

# A Phase I Trial of Bryostatin-1 in Children with Refractory Solid Tumors: A Pediatric Oncology Group Study

Steven Weitman,<sup>1</sup> Anne-Marie Langevin,  
Roger L. Berkow, Paul J. Thomas,  
Craig A. Hurwitz, Andrew S. Kraft,  
Ronald L. Dubowy, Debra L. Smith, and  
Mark Bernstein

University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-3217 [S. W., A.-M. L., P. J. T.]; University of Alabama, Birmingham, Alabama [R. L. B.]; Maine Children's Cancer Program, Barbara Bush Children's Hospital of the Maine Medical Center, Portland, Maine [C. A. H.]; University of Colorado Health Science Center, Denver, Colorado [A. S. K.]; SUNY-Health Science Center at Syracuse, Syracuse, New York [R. L. D.]; University of Kansas Medical Center, Kansas City, Kansas [D. L. S.]; and McGill University, Montreal, Quebec, Canada [M. B.]

## ABSTRACT

**Bryostatin-1, a macrocyclic lactone, appears to elicit a wide range of biological responses including modulation of protein kinase C (PKC). PKC, one of the major elements in the signal transduction pathway, is involved in the regulation of cell growth, differentiation, gene expression, and tumor promotion. Because of the potential for a unique mechanism of interaction with tumorigenesis, a Phase I trial of bryostatin-1 was performed in children with solid tumors to: (a) establish the dose-limiting toxicity (DLT) and maximum-tolerated dose (MTD); (b) establish the pharmacokinetic profile in children; and (c) document any evidence of antitumor activity.**

**A 1-h infusion of bryostatin-1 in a PET formulation (60% polyethylene glycol 400, 30% ethanol, and 10% Tween 80) was administered weekly for 3 weeks to 22 children (age range, 2–21 years) with malignant solid tumors refractory to conventional therapy. Doses ranged from 20 to 57  $\mu\text{g}/\text{m}^2/\text{dose}$ . Pharmacokinetics were performed in at least three patients per dose level. The first course was used to determine the DLT and MTD.**

**Twenty-two patients on five dose levels were evaluable for toxicities. At the 57  $\mu\text{g}/\text{m}^2/\text{dose}$  level dose-limiting myalgia (grade 3) was observed in three patients; two of those patients also experienced photophobia or eye pain, and one experienced headache. Symptoms occurred in all patients within 24–72 h after the second dose of bryostatin-1 with resolution within 1 week of onset. Other observed toxicities**

**(grades 1 and 2) included elevation in liver transaminases, thrombocytopenia, fever, and flu-like symptoms. The bryostatin-1 infusion was typically well tolerated. Although stable disease was noted in several patients, no complete or partial responses were observed.**

**The recommended Phase II dose of bryostatin-1 administered as a 1-h infusion weekly for 3 of every 4 weeks to children with solid tumors is 44  $\mu\text{g}/\text{m}^2/\text{dose}$ . Myalgia, photophobia, or eye pain, as well as headache, were found to be dose limiting.**

## INTRODUCTION

Bryostatin-1 is a macrocyclic lactone originally isolated from the marine organism *Bugula neritina* (1). Preclinical studies with bryostatin-1 suggest that this agent has *in vitro* and *in vivo* antitumor activity against several murine and human solid tumors and leukemias (2–6). With the use of murine tumors, bryostatin-1 substantially prolonged survival in mice bearing B16 melanoma, M5076 sarcoma, P388 leukemia, and L10A B-cell lymphoma. Bryostatin-1 was particularly active in reducing metastasis formation using the B16 melanoma tumor model (3). Against human tumors, bryostatin-1 demonstrated antitumor activity against myeloid and lymphoid leukemia as well as against lymphoma tumor xenografts (5, 6). In a pediatric tumor model, bryostatin-1 decreased *c-myc* expression and DNA synthesis, measured by thymidine incorporation, in neuroblastoma tumor cells (SH-SY5Y; Ref. 7). However, bryostatin-1 failed to induce morphological differentiation of neuroblastoma tumor cells (7).

Although the precise mechanism of the anticancer activity of bryostatin-1 is unclear, it appears to elicit a wide range of biological responses including modulation of PKC<sup>2</sup> (8). PKC, one of the major elements in the signal transduction pathway, is involved in the regulation of cell growth, differentiation, gene expression, and tumor promotion (8–10). Unlike some PKC interactive agents (*e.g.*, phorbol esters), bryostatin-1 does not appear to possess tumor-promoting activity (8, 9). Different biological responses elicited by PKC activators reflect the existence and selective activation of isoforms (*e.g.*,  $\alpha$ ,  $\beta$ ,  $\delta$ , and  $\theta$ ) of the enzyme and may, in part, explain the unique antitumor activity of bryostatin-1 (9).

Because of the unique properties of bryostatin-1 and its broad *in vitro* and *in vivo* antitumor activity, a Phase I study of this agent in children was developed. This is the first reported clinical trial of bryostatin-1 in the pediatric population. The objectives of this trial were: (a) to establish the toxicities and MTD of bryostatin-1 administered as a 1-h infusion weekly for

Received 12/19/98; revised 2/15/99; accepted 2/18/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> To whom requests for reprints should be addressed, at The Pediatric Oncology Group, Operations Office, 654 North Michigan Avenue, Suite 910, Chicago, IL 60611.

<sup>2</sup> The abbreviations used are: PKC, protein kinase C; MTD, maximum tolerated dose; NCI, National Cancer Institute; DLT, dose-limiting toxicity.

3 of every 4 weeks to children with solid tumors refractory to conventional therapy; (b) to establish the pharmacokinetic profile of bryostatin-1 in children; and (c) to document any antitumor activity.

## PATIENTS AND METHODS

### Eligibility Criteria

Patients <21 years of age with a histologically or cytologically documented solid tumor refractory to conventional treatment, or for which no effective therapy was known, were eligible for this trial. Other eligibility criteria included: (a) a predicted life expectancy  $\geq 6$  weeks; (b) adequate nutritional status; (c) a Karnofsky performance score of  $\geq 50\%$ ; (d) adequate bone marrow function (absolute neutrophil count  $\geq 1500/\text{mm}^3$  and platelets  $\geq 75,000/\text{mm}^3$ ); (e) adequate liver function (bilirubin  $\leq 1.5$  mg/dl and alanine aminotransferase  $< 2 \times \text{N}$ ); and (f) adequate renal function (creatinine  $\leq 1.5$  mg/dl). Patients could not receive any other anticancer agents or be on any other study during the course of this therapy, had to have recovered from toxicity of all previous chemotherapy, could not have severe uncontrolled infections, and could not be pregnant or lactating. At least 6 weeks had to have elapsed since administration of substantial radiation therapy or nitrosoureas. Patients must have been at least 6 months from the receipt of craniospinal irradiation, irradiation to  $\geq 50\%$  of the pelvis, or total body irradiation in the context of bone marrow transplantation. Informed consent was obtained from the patient or his/her legal guardian before entering onto the study in accordance with NCI and individual institutional policies.

### Drug Administration and Study Design

Bryostatin-1 was supplied by the Division of Cancer Treatment of the NCI (Bethesda, MD) in 6-ml vials that contained 0.1 mg of bryostatin-1 and 5 mg of povidone USP lyophilized from 40% *t*-butyl alcohol. The dry powder was reconstituted with 1 ml of sterile PET (60% polyethylene glycol 400, 30% ethanol, and 10% Tween 80) diluent. This solution was further diluted with 0.9% sodium chloride, for injection, to a final concentration of 10  $\mu\text{g}/\text{ml}$ . Bryostatin-1 was contained in glass bottles and was infused through nitroglycerin tubing. A central venous access device was used for drug administration in all patients.

The starting dose was 20  $\mu\text{g}/\text{m}^2/\text{dose}$  (80% of the adult MTD) administered as a 1-h infusion weekly for 3 of every 4 weeks. Dose escalation proceeded by 30% increments (26, 34, 44, and 57  $\mu\text{g}/\text{m}^2/\text{dose}$ ). No inpatient dose escalation was permitted. At least three patients were treated at each dose level. If one of the first three patients entered at any dose level experienced a DLT during the first course of therapy, an additional three patients were entered at that dose level. Toxicities were graded according to the NCI common toxicity criteria (11). The myalgia toxicity grading scale is shown in Table 1.

A minimum of two courses was recommended for patients who had tumor response or stable disease after the first course of treatment. However, treatment was aborted after a single course in the presence of progressive disease, and either terminated or dose reduced with grade 3 or grade 4 nonhematological toxicity, or any grade 4 hematological toxicity that lasted  $\geq 7$  days. If DLT was noted in two of three to six patients at any

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 $\leq 7$ days; patient remains ambulatory.
Grade 3	Moderate to severe pain, regular analgesics required (not morphine); patient's mobility is significantly restricted.
Grade 4	Very severe, incapacitating pain; patient requires constant bedrest and regular morphine.

given dose level or a patient died from toxicity, the MTD was exceeded, and three more patients were treated at the next lower dose level. The MTD was defined as the dosage immediately below the DLT.

Prior to treatment, patients were required to have a physical assessment including performance status, studies to assess tumor size, and laboratory measurements (complete blood count, blood urea nitrogen, serum creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, serum albumin, electrolytes, calcium, magnesium, prothrombin time, partial thromboplastin time, and urinalysis). Physical examination, evaluation of symptoms, and a complete blood count and chemistries were performed weekly. Tumor size was measured before the second and third course of treatment and every other course thereafter.

### Evaluation of Response

Complete response was achieved when all evidence of disease disappeared. Partial response was defined as  $\geq 50\%$  reduction in the sum of the products of the maximum perpendicular diameters of all measurable lesions without progression in any lesion and without any new lesions. Minor response was defined as  $\geq 25\%$  to  $< 50\%$  reduction in the sum of the products of the maximum perpendicular diameters of all measurable lesions without progression in any lesion and without any new lesions. Stable disease was defined as  $< 25\%$  reduction in the sum of the products of the maximum perpendicular diameters of all measurable lesions without progression in any lesion and without any new lesions. An increase of  $> 25\%$  in the sum of the products of the maximum perpendicular diameters or the appearance of new lesions was defined as progressive disease.

### Pharmacokinetics

**Sample Collection.** At least three patients at each dose level (except for at 34 and 57  $\mu\text{g}/\text{m}^2$ , where only one and two patients were evaluable for pharmacokinetics, respectively) had serial blood samples taken during their first treatment course for measurement of plasma bryostatin-1 concentrations and pharmacokinetic evaluation. Blood samples were collected in heparin-containing tubes at a venous site distant from the drug infusion site. Blood was collected immediately prior to drug administration, at 30 min during infusion, at the end of infusion, and at 10 min, 30 min, 4 h, 24 h, and 48 h after infusion. Each blood sample was divided into equal volumes and transferred to two polypropylene microcentrifuge tubes. The transferred blood was then microcentrifuged for 3 min. Plasma was aliquoted into

Table 2 Patient characteristics (n = 22)

Age (yr)	
Median	10.5
Range	2–21
Sex	
Girls	13
Boys	9
Diagnosis	
Osteosarcoma	4
Neuroblastoma	3
Hepatoma/Hepatocellular	3
Sarcoma <sup>a</sup>	3
Hodgkin's	2
Renal cell	2
Rhabdomyosarcoma	2
Wilms'	1
Germ cell	1
Adrenal cell carcinoma	1
Prior therapy	
None	0
Chemotherapy only	11
Chemotherapy + radiation	11
Autologous BMT	4

<sup>a</sup> Two Ewing's sarcoma/PNET and one nerve sheath sarcoma.

disposable polypropylene tubes and immediately frozen and stored at  $-70^{\circ}\text{C}$  until analysis.

**Bryostatin-1 Analysis.** Bryostatin-1 concentrations were determined by competition with the binding of tritiated phorbol-12,13-dibutyrate to a rat brain membrane preparation (12). The standard curve was performed in the presence of plasma collected in the same manner as patient samples and was performed on the same day with identical reagents. All determinations, when possible, were performed in duplicate. Sample-to-sample variation was  $<10\%$  in 90% of duplicates. The concentration of bryostatin-1 in the patient's plasma was determined from the standard curve plot of binding inhibition *versus* the logarithm of bryostatin-1 concentration. The lower limit of detection for bryostatin-1 using this assay is  $5 \times 10^{-9}$  M.

## RESULTS

### Patients

A total of 28 patients were entered onto the study. Six patients could not be evaluated for either toxicity or response; five patients had progressive disease before receiving the scheduled three weekly doses of bryostatin-1 during course 1 of therapy (three patients clinically and radiologically progressed after the first dose, and two patients progressed after the second dose). One additional patient was removed from study for administrative reasons prior to receiving a complete course of therapy. Our report is generated from 22 fully evaluable patients ( $20 \mu\text{g}/\text{m}^2$ ,  $n = 3$ ;  $26 \mu\text{g}/\text{m}^2$ ,  $n = 3$ ;  $34 \mu\text{g}/\text{m}^2$ ,  $n = 4$ ;  $44 \mu\text{g}/\text{m}^2$ ,  $n = 6$ ;  $57 \mu\text{g}/\text{m}^2$ ,  $n = 6$ ) whose characteristics are shown in Table 2.

### Toxicity

**Nonhematological Toxicity: Dose Limit.** Myalgia, headache, and photophobia or eye pain were the DLTs in this trial (Tables 3 and 4). Three of six patients treated at dose level 5 ( $57 \mu\text{g}/\text{m}^2/\text{dose}$ ) had dose-limiting (grade 3) myalgia. Symp-

Table 3 Myalgia, headache, and eye pain at all dose levels

Dose level	No. eligible	No. of patients with toxicity			
		Grade 1	Grade 2	Grade 3	Grade 4
$20 \mu\text{g}/\text{m}^2$	3				
$26 \mu\text{g}/\text{m}^2$	3		2		
$34 \mu\text{g}/\text{m}^2$	4	1	2		
$44 \mu\text{g}/\text{m}^2$	6	1	1		
$57 \mu\text{g}/\text{m}^2$	6	1	1	3	

toms occurred in all patients within 24–72 h after the second or third dose ( $>$  day 7) of bryostatin-1 with resolution within 1 week of onset. One patient required hospitalization for grade 3 myalgia that was managed with acetaminophen with codeine. An additional patient with grade 3 myalgia was also being treated as needed with fentanyl for tumor-related bone pain. Generalized body aches were described in two patients, and leg pain occurred in a third. Grade 3 photophobia or eye pain was also noted in two patients, who also had dose-limiting myalgia, at the  $57 \mu\text{g}/\text{m}^2/\text{dose}$  level. Both patients underwent ophthalmological examination, the results of which were normal. One additional patient at the  $57 \mu\text{g}/\text{m}^2$  dose level with myalgia also had grade 3 headache. Grade 1 or 2 myalgia, headache, or eye pain was observed at all lower dose levels except  $20 \mu\text{g}/\text{m}^2$ .

**Other Nonhematological Toxicity.** Grade 1 (three patients at  $20 \mu\text{g}/\text{m}^2$  and two patients at  $57 \mu\text{g}/\text{m}^2$ ) and grade 2 (one patient at  $44 \mu\text{g}/\text{m}^2$ ) elevations in transaminases were observed in six patients. Fever and flu-like symptoms were mild and described in several patients at all dose levels. The bryostatin-1 infusion was well tolerated in all patients with three possible exceptions. One patient described an odd taste in the mouth and exhibited slight flushing during infusion of bryostatin-1, and a second complained of difficulty with breathing that improved with relaxation. A third patient experienced grade 1 hypotension at the  $34 \mu\text{g}/\text{m}^2$  dose level that was not associated with any symptoms, for which no intervention or interruption in therapy was required.

**Hematological Toxicity.** Grade 1 thrombocytopenia was noted in a total of six patients and occurred at all dose levels of bryostatin-1. A seventh patient, at the  $34 \mu\text{g}/\text{m}^2$  dose level, was treated with a platelet count of 90,000 and developed grade 2 thrombocytopenia. Thrombocytopenia always occurred  $<7$  days after initiation of the bryostatin-1 infusion and frequently persisted throughout the study period. No patient required a platelet transfusion. Grade 1 neutropenia at day 8 after infusion was noted in one patient at  $26 \mu\text{g}/\text{m}^2$ .

### Pharmacokinetics

Patients at each dose level (five patients at  $20 \mu\text{g}/\text{m}^2$ , three patients at  $26 \mu\text{g}/\text{m}^2$ , 1 patient at  $34 \mu\text{g}/\text{m}^2$ , 3 patients at  $44 \mu\text{g}/\text{m}^2$ , and 2 patients at  $57 \mu\text{g}/\text{m}^2$ ) had plasma samples analyzed for bryostatin-1 concentrations using the competitive binding bioassay. End-of-infusion concentrations of bryostatin-1 were at or below the lower level of detection ( $5 \times 10^{-9}$  M) for most patients, with the exception of one patient each at dose levels 1 ( $20 \mu\text{g}/\text{m}^2$ ), 2 ( $26 \mu\text{g}/\text{m}^2$ ), and 5 ( $57 \mu\text{g}/\text{m}^2$ ). These patients achieved peak plasma concentrations of  $1-2 \times 10^{-8}$  M during or shortly after the end of the bryostatin-1 infusion. In

Table 4 Myalgia, headache, and eye pain at 57  $\mu\text{g}/\text{m}^2$ 

Patient	1	2	3	4	5
Age (yr)	8	19	13	19	15
Symptom	Myalgia/eye pain	Myalgia/eye pain	Myalgia/headache	Myalgia	Myalgia
Grade	3	3	3	2	1
No. of doses	2	3	2	3	2
Day of cycle	8	15	10	15	8
Duration (days)	3	5	7	2	7
Treatment	Acetaminophen/codeine	Fentanyl	Acetaminophen/codeine	Acetaminophen	Ibuprofen

these three patients, plasma concentrations of bryostatin-1 were undetectable within 4 h after peak concentrations were achieved.

### Response

There were no partial or complete responses to bryostatin-1 on this schedule. One patient with renal cell carcinoma had stable disease through four courses and one with neuroblastoma similarly received two courses at 20  $\mu\text{g}/\text{m}^2$ . Both patients then elected to seek other therapeutic options. Two patients (1 germ cell tumor, 34  $\mu\text{g}/\text{m}^2$  for five courses; 1 renal cell carcinoma, 34  $\mu\text{g}/\text{m}^2$  for three courses) with microscopic disease at initiation of bryostatin-1 were free of progression while on study.

### DISCUSSION

Two Phase I studies of short infusions of bryostatin-1 in adults with refractory tumors have been completed. In one, a 1-h infusion was given every 2 weeks. In the second, the infusion was administered weekly for 3 of every 4 weeks (13, 14). The main DLTs have been cellulitis and phlebitis at the site of infusion and myalgia. When bryostatin-1 was administered weekly for 3 of every 4 weeks, the recommended Phase II dose was 25  $\mu\text{g}/\text{m}^2/\text{dose}$ . Partial responses were observed in two patients with melanoma. In addition, the 25  $\mu\text{g}/\text{m}^2/\text{dose}$  has recently been shown in a Phase Ib study to induce *in vivo* down-regulation of PKC in peripheral blood mononuclear cells (15). Using a prolonged (24- or 72-h) infusion, partial responses to bryostatin-1 have been reported in patients with lymphoma, leukemia, and ovarian and cervical carcinoma (16, 17). The recommended Phase II dose was 25 and 40  $\mu\text{g}/\text{m}^2/\text{day}$  for the 24- and 72-hour continuous infusion schedules, respectively.

The Pediatric Oncology Group evaluated the clinical toxicities and pharmacokinetics of bryostatin-1 given as a 1-h infusion weekly for 3 of 4 weeks to children with refractory solid tumors. Myalgia, photophobia or eye pain, and headache were dose limiting at the 57  $\mu\text{g}/\text{m}^2/\text{dose}$ . The DLTs observed in this initial pediatric Phase I study of bryostatin-1 were similar to those reported from adult trials of this agent, although children tolerated considerably more drug. In addition, because the DLTs were observed in older children (8–19 years of age), one could speculate that younger patients would tolerate even higher doses. Our study also suggests that myalgias are dose dependent and possibly cumulative, as was reported recently in an adult Phase I trial of bryostatin-1 (17). The duration of myalgias in children were short, usually  $\leq 1$  week, with some improvement with oral narcotics or acetaminophen. No further dose escalation was attempted with the use of scheduled narcotics.

Although myalgias, including eye pain, have been dose-

limiting in earlier adult Phase I studies of bryostatin-1, this is the first trial with this agent in which photophobia was observed. The relation of photophobia to the retro-orbital pain associated with eye movements, as observed in adult Phase I studies of bryostatin-1, is unclear. However, in at least one patient, pain was clearly described as associated with bright light. Moreover, the pain decreased significantly with the use of dark sunglasses. Because no structural abnormality was noted on ophthalmological examination, the most common causes of photophobia in children, including alterations of the cornea (*e.g.*, buphthalmos), uvea, lens, optic nerve, or retina, seem unlikely (18). It is of interest to note that myalgias associated with bryostatin-1 typically involve the larger muscles of the lower extremities. Why the small ocular fibers would be targeted is unknown (17).

It is of interest to note that the toxicities of bryostatin-1 occurred days after the infusion and lasted for prolonged periods. It is possible that this reflects the activation of PKC or increases in cytokines such as tumor necrosis factor  $\alpha$  or interleukin 6 (2–4, 13, 14). Alternatively, it is possible that these side effects are secondary to a bryostatin-1 metabolite(s).

Prior studies in animals have suggested that bryostatin-1 is removed rapidly from plasma with a half-life of elimination from plasma of  $< 10$  min and a clearance of 3.53 ml/min (12, 19). Data presented in this present study suggest that the pharmacokinetic behavior of bryostatin-1 in humans may be similar to that reported in animals. Bryostatin-1 appears to be rapidly eliminated from plasma in children; however, the limited sensitivity of present methods of detection of this agent in plasma prevents further description of its pharmacology. Assays of PKC levels in leukocytes from adults treated with bryostatin-1 also demonstrate changes immediately at the end of the infusion<sup>3</sup> (data not shown) and not at 4 h after the infusion is completed, suggesting that concentrations of this agent, and hence biological effects, are dropping rapidly.

Peak concentrations of bryostatin-1 ( $1\text{--}2 \times 10^{-8}$  M) achieved in several patients in this study were in the range associated with down-regulation of PKC in neuroblastoma and leukemia tumor cells (6, 7). Antitumor activity using several murine and human tumor cell lines has also been observed at these concentrations of bryostatin-1 (2, 6). However, preclinical studies suggest that antitumor activity may require maintenance of these concentrations for a more prolonged (hours to days) period of time than what was achievable in this clinical trial. For

<sup>3</sup> A. S. Kraft, personal observation.

example, maximal antiproliferative activity with *in vitro* exposure of lymphoma tumor cells to bryostatin-1 at  $1 \times 10^{-9}$  M occurred after several days of exposure (2). The necessity for prolonged exposure to bryostatin-1 supports its use as a cytostatic agent and the importance of possible subsequent pediatric Phase I studies using continuous infusion.

Future clinical studies of bryostatin-1 in children could also include combination Phase I/II studies with agents such as cisplatin, 2-chlorodeoxyadenosine, vincristine, or cytarabine, which have been shown to interact beneficially with bryostatin-1 *in vitro* (20–25). The precise mechanism by which bryostatin-1 enhances the antitumor activity of standard cytotoxics is unclear; however, several potential hypotheses have been postulated. Bryostatin-1 has been shown to cause down-regulation of *mdr1*, which can lead to increased cellular accumulation of agents such as vincristine and daunorubicin (24). Bryostatin-1 may also potentiate the activity of other oncolytics by altering the expression of genes such as *bcl2*, *p53*, and *c-Myc* (21, 26). In regard to 2-chlorodeoxyadenosine, bryostatin-1 has been shown to increase the ratio of deoxycytidine kinase to 5'-nucleotidase, enzymes responsible for the activation and detoxification, respectively, of this antimetabolite (25, 27). It is likely that the positive drug-drug interaction between bryostatin-1 and other anticancer agents is complex and multifactorial.

In summary, myalgia, photophobia or eye pain, and headache were the DLTs of bryostatin-1. The recommended dose for Phase II clinical trials in children is  $44 \mu\text{g}/\text{m}^2/\text{dose}$  administered as a 1-h infusion every week for 3 of every 4 weeks.

## REFERENCES

- Pettit, G., Herald, C., Doubek, D., and Herald, D. Isolation and structure of bryostatin 1. *J. Am. Chem. Soc.*, *104*: 6846–6848, 1982.
- Hornung, R., Pearson, J., Beckwith, M., and Longo, D. Preclinical evaluation of bryostatin as an anticancer agent against several murine tumor cell lines: *in vitro* versus *in vivo* activity. *Cancer Res.*, *52*: 101–107, 1992.
- Schuchter, L., Esa, A., May, W., Laulis, M., Pettit, G., and Hess, A. Successful treatment of murine melanoma with bryostatin 1. *Cancer Res.*, *51*: 682–687, 1991.
- Pettit, G., Day, J., Hartwell, J., and Wood, H. Antineoplastic components of marine animals. *Nature (Lond.)*, *227*: 962–963, 1970.
- Mohammad, R., Al-Katib, A., Pettit, G., and Sensenbrenner, L. Successful treatment of human Waldenstrom's macroglobulinemia with combination biological and chemotherapy agents. *Cancer Res.*, *54*: 165–168, 1994.
- Jones, R., Sharkis, S., Miller, C., Rowinsky, E., Burke, P., and May, S. Bryostatin-1, a unique biologic response modifier: anti-leukemic activity *in vitro*. *Blood*, *75*: 1319–1323, 1990.
- Jalava, A., Heikkila, J., Akerlind, G., Pettit, G., and Akerman, K. Effects of bryostatin 1 and 2 on morphological and functional differentiation of SH-SY5Y human neuroblastoma cells. *Cancer Res.*, *50*: 3422–3428, 1990.
- Gescher, A. Towards selective pharmacological modulation of protein kinase C—opportunities for the development of novel antineoplastic agents. *Br. J. Cancer*, *66*: 10–19, 1992.
- Basu, A. The potential of protein kinase C as a target or anticancer treatment. *Pharmacol. Ther.*, *59*: 257–280, 1993.
- Maki, A., Diwakaran, H., Redman, B., Al-Asfar, S., Pettit, G., Mohammad, R., and Al-Katib, A. The *bcl-2* and *p53* oncoproteins can be modulated by bryostatin 1 and dolastatins in human diffuse large cell lymphoma. *Anti-Cancer Drugs*, *6*: 392–397, 1995.
- National Cancer Institute. Guidelines for reporting of adverse drug reactions. Bethesda, MD: Division of Cancer Treatment, National Cancer Institute, 1988.
- Kraft, A., Woodley, S., Pettit, G., Gao, F., Coll, J., and Wagner, F. Successful treatment of murine melanoma with bryostatins 5 and 8. *Cancer Chemother. Pharmacol.*, *37*: 271–278, 1998.
- Philip, P., Rea, D., Thavasu, P., Carmichael, J., Stuart, N., Rockett, H., Talbot, D., Ganesan, T., Pettit, G., Balkwill, F., and Harris, A. Phase I study of bryostatin 1: assessment of interleukin 6 and tumor necrosis factor  $\alpha$  induction *in vivo*. *J. Natl. Cancer Inst.*, *85*: 1812–1818, 1993.
- Prendiville, J., Crowther, D., Thatcher, N., Woll, P., Fox, B., McGown, A., Testa, N., Stern, P., McDermott, R., Potter, M., and Pettit, G. A Phase I study of intravenous bryostatin 1 inpatients with advanced cancer. *Br. J. Cancer*, *68*: 418–424, 1993.
- Grant, S., Roberts, J., Poplin, E., Tombes, M., Kyle, B., Welch, D., Carr, M., and Bear, H. Phase Ib trial of bryostatin 1 in patients with refractory malignancies. *Clin. Cancer Res.*, *4*: 611–618, 1998.
- Jayson, G., Crowther, D., Prendiville, J., McGown, A., Scheid, C., Stern, P., Young, R., Brenchley, P., Chang, J., and Owens, S. A Phase I trial of bryostatin 1 inpatients with advanced malignancy using a 24 hour intravenous infusion. *Br. J. Cancer*, *72*: 461–468, 1995.
- Varterasian, M., Mohammad, R., Eilender, D., Hulburd, K., Rodriguez, D., Pemberton, P., Pluda, J., Dan, M., Pettit, G., Chen, B., and Al-Katib, A. Phase I study of bryostatin 1 inpatients with relapsed non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *J. Clin. Oncol.*, *16*: 56–62, 1998.
- Day, S. In: D. Taylor (ed.), *Paediatric Ophthalmology*, Chapter P9: Photophobia. pp. 1034–1036. London: Blackwell Science, 1997.
- Berkow, R., Schlabach, L., Dodson, R., Benjamin, W., Pettit, G., Rustagi, P., and Kraft, A. *In vivo* administration of the anticancer agent bryostatin 1 activates platelets and neutrophils and modulates protein kinase C activity. *Cancer Res.*, *53*: 2810–2815, 1993.
- Basu, A., and Lazo, J. Sensitization of HeLa cells to *cis*-diamminedichloroplatinum by bryostatin 1. *Proc. Am. Assoc. Cancer Res.*, *33*: A3036, 1992.
- Mohammad, R., Diwakaran, H., Maki, A., Emara, M., Pettit, G., Redman, B., and Al-Katib, A. Bryostatin 1 induces apoptosis and augments inhibitory effects of vincristine in human diffuse large cell lymphoma. *Leuk. Res.*, *19*: 667–673, 1995.
- Grant, S., Jarvis, W., Swerdlow, P., Turner, A., Traylor, R., Wallace, H., Lin, P., Pettit, G., and Gewirtz, D. Potentiation of the activity of 1- $\beta$ -D-arabinofuranosylcytosine by the protein kinase C activator bryostatin 1 in HL-60 cells: association with enhanced fragmentation of mature DNA. *Cancer Res.*, *52*: 6270–6278, 1992.
- Katato, K., Mohammad, R., and Dugan, M. Sequential treatment of chronic lymphocytic leukemia (CLL) with bryostatin 1 and 2-chlorodeoxyadenosine: a preclinical study. *Exp. Hematol.*, *24*: A693, 1996.
- Al-Katib, A., Smith, M., Kamanda, W., Pettit, G., Hamdan, M., Mohamed, A., Chelladurai, B., and Mohammad, R. Bryostatin 1 down-regulates *mdr1* and potentiates vincristine cytotoxicity in diffuse large cell lymphoma xenografts. *Clin. Cancer Res.*, *4*: 1305–1314, 1998.
- Mohammad, R., Katato, K., Almatchy, V., Wall, N., Liu, K., Schultz, C., Mantsch, H., Varterasian, M., and Al-Katib, A. Sequential treatment of human chronic lymphocytic leukemia with bryostatin 1 followed by 2-chlorodeoxyadenosine: preclinical studies. *Clin. Cancer Res.*, *4*: 445–453, 1998.
- Chelliah, J., Freerman, A., Wu Pong, S., Jarvis, W., and Grant, S. Potentiation of ara-C-induced apoptosis by the protein kinase C activator bryostatin 1 in human leukemia cells (HL-60) involves a process dependent upon c-Myc. *Biochem. Pharmacol.*, *54*: 563–573, 1997.
- Kawasaki, H., Carrera, C., Piro, L., Saven, A., Kipps, T., and Carson, D. Relationship of deoxycytidine kinase and cytoplasmic 5'-nucleotidase to the chemotherapeutic efficacy of 2-chlorodeoxyadenosine. *Blood*, *81*: 597–601, 1993.

# Clinical Cancer Research

## A Phase I Trial of Bryostatins-1 in Children with Refractory Solid Tumors: A Pediatric Oncology Group Study

Steven Weitman, Anne-Marie Langevin, Roger L. Berkow, et al.

*Clin Cancer Res* 1999;5:2344-2348.

**Updated version** Access the most recent version of this article at:  
<http://clincancerres.aacrjournals.org/content/5/9/2344>

**Cited articles** This article cites 24 articles, 12 of which you can access for free at:  
<http://clincancerres.aacrjournals.org/content/5/9/2344.full#ref-list-1>

**Citing articles** This article has been cited by 4 HighWire-hosted articles. Access the articles at:  
<http://clincancerres.aacrjournals.org/content/5/9/2344.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](mailto: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](mailto:permissions@aacr.org).