Phase II Trial of Bryostatin 1 in Patients with Relapsed Low-Grade Non-Hodgkin’s Lymphoma and Chronic Lymphocytic Leukemia

Mary L. Varnerasian, Ramzi M. Mohammad, Muhammad S. Shurafa, Kim Hulburd, Pamela A. Pemberton, Dorothy H. Rodriguez, Virginia Spadoni, David S. Elender, Anthony Murgo, Nathan Wall, Maria Dan, and Ayad M. Al-Katib

Karmanos Cancer Institute and Wayne State University, Detroit, Michigan 48201 [M. L. V., R. M. M., K. H., P. A. P., D. H. R., V. S., D. S. E., N. W., M. D., A. M. A-K.]; Henry Ford Hospital, Detroit, Michigan 48202 [M. S. S.]; and Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, Maryland 20852 [A. M.]

ABSTRACT
Bryostatin 1 is a natural product isolated from the marine bryzoan Bugula neritina in 1982 and is currently undergoing evaluation in a number of malignancies. Twenty-five patients with relapsed, low-grade non-Hodgkin’s lymphoma or chronic lymphocytic leukemia (CLL) received bryostatin 1 by 72-h continuous infusion every 2 weeks at a dose of 120 μg/m² per course. Patients who progressed while receiving bryostatin 1 alone could participate in a feasibility study by receiving vincristine administered by bolus i.v. injection immediately after the completion of the bryostatin 1 infusion. The dose of vincristine was escalated in groups of three patients as follows: level 1, 0.5 mg/m²; level 2, 1.0 mg/m²; and level 3, 1.4 mg/m² with vincristine doses capped at 2.0 mg for all patients. Bryostatin 1 alone resulted in one complete remission and two partial remissions. Nine patients received sequential treatment with bryostatin 1 and vincristine. The addition of vincristine at a dose of 2 mg was feasible and caused the expected dose-related sensory neuropathy. Phenotypic analysis by flow cytometric analysis on pre- and post-bryostatin 1-treated peripheral blood lymphocytes revealed up-regulation in the coexpression of CD11c/CD22 on CD20⁺ B cells in two of four CLL patients studied, which is consistent with in vitro findings of differentiation of CLL cells to a hairy cell phenotype.

INTRODUCTION
Bryostatin 1 was isolated from the marine bryozoan Bugula neritina in 1982 by Pettit et al. (1) as part of the National Cancer Institute’s Natural Products Program and is one of a family of more than 17 compounds with a multiringed macrocyclic lactone structure. Its ability to modulate the family of PKC enzymes, which are involved in regulating cellular proliferation and differentiation, is thought to account for many of its biological actions (2). In preclinical in vitro and in vivo models of human lymphoid tumors, bryostatin 1 has antitumor activity, induces apoptosis and potentiates the activity of the Vinca alkaloid vincristine (3, 4), down-regulates MDR1 gene expression (5), and modulates bcl-2 and p53 gene expression (6).

Bryostatin 1, like the PKC-activating phorbol esters, is capable of inducing the differentiation of several B cell tumors in vitro to a terminal nonproliferative state (7). Specific studies in CLL have shown that bryostatin 1 can induce the differentiation of CLL cells in vitro (8). These differentiated CLL cells resembled hairy cells both phenotypically with the up-regulation of the surface markers CD22 and CD11c and enzymatically with the induction of tartrate-resistant acid phosphatase. Both of these findings are major indicators of the differentiation of CLL cells toward hairy cells according to an established B cell differentiation schema (9).

Our Phase I clinical evaluation of bryostatin 1 in patients with relapsed NHL and CLL defined the maximum tolerated dose of bryostatin 1, when administered over 72 h every 2 weeks, to be 120 μg/m² (40 μg/m²/day for 3 days; Ref. 10). Generalized myalgia was the dose-limiting toxicity. We now report our results of a Phase II evaluation of a 72-h continuous infusion of bryostatin 1 in patients with relapsed LG-NHL or CLL and of a pilot feasibility study of vincristine administered immediately after bryostatin 1.

PATIENTS AND METHODS
Study Population. Adults with B cell CLL [intermediate risk with active disease or high risk by the modified three-stage RAI classification (11)] or LG-NHL who had relapsed after one or two regimens were eligible for this study. Other eligibility requirements were a Zubrod performance status of ≤2, time lapse of 4 weeks from prior therapy, age ≥18, and adequate hematological, hepatic, and renal function. The protocol was...
approved by the Institutional Review Boards of Wayne State University, Henry Ford Hospital, and the Cancer Therapy Evaluation Program of the National Cancer Institute. Informed consent was obtained from all patients.

**Treatment Schedule.** Patients received bryostatin 1 via a portable infusion pump at 120 μg/m² over 72 h (40 μg/m²/day × 3 days) every 14 days with reevaluation every four cycles. Patients with stable disease or partial response continued to receive bryostatin 1 alone. Patients who progressed on bryostatin 1 could continue to participate in a feasibility study and receive vincristine by bolus i.v. injection immediately after the completion of the bryostatin 1 infusion. The dose of vincristine was escalated in groups of three patients as follows: level 1, 0.5 mg/m²; level 2, 1.0 mg/m²; and level 3, 1.4 mg/m². The dose of vincristine was capped at 2.0 mg for all patients.

**Formulation.** Bryostatin 1 (NSC 339555) was supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute along with PET diluent [PET 60/30/10, NSC 641159, is a 60%, 30%, and 10% (v/v) mixture of polyethylene glycol 400, dehydrated alcohol, U.S. Pharmacopeia, and polysorbate 80 (Tween 80)]. The 72-h dosage was prepared by dilution with benzyl alcohol-preserved normal saline in a polypropylene bag to a drug concentration between 1 and 10 μg/ml.

**Response and Toxicity Criteria.** NHL: a CR was defined as complete disappearance of all measurable and evaluable disease, no new lesions, no disease-related symptoms, and no evidence of nonevaluable disease maintained for at least 4 weeks. A PR was defined as a decrease of ≥50% in the product of the perpendicular diameters of all measurable lesions maintained for at least 4 weeks with no new lesions and no progression of evaluable disease. Progression of disease was defined as an increase of ≥25% in the sum of the products of the perpendicular diameters of all measured lesions or appearance of new lesions or worsening of any evaluable disease. Patients with stable disease are those not meeting the criteria for CR, PR, or progression of disease. CLL: standard response criteria for CLL were used (11). Toxicity was graded on a scale of 0–4 by the National Cancer Institute criteria (12).

**Flow Cytometric Analysis.** Peripheral blood mononuclear cells from CLL patients were obtained by Ficoll-Hypaque separation prior to treatment and immediately after completion of the first cycle of therapy. Cells were stained simultaneously with three antibodies targeting CD20, CD11c, and CD22 directly conjugated to peridinin chlorophyll protein, PE, and FITC, respectively. CD20⁺ B cells were isolated and the proportion of cells coexpressing CD11c/CD22 was determined.

**RESULTS**

Between December 1996 and March 1999, 25 patients with relapsed LG-NHL or CLL were entered into this protocol. Bryostatin 1, in the PET formulation, was administered at a dose of 120 μg/m² by 72-h continuous infusion (40 μg/m²/day for 3 days) through a central venous access device every 2 weeks. Patient characteristics are shown in Table 1. The seven patients with CLL and the 18 patients with LG-NHL had a median disease duration of 72 months (range, 9 months–18 years) and 45 months (range, 10 months–8 years), respectively, prior to study enrollment.

**Response.** There was one CR in a 41-year-old woman with a follicular small-cell cleaved NHL, stage 4B, who achieved a remission with alkylator-based combination therapy 5 years prior to study entry. At the time of study entry, she had a biopsy-documented recurrence at multiple cutaneous sites. There were no other sites of disease. She achieved a CR gradually after 8 cycles of bryostatin 1 and remains in a CR now, 18 months after coming off the study. Two patients with small lymphocytic lymphoma achieved a PR, both after four cycles of therapy (one for 13 months and the other for 6 months). Two patients had stabilization of disease for 4 and 5 months.

**Toxicity.** In general, bryostatin 1 was well tolerated. Six patients experienced grade 3 myalgias (severe pain with either pain or analgesics interfering with daily activities) requiring dose reductions. As in previous studies, myalgias became increasingly severe, prolonged, and generalized with increase in cumulative dose (median, seven cycles administered prior to development of grade 3 myalgias; range, four to fifteen cycles) but could be controlled by dose reductions and/or dose delays. Eight patients experienced grade 2 fatigue, and two patients experienced grade 2 infectious complications (one catheter-
related and one cellulitis). There was no significant hematological toxicity.

**Addition of Vincristine.** Nine patients received sequential treatment with bryostatin 1 and vincristine (Table 2). The addition of vincristine caused grade 3 sensory neuropathy noted in the four patients receiving a total dose of greater than 10 mg.

**Phenotypic Analysis in Vivo.** Bryostatin 1 treatment up-regulated the expression of CD11c and CD22 in two of four CLL patients studied (Fig. 1) but was not associated with response. The coexpression of CD22/CD11c is unique to hairy cell leukemia and monocytoid B cell lymphoma (8, 13).

**DISCUSSION**

Our preclinical Phase I and Phase II studies demonstrate that bryostatin 1 has biological activity against LG-NHL and CLL. Bryostatin 1 produced objective responses in a broad spectrum of tumors, including malignant melanoma, ovarian carcinoma, and low-grade NHL, in initial Phase I studies. This finding spawned a number of single-agent Phase II and combination Phase I studies in a variety of tumor types. Because there is currently no assay for plasma bryostatin 1 levels and because data regarding PKC modulation, a potential biological surrogate, has not been consistent, the optimal dose and schedule of administration of the drug has not yet been determined. The most commonly used schedules of administration are 25 μg/m² over 1 h or continuous infusion over 24 h each week for 3 weeks, repeated every 4 weeks, and 40 μg/m²/day for 3 days continuous infusion every 2 weeks. The first published Phase II study of bryostatin 1 as a single agent has failed to demonstrate activity in previously treated patients with metastatic malignant melanoma (14).

Our observation in two CLL patients that bryostatin 1 induced the coexpression of CD11c and CD22 on malignant B cells is consistent with the in vitro findings of differentiation of CLL cells. There was no correlation with response, but the number of patients studied was small. The further development of bryostatin 1, which works through the modulation of signal transduction pathways resulting in a predominantly cytostatic effect and for which there is currently no assay, should be aided by the incorporation of the demonstration of biological effects into clinical trials. In an effort to capitalize upon its ability to differentiate CLL cells to hairy cells and sensitize them to 2-chloro-deoxyadenosine (15), a sequential study of these two drugs in CLL patients is ongoing at our institution.

In the present study, we have demonstrated that sequential therapy with vincristine in doses up to 2 mg is feasible and well tolerated. Vincristine was chosen for sequential therapy in this trial because it had been shown that in SCID mouse xenografts bearing human diffuse large cell lymphoma, administering vincristine 24 h after bryostatin 1 resulted in improved antitumor activity compared with administration of either agent alone (5). Furthermore, the improvement in antitumor activity in that model was associated with a decrease in p-glycoprotein and a down-regulation of mdrl RNA expression, suggesting a possible mechanism by which bryostatin 1 potentiates the activity of vincristine. On the basis of this preclinical data and the fact that the combination of bryostatin 1 and vincristine can safely be administered, a Phase II study of sequential therapy seems warranted. Combination therapy with other cytotoxic agents should be feasible because bryostatin 1 does not have myelosuppressive properties.
As part of the National Cancer Institute’s Natural Products Program, a number of novel agents derived from marine products with antilymphoid activity have been identified and are now in clinical trials. Preclinically, we have also tested the activity of dolastatin 10 (a natural product derived from the shell-less marine mollusk Dolabella auricularia that is currently in Phase II clinical trials) and its structural homologue, auristatin PE, alone and in combination with bryostatin 1. The combinations auristatin PE + bryostatin 1 and dolastatin 10 + bryostatin 1 produced cures in five of five and two of five treated SCID mice xenografts harboring human CLL (WSU-CLL-SCID), respectively, providing a compelling rationale for these natural products to be explored clinically in the treatment of CLL (16).

In summary, bryostatin 1 as administered in this study at the MTD by 72-h continuous infusion resulted in modest although definite activity as a single agent. It can be given safely in sequence with vincristine at the standard dose of 2 mg. Further clinical studies of bryostatin 1 in lymphoid malignancies should focus on combination therapies. Preclinical studies suggest that vincristine, 2-CdA, and the natural products dolastatin 10 and auristatin PE would be logical candidates.

ACKNOWLEDGMENTS

We thank Pamela Sorice for excellent technical assistance in the flow cytometric analysis and Dr. Gregory Kalemkerian for review of the manuscript.

REFERENCES

Phase II Trial of Bryostatin 1 in Patients with Relapsed Low-Grade Non-Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia

Mary L. Varterasian, Ramzi M. Mohammad, Muhammad S. Shurafa, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/3/825

Cited articles
This article cites 13 articles, 7 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/3/825.full#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/3/825.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.