr>
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<td>Alkaline phosphatase</td>
<td>≤3</td>
<td>2</td>
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</table>

Patients initially receiving bryostatin 1 as a 24-h continuous infusion also experienced grade 2 or lower myalgias (one of four patients), fever (three of four patients), and anemia (four of four patients). The anemia did not require transfusions and resolved after discontinuation of treatment. In addition, two of four patients experienced significant fatigue, leading to a decline in performance status (to 3) in one patient (grade 3 toxicity). This symptom resolved after the bryostatin 1 was discontinued as a consequence of disease progression. Other toxicities encountered in this cohort were of grade 2 or lower and included leukopenia, anorexia, ectopy, thrombocytopenia, phlebitis, headache, and neuromotor dysfunction.

Patients receiving bryostatin 1 as a 0.5-h infusion also experienced grade 2 or lower myalgias (one of four patients), fever (one of four patients), and anemia (one of four patients). In addition, two patients in this cohort developed grade 3 liver function abnormalities, including hyperbilirubinemia (one of four patients) and elevated alkaline phosphatase levels (one of four patients). Grade ≤ II liver function abnormalities included elevated aspartate aminotransferase (two of four patients), alanine aminotransferase (two of four patients), and alkaline phosphatase (one of four patients). The latter toxicities were self-limited and resolved following drug discontinuation after disease progression. It should be noted that each of the patients exhibiting liver function abnormalities had preexisting liver metastases.

Responses. There were no objective complete or partial responses in this group of patients. Three patients (with carcinoma of the pancreas, breast, and soft tissue sarcoma) exhibited stable disease during treatment that persisted for at least 8 weeks before disease progression was noted. Eight patients developed disease progression during or after the first cycle of therapy, and one patient elected to discontinue treatment before being evaluated.

PBMNC PKC Activity. The effects of bryostatin 1 on PBMNC PKC activity are shown in Fig. 1. Patients receiving bryostatin 1 as a 1-h bolus infusion (schedule 1) displayed an initial increase in PBMNC PKC activity immediately after drug administration. By 24 h, a decline in PBMNC PKC activity of 50% was observed in two of four patients and ~30% in a third (P ≤ 0.05 in each case). These declines were sustained for 72–120 h, a pattern similar to that previously reported in an animal model (31). In one patient, PBMNC PKC activity remained elevated at 24 h, fell to pretreatment control levels at 72 h, and subsequently showed a delayed increase in activity before declining to baseline at 144 h.

Patients receiving bryostatin 1 as a 24-h continuous infusion (schedule 2) showed a heterogeneous pattern of PBMNC PKC activity. Responses at 24 h ranged from a significant decline in activity (~65%) in one patient, a more modest decline (~30%) in a second (P ≤ 0.05 in each case), no change in a third, and a 100% increase in the fourth. The latter patient subsequently displayed a late decline in activity that approached 50% at 96 h (P ≤ 0.05). Activity returned to control levels at 72–120 h in all but the first patient, whose cells displayed a more prolonged recovery phase.

Patients receiving bryostatin 1 as a split dose (12.5 μg/m2 on days 1 and 4; schedule 3) also exhibited a heterogeneous response. In one patient, a modest decline in PBMNC PKC activity was observed postinfusion on day 1 (~40%; P ≤ 0.05), whereas an increase was noted in one of four patients. At 24 h,
activity declined modestly (~30%) in one patient (P ≤ 0.05), remained unchanged in two patients, and increased (~60%) in a fourth. After the second dose of bryostatin 1, PBMNC PKC activity did not change appreciably in three of four patients and increased in one patient. In this patient, PBMNC PKC activity remained elevated at the 144-h interval.

For the three patient cohorts as a whole, comparison of 72 h with baseline PBMNC PKC activities showed a trend toward down-regulation (P = 0.06; signed rank test). However, no significant differences among the three cohorts could be identified.

Pharmacokinetics Studies. Bryostatin 1 plasma levels were undetectable in samples obtained during or after infusion of bryostatin 1 for all of the schedules used. Based on the level of sensitivity of the platelet aggregation-based assay (34), this finding suggests that at the 25 μg/m² bryostatin 1 dose level, plasma bryostatin 1 levels are <5 nm.

IL-2 Response and Lymphocyte Markers. Analysis of phenotypic markers failed to demonstrate consistent changes in the surface markers of PBMNC (data not shown). For example, 1-25% of PBMNC were CD25+ before treatment, and this value decreased in 4 of 11 patients tested, increased in 2 of 11 patients, and remained the same in 5 of 11 patients. In addition, DR expression, observed in 7-25% of PBMNC before treatment, did not change significantly after administration of bryostatin 1, nor did the expression of other phenotypic markers. Comparison of phenotypes among different patient cohorts also did not reveal consistent patterns (data not shown).

When IL-2-stimulated proliferation was assessed, no significant change was detected in 7 of the 10 samples assessed at any time after administration of bryostatin 1 (Fig. 2). However, in three of the specimens assayed, increases in [%H]thymidine incorporation ranging from 100-1000% were noted (Fig. 2). Interestingly, two of the three patients demonstrating an increase in IL-2-stimulated proliferation were in cohort 3 (split dose), whereas the third patient was treated with a 1-h infusion of bryostatin 1.

Lastly, cytotoxicity studies also failed to detect significant bryostatin 1 effects in the majority of patients (data not shown). In one patient treated according to schedule 2, Ab-redirected T-cell cytotoxicity versus P815 target cells rose from 42%
before treatment to 77% on day 2 (data not shown); values subsequently declined to basal levels by day 8. In 3 of 11 patient samples assayed, significant increases in LAK-mediated killing was observed, most notably on days 4 or 5. An example of such a response in one of these patients is shown in Fig. 3.

**DISCUSSION**

Bryostatin 1 has shown a variety of interesting biological properties both in vitro and in vivo. At very low (i.e., nanomolar) concentrations, it stimulates the in vitro growth of normal human hematopoietic progenitors (8), possibly through an accessory cell mechanism (9). Comparable concentrations inhibit the growth of leukemic progenitors (7, 38), raising the possibility of a selective antileukemic effect. In animals, bryostatin 1 exhibits activity against a variety of tumor types, including carcinoma of the breast (3), lung (4), melanoma (5), lymphoma (6), and in a xenobiologic model, Waldenstrom’s macroglobulinemia (39). It has been assumed that the antineoplastic activity of bryostatin 1 stems from its ability to activate PKC, although recent studies bring this concept into question (40). In human trials to date, bryostatin 1 has been administered by several schedules, and limited responses have been observed in patients with malignant melanoma, renal cell carcinoma, and lymphoma (10–13). The dose-limiting toxicity in each of these studies has been myalgias, a phenomenon that seems to be cumulative (10–13). This has led to a recommended Phase II dose of 25–35 μg/m² every 1–2 weeks for a 1-h bolus or 24-h continuous infusion schedule, although recent studies suggest that when administered as a 72-h infusion, considerably higher bryostatin 1 doses may be achieved (41).

In addition to a potential activity as a single agent, bryostatin 1 may also have a clinical role as a facilitator of nonplastic cell apoptosis (23, 24). One of the known actions of PKC is to oppose programmed cell death induced by growth factor deprivation (25) or cytotoxic agents (26, 27). Whereas the mechanism by which PKC opposes apoptosis is uncertain, recent attention has focused on the opposing influences of the PKC and sphingomyelinase/ceramide pathways on cell viability (42). It has recently been proposed that factors that modulate the PKC/ceramide “rheostat” may shift the balance between cell survival and death (43, 44). The apparent paradox that bryostatin 1, a known PKC activator, promotes apoptosis, may be explained by its delayed ability to induce profound PKC down-regulation (20) through a mechanism involving ubiquitination and proteosomal degradation of the enzyme (21). Consistent with this concept, the effect of acute exposure of human leukemia cells to bryostatin 1, which leads to increased PKC activity, inhibits ara-C-induced apoptosis, whereas a more prolonged incubation (i.e., 24 h) results in a decrease in enzyme activity and potentiation of apoptosis (24).

In animal studies, in vivo administration of bryostatin 1 has been shown to induce rapid reductions in cytosolic PKC activity in peritoneal neutrophils (45). However, this action, which stems from translocation of the enzyme to the cell membrane, is distinct from the decline in total cellular activity that results from enzyme down-modulation (22). In a recent study involving C57BL/6 mice receiving a single i.v. injection of bryostatin 1 (1 μg), significant down-regulation of total splenocyte PKC activity was observed, which persisted for periods as long as 96 h (31). This finding indicates that the ability of bryostatin 1 to induce PKC down-regulation is not restricted to the in vitro setting. The central goal of this study was to determine whether in vivo administration of bryostatin 1 in humans by one or more schedules could reduce PKC activity in a hematopoietic target tissue, analogous to results obtained in a murine model (31). Once identified, such a bryostatin 1 schedule could then be combined with a cytotoxic agent (e.g., ara-C) to mimic in vitro regimens displaying enhanced antileukemic activity (23, 24). Although responses in the current trial were quite heterogeneous, marked reductions in PBMC PKC activity were observed in several patients, and there was a trend (P = 0.06, signed rank test) toward reduction at 72 h for the three cohorts as a whole. Both the magnitude and duration of these reductions were comparable to those encountered in an antecedent murine study (31). It is possible that down-regulation of PBMC PKC activity was limited by the bryostatin 1 dose used in this trial. In this regard, Jayson et al. (12) measured total PKC activity in PBMC from three patients at various intervals during the course of a 24-h continuous infusion of 25 μg/m² of bryostatin 1. Although changes in PKC activity were noted in these patients, consistent depletion of activity at the end of the 24-h infusion could not be documented. Effects on PBMC PKC activity at later time points were not examined in that study. It should be noted that the dose of bryostatin 1 used in this and in previous trials (25 μg/m²; Refs. 10–13) has been limited by cumulative toxicity (chiefly myalgias) that accompanies chronic administration. It is conceivable that less frequent administration of bryostatin 1 (e.g., every 4–6 weeks) might permit higher individual doses to be given, with greater effects on PKC activity. Studies designed to test this hypothesis are currently under development.

Plasma levels of bryostatin 1, regardless of schedule of
administration, were below the level of detection of the platelet aggregation-based bioassay, which is sensitive to bryostatin 1 concentrations as low as 2–5 nm (34). These results, as well as those of several earlier studies in animals, suggest that the failure to achieve high plasma bryostatin 1 concentrations stems from rapid plasma clearance of this compound. For example, Berkow et al. (45), using a neutrophil activation assay, reported that in mice receiving a single i.v. injection of bryostatin 1 (1 μg), plasma concentrations declined to undetectable levels (e.g., < 60 nm) within minutes of drug administration. More recently, Zhang et al. (46) described the pharmacokinetics of [C26-3H]bryostatin 1 (40 μg/kg) in CD1/F2 mice after i.v. or i.p. administration. When given by the i.v. route, bryostatin 1 was rapidly cleared from the plasma, with concentrations falling to ~15 nm after 4 h, to <10 nm after 12 h, and to subnanomolar concentrations thereafter. Both urinary and gastrointestinal excretion were detected, and bryostatin 1 was widely distributed in tissues including lung, liver, heart, lymph nodes, and fat. If the disposition of bryostatin 1 in humans is similar, rapid plasma clearance secondary to excretion in conjunction with widespread tissue distribution could account for our failure to detect plasma bryostatin 1 levels of ≥5 nm in any of the patient cohorts.

No consistent pattern of alterations in lymphocyte phenotype, IL-2-induced proliferation, or cytotoxicity against a variety of targets was observed in the majority of subjects after treatment with bryostatin 1. In three patients, however, a striking increase in IL-2-induced proliferation was noted. Our results differ somewhat from those of previous trials in which increases in IL-2 responsiveness and LAK activity were noted (12, 13). However, in one of these studies, which involved a 24-h infusion schedule, peak increases in IL-2-induced proliferation were observed 2 h after initiation of drug infusion (13). If such maximal responses typically occur within this time frame, they would not have been detected at the later intervals examined in the present study. It may be significant that two of three patients displaying a significant increase in IL-2-induced lymphocyte proliferation received bryostatin 1 as a split dose on days 1 and 4. Taken in conjunction with evidence that administration of bryostatin 1 at or near the maximum tolerated dose in mice inhibits their resistance to bacterial infection (47), these findings raise the possibility that lower bryostatin 1 doses may be more appropriate when this agent is intended as an immunomodulator. In any case, based on the small number of patients in each cohort, definitive conclusions regarding the schedule-dependent immunomodulatory effects of bryostatin 1 cannot be drawn.

In summary, the results of the present study suggest that administration of bryostatin 1 at a fixed dose of 25 μg/m² according to several schedules may be capable of down-regulating PBMC PKC activity in a subset of patients, and that in these individuals, down-modulation is qualitatively and quantitatively similar to that observed in a preclinical animal model. In this study of limited power, response patterns seemed to favor the 1-h bolus and 24-h infusion over the split-course schedule if down-regulation of PKC activity is a targeted goal. However, based on the inconstancy of the PKC modulatory effects, it is unclear which of the tested schedules will prove optimal for projected Phase II successor trials. It also remains possible that higher bryostatin 1 doses could be more effective than the 25-μg/m² dose used in the present study. In view of evidence that bryostatin 1 doses as high as 120 μg/m² are tolerable when administered as a 72-h continuous infusion (41), it is conceivable that such schedules might result in more profound and/or sustained PKC down-regulation than that observed in the present study. Other issues remaining to be addressed include: (a) whether bryostatin 1 is capable of down-regulating PKC in neoplastic target tissues such as leukemic blasts; (b) whether this action increases the in vivo susceptibility of such cells to cytotoxic drugs (i.e., ara-C); and (c) whether the combination of bryostatin 1 and a cytotoxic agent offers the prospect of improved therapeutic selectivity. To answer these and related questions, a Phase I trial of escalating doses of bryostatin 1 administered in conjunction with high-dose ara-C in patients with refractory hematological malignancies is currently under development.

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Phase Ib Trial of Bryostatin 1


Phase Ib trial of bryostatin 1 in patients with refractory malignancies.

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