Weekly Bryostatin-1 in Metastatic Renal Cell Carcinoma: A Phase II Study[1]

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ABSTRACT

Purpose: **We conducted a Phase II trial of bryostatin-1, an inhibitor of protein kinase C, in advanced renal cell carcinoma to measure toxicity, response rate, time to progression, and induction of cytokines.**

Experimental Design: **A total of 32 patients (26 male and 6 female) received bryostatin-1 at 35–40 μg/m^2 i.v. over 1 h on days 1, 8, and 15 of each 4-week cycle. Plasma interleukin-6, tumor necrosis factor-α, and C-reactive protein levels were assayed pretreatment, 1 and 23 h after completion of bryostatin-1 infusion at weeks 1 and 5.**

Results: **Cycles (102) of bryostatin-1 were given (median 2, range 1–8). The most common grade 1 or 2 toxicities were myalgias (46.8%), fatigue (59.3%), and dyspnea (18.8%). Grade 3–4 toxicity included myalgias (40.6%), ataxia (9.3%), and dyspnea (15.6%). Four (12%) patients experienced cardiac events while on study (cardiac arrhythmias and congestive heart failure occurred in 2 patients, and 2 patients had fatal cardiac arrests). Of 32 patients evaluable for response, 2 (6.3%) had partial responses lasting 9 with 6 months. A total of 15 patients (46.8%) had stable disease, and 6 (18.8%) patients had stable disease for ≥6 months. Plasma interleukin-6 increased ≥2-fold over baseline measurements in 5 of 17 patients (29.4%) but did not correlate with response or toxicity.**

Conclusions: **Although weekly bryostatin-1 at 35–40 μg/m^2 produced a low proportion of objective responses, prolonged (>6 months) stable disease or partial remission in 25% of patients suggests that this agent, or other inhibitors of protein kinase C, may have a role in the treatment of renal cell carcinoma, perhaps in combination with other agents.**

INTRODUCTION

An estimated 37,000 new kidney cancers were diagnosed in 2001, and ~12,000 deaths will occur from this disease (1). One-third of the patients will present with advanced or metastatic disease (2) for which therapeutic options are limited. Chemotherapy and hormonal therapy for metastatic renal cell cancer have been largely ineffective (3–6). Biological therapies for renal cell cancer, such as IL-2 and IFN-α, have produced objective response proportions of only 10–15% (5, 7–9). Although responses have been durable in rare cases (8), most patients develop progressive disease within 4–6 months. Because of limited activity and significant toxicity of these therapies, there is an urgent need to identify new, active therapies for this disease.

Bryostatin-1, a macrocytic lactone isolated from the marine bryozoan *bugula neritina* (10), has shown antitumor activity against both murine and human tumor cell lines *in vitro* (11, 12). Bryostatin-1 is a partial agonist of PKC,[3] a mechanism that is thought to be responsible for both immunomodulatory and antitumor activity (13). In the absence of ligands, bryostatin-1 activates plasma membrane PKC, which phosphorylates other downstream proteins important in intracellular signaling. In the presence of ligands, bryostatin-1 inhibits cell growth and differentiation by interacting with other activators of PKC (such as phorbol esters; Ref. 13). *In vitro* studies have suggested that bryostatin-1 may compete with phorbol esters whose interaction with PKC may be tumorigenic (14). Ultimately, prolonged exposure to bryostatin-1 down-regulates PKC kinase activity (13). One potentially important mechanism of PKC activation involves the epidermal growth factor receptor. The epidermal growth factor receptor is strongly expressed in the majority of RCCs and might drive tumor growth or survival through PKC-dependent signaling (15, 16).

To test the hypothesis that an inhibitor of PKC-dependent signaling has therapeutic activity against RCC, we conducted a Phase II trial of bryostatin-1 in patients with this tumor. The selected schedule of bryostatin-1 was a 1-h infusion weekly for 3 of 4 weeks at a dose determined from a previous Phase I trial (17). Because a reliable assay for serum bryostatin-1 is unavailable, we measured plasma levels of IL-6 and TNF-α before and after drug administration. A dose-response relationship between weekly bryostatin-1 and increased plasma levels of IL-6 and TNF-α had been demonstrated previously (18).

Received 4/25/02; revised 7/29/02; accepted 8/12/02.
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[1] Data from this Phase II Trial were presented at the Annual Meeting of the American Society of Clinical Oncology, New Orleans, LA, 2001.

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[3] The abbreviations used are: PKC, protein kinase C; IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein; ECOG, Eastern Cooperative Oncology Group; PR, partial remission; RCC, renal cell carcinoma; DLT, dose-limiting toxicity; CR, complete remission.

The primary objectives of this trial were to assess the objective response rate, time to progression, and toxicities of bryostatin-1 in advanced RCC. A secondary objective was to evaluate the effects of bryostatin-1 administration on the levels of plasma IL-6, TNF-α, and CRP.

PATIENTS AND METHODS

Patient Eligibility. Patients were ≥18 years of age and had histologically confirmed metastatic RCC with bi-dimensionally measurable soft tissue or bone disease not <1 cm^2. Entry criteria included an ECOG performance status of 0 or 1, life expectancy >3 months, and adequate organ function, including WBC ≥3000 cells/mm^3, platelet count ≥100,000/mm^3, bilirubin ≤1.5 mg/dl, and creatinine ≤2 mg/dl (or creatinine clearance of >50 ml/min). One previous therapy with a biological response modifier regimen was allowed, but previous chemotherapy for the treatment of RCC was not permitted. Radiotherapy or major surgery, including nephrectomy, must have been completed ≥4 weeks before protocol entry. Patients with irradiated or resected brain metastases stable for a minimum of 3 months and not on corticosteroids were also eligible. All patients provided written informed consent in accordance with federal, state, and institutional guidelines.

Drug Administration. Bryostatin-1 (NSC 339555) was provided by the Division Cancer Treatment and Diagnosis of the National Cancer Institute. Bryostatin-1 was supplied in a formulation of two parts: a 6-cc vial containing 0.1 mg of bryostatin and 5 mg of povidone USP (as a bulking agent) lyophilized from 40% *t*-butanol was reconstituted with 1 ml of sterile positron emission tomography diluent (60% polyethylene glycol 400/30% ethanol/10% Tween 80). The resulting solution was further diluted with 9 ml of 0.9% sodium chloride injection, producing a 10 μg/ml solution of bryostatin-1. The drug was further diluted in glass or polyolefin containers with 0.9% sodium chloride or 5% dextrose in water to a final concentration in the range of 0.15–0.75 μg/ml. Bryostatin-1 was administered as a 1-h infusion on days 1, 8, and 15 of each 4-week cycle.

Evaluation of Patients. Patients were evaluated at baseline with complete blood count and chemistry profile, including electrolytes, alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, calcium, phosphorus, lactate dehydrogenase, magnesium, prothrombin time, partial thromboplastin time, urinalysis, and serum pregnancy test (if applicable) within 2 weeks of registration. Physical examination, height, weight, ECOG performance status, EKG, chest X-ray, and tumor measurements were required at baseline. A complete blood count was drawn weekly, and history, physical, performance status, weight, and serum chemistries were assessed every 4 weeks. Tumor measurements were taken every 8 weeks.

Toxicity was graded by National Cancer Institute Common Toxicity Criteria, version 2.0.[4] Grade 3 or 4 hematologic (other than anemia) toxicity required a dose reduction to 75% of the starting dose for the next course. The dose was omitted if the absolute neutrophil count on day 8 or 15 was $<1{,}000$ cells/mm^3 or the platelet count was $<50{,}000$/mm^3, with subsequent dose reduction to 75% for retreatment (day 29) and all subsequent doses. Grade 3 or 4 nonhematologic toxicity led to a dose reduction of 75% of the starting dose for the next course and omission of treatment on that day if a patient had grade 3 or 4 nonhematologic toxicity on day 8 or 15. If the dose on a scheduled dosing day was omitted, the patient could be retreated on the next scheduled dosing day in the course, provided toxicity had resolved to a grade 2 or less. Myalgia was assessed before each dose of bryostatin-1 using the following scale: 0 = none, 1 = mild pain not interfering with daily activities, 2 = moderate pain (pain or analgesics produce some interference with daily activities), 3 = severe pain (pain or analgesics severely interfere with daily activities), or 4 = disabling. Grade 3 or 4 myalgia during a course prompted a dose reduction of 75% of the starting dose for the next course. Response assessment was defined by WHO criteria (19).

Correlative Laboratory Studies. One red top tube of blood was obtained immediately before week 1 treatment and at 1 and 23 h after completion of the first infusion, during cycles 1 and 2 from patients participating in the study at Fox Chase Cancer Center. Specimens were drawn, plasma was separated by centrifugation within 30 min, and plasma was frozen at −20°C and sent for measurement of IL-6 and TNF-α. Plasma IL-6 was determined by ELISA, according to the manufacturer's instructions (hIL-6; ELISA kit procedure; Biosource International, Inc., Camarillo, CA). Briefly, 100 μl of plasma were incubated for 2 h with 50 μl of biotin conjugate (anti-hIL-6) in hIL-6 antibody-coated wells, 96-well plates, at room temperature. After aspirate and washing four times with the second antibody, streptavidin-horseradish peroxidase was added and incubated for 30 min at room temperature and then processed in a similar fashion with stabilized chromogen, and after the addition of stop solution, it was photometrically assayed (450 nm; Cytoscreen ultrasensitive human IL-6, sensitivity <0.1 pg/ml). Plasma TNF-α was assayed in a similar fashion with biotinylated anti-TNF-α (BioSource International, Inc.). Plasma was assayed by ELISA in a similar fashion duplicate for TNF-α (Cytoscreen ultrasensitive human TNF-α sensitivity <0.1 pg/ml). CRP was assayed by nephelometry using Beckman Array commercially available assay (Beckman Coulter, Inc., Palo Alto, CA). Plasma levels of these proteins were drawn immediately before week 1 bryostatin-1 and repeated at 2 and 24 h poststart of infusion of bryostatin-1 in cycles 1 and 2 only.

Statistical Considerations. The primary endpoints for this Phase II study were the proportion of patients with partial and complete responses using the WHO response criteria (19) and time to progression. The new treatment would be of interest if the proportion of patients with objective PR or CR was ≥20%. With 32 evaluable patients, the objective response rate of interest could be distinguished from a response proportion of <5% with 80% power. If ≥5 patients had PR or CR, then the null hypothesis would be rejected at the 2% level of significance (one-sided test). Conversely, if ≤4 patients had a PR or CR, then the null hypothesis would be accepted. If no patients with PR or CR were observed, or conversely, if ≥3 patients with tumor responses were observed when 24 patients were evalu-

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Table 1 Demographics

No. registered/evaluable	34/32
Age, median (range)	64 (42–81)
Gender	
Male	28
Female	6
Race	
Caucasian	33
African-American	1
ECOG performance score	
0	16
1	18
Previous biologic response modifiers	1
Previous nephrectomy	24
LDH[a] $>1.5 \times$ normal	3
Time from diagnosis <1 year	17
Hemoglobin less than lower limit of normal	21
Corrected serum calcium greater than normal	2
Tumor sites	
Lung	23
Mediastinum	3
Lymph nodes	14
Bone	8
Liver	6
Kidney	10
No. adverse prognostic factors[21,29]	
0 (Good)	6
1–2 (Intermediate)	19
≥3 (Poor)	7

[a] LDH, lactate dehydrogenase.

Table 2 Toxicity for all cycles (worst grade)[a]

	1	2	3	4	(% Grades 3–4)
Myalgias	6	9	13 (6)	0	40.6
Ataxia[b]	0	0	3	0	9.3
Headache	5	1	0	0	
Fatigue	17	2	2	0	6.3
Urinary frequency	1	2	2	0	6.3
Dyspnea	3	3	3	2	15.6
Anemia	3	6	2	0	6.3
Leg weakness	0	0	1	0	3.1
Allergic reaction	0	0	1	0	3.1
GI (N/V)[c]	6	4	0	0	
Anorexia	7	3	1	0	3.1
Edema/weight gain	0	0	1	0	3.1

[a] Six of the 13 patients had grade 3 myalgias after three or greater cycles.
[b] Reported ataxia was noted in two, three, and seven cycles.
[c] GI (N/V), Gastrointestinal (Nausea/Vomiting).

able, the trial would be terminated early on the grounds that the null or alternative hypothesis was accepted.

For time to progression, 32 patients were needed to test the null hypothesis that the proportion of patients progression free at 6 months is $<10\%$ against the alternative hypothesis that the proportion of patients free of progression is $\geq 30\%$ with 88.7% power. Using this design, if ≥ 7 patients with favorable response were observed, then the null hypothesis would be rejected at the 3.6% level of significance (one-sided test).

Repeated measure ANOVA was implemented to examine the change of the plasma levels of TNF-α, IL-6, and CRP from baseline to 24 h after bryostatin-1 administration and the effect of first cycle dose levels on these measures in the 15 patients treated at Fox Chase Cancer Center. One-sided *t* tests from the ANOVA were used to test the hypothesis that plasma cytokine levels increased after bryostatin-1 administration. Repeated measure ANOVA was also used to study the relationships among TNF-α, IL-6, and CRP. Logistic regression was conducted to examine the relationship of IL-6, TNF-α, or CRP with response to therapy.

Although the major end point in Phase II trials is efficacy, the search for the optimal dose is usually restricted to the Phase I setting. The assumption that the dose selected in a Phase I patient population will be optimal for Phase II or III populations may be incorrect, and maximizing dose intensity is still regarded as important to achieve an optimal therapeutic effect. Therefore, the study design permitted dose escalation depending on the frequency of DLT observed in an initial patient sample. Thus, the adequacy of the initial dose was assessed after 15 patients had been treated, and the dose was modified such that the probability of DLT was 0.2. This dose modification design was based on methodology described by Babb *et al.* (20).

RESULTS

A total of 34 patients with metastatic RCC was enrolled at Fox Chase Cancer Center ($n = 15$) and six Fox Chase Network institutions ($n = 19$) from June 30, 1999 to March 5, 2001. Two patients never received protocol treatment because of rapid declines in performance status and were not included in toxicity or response analysis. A total of 102 cycles (range 1–8) of bryostatin-1 was administered to the 32 evaluable patients. Patient characteristics are summarized in Table 1. Nineteen (59.4%) and 7 (21.8%) of our patients had an intermediate or poor risk of death, respectively, with predicted median survivals of 10 and 4 months based on pretreatment characteristics depicted in Table 1 (21). The first 15 patients received bryostatin-1 at 35 $\mu g/m^2$ over 1 h i.v. weekly for 3 of every 4 weeks. On the basis of the occurrence of DLT during the first 4-week cycle in 2 of these patients, the estimated probability of DLT was <0.2 after first-stage accrual. Accordingly, the dose of bryostatin-1 was increased to 40 $\mu g/m^2$ for the subsequent 17 patients accrued.

Toxicity. Thirty-two patients were evaluable for toxicity. Toxicities are shown in Table 2. The predominant toxicity was myalgia, present in 87% ($n = 28$) of patients at some point during treatment. Seven patients (22%) had grade 3 myalgia in the first two cycles of treatment. Most of these ($n = 5$) occurred in the 40 $\mu g/m^2$ dose group. However, 3 additional patients receiving bryostatin-1 at the 35 $\mu g/m^2$ dose had grade 3 myalgias at four, six, or seven cycles, and 3 additional patients receiving bryostatin-1 at the 40 $\mu g/m^2$ dose had grade 3 myalgias at three or four cycles. Four patients already receiving reduced doses (26–30 $\mu g/m^2$) for myalgias required an additional dose reduction for recurrent myalgias.

Three cases of transient grade 3 ataxia were seen at the 35 $\mu g/m^2$ dose, not reported previously as a toxicity of bryostatin-1. Brain magnetic resonance imaging studies performed on these patients were normal. One patient had a pulmonary embolus from inferior vena cava tumor thrombus after one treat-

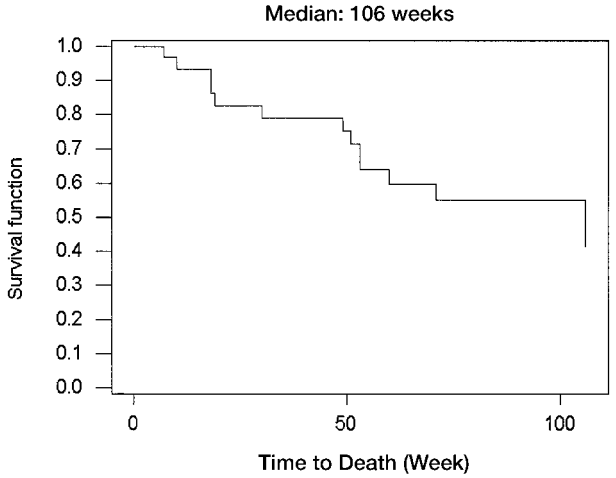

Fig. 1 Kaplan-Meier curve for survival, according to weeks from time of registration to trial.

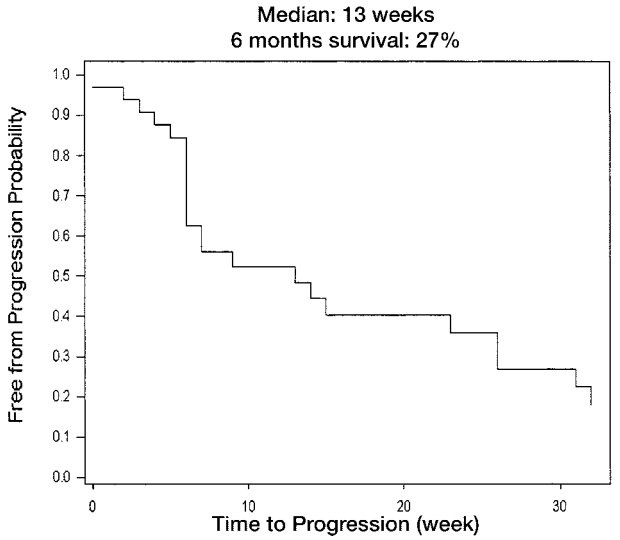

Fig. 2 Kaplan-Meier curve for time to progression in weeks from time of registration to date of radiological documentation of disease progression.

ment. Three patients died while on study, 1 from a perforated gastric ulcer and 2 from cardiac arrests that occurred several days after drug administration. One of these 2 patients had observed ventricular tachycardia. Neither had cardiac disease diagnosed previously. Two additional patients experienced nonfatal cardiac arrhythmias and congestive heart failure while on study. We cannot be certain that these toxicities were unrelated to bryostatin-1 treatment.

Response to Treatment. Thirty-two patients were evaluable for response. Two patients had partial responses that lasted 9 and 6 months. A total of 15 patients (46.8%) had stable disease, and 15 patients (46.8%) had progressive disease after two cycles of treatment. A 65-year-old female with multiple pulmonary lesions achieved a partial response after four cycles. A 45-year-old male with pulmonary nodules and mediastinal and retroperitoneal lymph nodes achieved a partial response after two cycles. A 3rd patient, a 71-year-old male with multiple pulmonary nodules and retroperitoneal lymph nodes, had a 50% decrease in the size of the lung nodules but concurrently developed a new pelvic mass and therefore had progressive disease as his best response

Time to progression was ≥6 months (26 weeks) in 8 of 32 (25%) patients (95% confidence interval was 11.8–44.6). This result rejects the null hypothesis for time to progression. Overall and progression-free survival distribution are shown in Figs. 1 and 2. Nineteen of the patients (59.4%) were deceased at the time of this analysis. The median survival was 106 weeks, and the median time to progression was 13 weeks.

Serum IL-6, TNF-α, and CRP. Baseline, 2-, and 24-h poststart of bryostatin-1 infusion mean ± SD plasma levels of IL-6, TNF-α, and CRP levels are shown in Table 3. Adjusted for cycle and dose level, IL-6 level increased 24 h after first dose initiation compared with baseline ($P = 0.02$). For TNF-α level or CRP, the data did not show any difference between baseline and 24 h afterwards ($P = 0.86$ and 0.67, respectively). However, TNF-α level decreased 2 h postinitiation of treatment ($P = 0.02$) and increased back 24 h postinitiation of infusion ($P = 0.01$). Adjusted for cycle, time, and dose levels of CRP were significantly related to plasma IL-6 ($P = 0.001$), but there were no other pair-wise correlations of these cytokines. Changes in plasma IL-6, TNF-α, and CRP levels did not correlate with response to therapy or toxicity.

DISCUSSION

Bryostatin-1 has undergone Phase I evaluation on several schedules, including 1-h infusion every other week (22), 1-h infusion q weekly × 3 (18), 24-h infusion weekly × 3, (23), and 72-h continuous infusion q 2 weeks (24). The doses recommended for Phase II trials were 25–35 μg/m^2/week for the weekly 1-h infusion schedules, 25 μg/m^2/week for 24 h weekly, and 40 μg/m^2/day for the 72-h infusion q 2 weeks (17, 18, 23, 24). The DLT for all schedules was myalgia. Myalgias were reported to be cumulative in a previous publication (18), and we confirmed substantial cumulative toxicities of myalgias at the 35 μg/m^2 dose level. This toxicity occurred earlier in patients receiving the 40 μg/m^2 dose. Thus, although only two DLTs were initially observed in the first 15 patients, resulting in dose escalation to 40 μg/m^2, there were additional patients in the 35 μg/m^2 cohort who developed grades 2 and 3 myalgia in later cycles of treatment. These observations lead us to conclude that both the 35 and 40 μg/m^2 doses exceed the maximally tolerated dose for multiple consecutive weeks of treatment. An earlier report by Philip *et al.* (18) of bryostatin-1 administered as a 1-h infusion on a weekly schedule recommended 25 μg/m^2 as a single agent Phase II dose. Our results support the use of a lower weekly dose of bryostatin 1.

We used a Phase II dose escalation design, in which lack of toxicity in the initial cycle of treatment resulted in further dose escalation (20). Although this design may be valuable in optimizing the dose of antitumor agents based on acute toxicity, it does not account for cumulative toxicity that may emerge after multiple cycles of treatment, as in the present trial of bryostatin 1. Thus, when cumulative toxicity is a concern, we recommend

Table 3 Effect of bryostatin-1 on plasma cytokine levels; pg/ml (mean ± SD)

	Cycle 1: mean ± SD			Cycle 2: mean ± SD		
Time from start of infusion	Baseline 0	2 h	24 h	Baseline	2 h	24 h
IL-6	14.75 ± 17.02	19.36 ± 23.5	21.89 ± 25.2[a]	13.12 ± 15.6	16.52 ± 19.2	29.97 ± 58[a]
TNF-α	13.24 ± 6.6	9.31 ± 4.4[b]	16.1 ± 13.2	13.53 ± 9.3	10.16 ± 7.26[b]	11.4 ± 8.5
CRP	3.87 ± 5.8	3.75 ± 5.6	5.86 ± 8.6	4.47 ± 6.6	4.17 ± 6.6	3.06 ± 6.1

[a] Significantly different from pretreatment level ($P = 0.02$).
[b] Significantly different from pretreatment level ($P = 0.02$) and 24-h level ($P = 0.01$).

modification of this design such that the decision to escalate the dose is based on the toxicity experienced in two or more cycles.

The schedule of a 1-h infusion of bryostatin-1 given weekly for 3 of 4 weeks was selected based on data that demonstrated a dose-response relationship between weekly bryostatin-1 and increased plasma levels of IL-6 and TNF-α (18). These findings and the fact that the measurement of serum bryostatin-1 levels has been unreliable by present analytical methods (25) prompted us to measure IL-6, TNF-α, and CRP levels to further evaluate correlation with bryostatin-1 biological activity. We saw no correlation between changes in plasma IL-6, TNF-α, or CRP levels to bryostatin-1 dose, response to therapy, occurrence of myalgias, or other toxicity, such as anemia or fever. Plasma IL-6 levels were reported previously to correlate with the presence of anemia or fever in patients with metastatic RCC, although not to response (to IL-2; Refs. 26 and 27).

We observed limited antitumor activity of single-agent bryostatin-1 on a 3- of 4-week schedule in a group of patients with RCC, leading us to accept the null hypothesis for the efficacy end point of objective response (objective response rate $< 20\%$). These results were consistent with a previous Phase II trial in RCC using the same schedule of bryostatin-1, but at a lower dose of 25 $\mu g/m^2$, in which two objective responses were seen in 30 patients (28). However, for the second efficacy end point of time to progression, we were able to reject the null hypothesis based on a time to progression of $\geq$6 months in 8 of the patients. Using the criteria from two publications that analyzed survival of patients with RCC, we determined that 81% of our patients had an intermediate or poor prognosis, with a predicted median survival of 10 or 4 months, respectively, based on pretreatment characteristics (21, 29). These risk factors, including weight loss, number of metastatic sites, previous chemotherapy, and performance status, were used to divide patients into prognostic subtypes (29). Good risk patients had none of these adverse factors (median survival 20 months; Ref. 21). There was a 10% progression-free, 6-month survival in patients receiving no treatment and an estimated 6-month, 20% progression-free survival in patients receiving biological response modifiers (21). Thus, despite the low proportion of objective responses, our patient population, of which the majority had one to two risk factors, nevertheless had a median overall survival of 25 months. This was substantially longer than overall survival of 13.1 months in a previous Phase II trial of 25 $\mu g/m^2$ bryostatin-1 using a similar schedule in which 93% of patients appeared to have similar pretreatment characteristics (28).

The median survival was longer than expected for patients treated on this trial. We can attribute this finding to three possibilities: (*a*) although most of the patients would be considered intermediate or poor risk as noted above, patient selection may still have contributed to this finding in a small Phase II study (21, 29); (*b*) a number of patients on this treatment went on to receive biological therapy or additional investigational new drugs that may have had an impact on survival; and (*c*) although bryostatin-1 activity was limited in terms of inducing tumor regression, the drug may still have reduced the rate of tumor cell proliferation or increased the rate of cell death to an extent sufficient to restrain net tumor growth. If the latter possibility is true, then investigation of this PKC inhibitor in combination with other agents may produce greater antitumor effects.

REFERENCES

1. Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M. Cancer statistics, 2001. CA-Cancer J. Clin., *51:* 15–36, 2001.

2. Linehan, W. M., Zbar, B., Bates, S. E., Zelefsky, M. J., and Yang, J. C. Cancer of the kidney and ureter. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Cancer: Principles and Practice of Oncology, Ed. 6, pp. 1362–1396, 2001.

3. Buzaid, A. C., and Todd, M. B. Therapeutic options in renal cell carcinoma. Semin. Oncol., *16:* 12–19, 1989.

4. Yagoda, A., Petrylak, D., and Thompson, S. Cytotoxic chemotherapy for advanced renal cell carcinoma. Urol. Clin. North Am., *20:* 303–323, 1993.

5. Haas, N., and Hudes, G. Genitourinary Malignancies. *In:* H. M. Pinedo, D. L. Longo, and B. A. Chabner (eds.), Cancer Chemotherapy, and Biologic Response Modifiers, Vol. 17, pp. 504–535. Amsterdam: Elsevier Science, 1997.

6. Bloom, H. J. Hormone induced and spontaneous regression of metastatic renal cancer. Cancer (Phila.), *32:* 1066–1071, 1973.

7. Rosenberg, S. A. Immunotherapy of patients with advanced cancer using interleukin-2 alone or in combination with lymphokine activated killer cells. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Important Advances in Oncology, pp. 217–257, Philadelphia: Lippincott, 1988.

8. Fisher, R. I., Coltman, C. A., and Doroshow, J. H. Phase II clinical trial of interleukin II plus lymphokine activated killer cells in metastatic renal cancer. Proc. Am. Soc. Clin. Oncol., *6:* 244, 1987.

9. Negrier, S., Escudier, B., Lasset, C., Douillard, J-Y., Savary, J., Chevreau, C., Ravaud, A., Mercatello, A., Peny, J., Mousseau, M., Philip, T., and Tursz, T. For the Groupe Francais d'Immunotherapie. Recombinant human interleukin-2, recombinant human interferon α-2a, or both in metastatic renal-cell carcinoma. N. Engl. J. Med., *338:* 1272–1275, 1998.

10. Pettit, G. R., Herald, C. L., and Doubek, D. L. Isolation and structure of bryostatin I. J. Am. Chem. Soc., *104:* 6846–6848, 1982.

11. Homong, R. L., Pearson, J. W., Beckwith, M., and Longo, D. L. 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.

12. Dale, I. L., Bradshaw, T. D., Gescher, A., and Pettit, G. R. Comparison of effects of bryostatins 1 and 2 12–0-tetradecanoylphorbol-13 acetate on PKC activity in A549 human lung carcinoma cells. Cancer Res., *49:* 3242–3245, 1989.

13. Trenn, G., Pettit, G. R., Takayama, H., Hu-Li, J., and Sitkovsky, M.V. Immunomodulating properties of a novel series of protein kinase C activators: the bryostatins. J. Immunol., *140:* 433–439, 1988.

14. Kraft, A. S., Reeves, J. A., and Ashendel, C. L. Differing modulation of protein kinase C by bryostatin 1 and phorbol esters in JB6 mouse epidermal cells. J. Biol. Chem., *263:* 8437–8442, 1988.

15. Uhlman, D. L., Nguyen, P., Manivel, J. C., Zhang, G., Hagen, K., Fraley E., Aeppli, D., and Niehans, G. A. Epidermal growth factor receptor and transforming growth factor-α expression in papillary and non-papillary renal cell carcinoma: correlation with metastatic behavior and prognosis. Clin. Cancer Res., *1:* 913–920, 1995.

16. Mydlo, J. H., Michaeli, J., Cordon-Cardo, C., Goldenberg, A. S., Heston, W. D., and Fair, W. R. Expression of transforming growth factor α and epidermal growth factor receptor messenger RNA in neoplastic and non-neoplastic human kidney tissue. Cancer Res., *49:* 3407–3411, 1989.

17. Kaubisch, A., Kelsen, D. P., Saltz, L., Kemeny, N., O'Reilly, E., Ilson, D., Endres, S., Barazzuol, J., Piazza, A., and Schwartz, G. K. Phase I trial of weekly sequential bryostatin-1 (Bryo), cisplatin, and paclitaxel in pts with advanced solid tumors. Proc. Am. Soc. Clin. Oncol., *19:* 230a, 2000.

18. Philip, P. A., Rea, D., Thavasu, P., Carmichael, J., Stuart, N. S., Rockett H., Talbot, D. C., Ganesan, T., Pettit, G. R., and Balkwill, F. Phase I study of bryostatin I assessment of interleukin 6 and tumor necrosis factor α induction *in vivo*. The Cancer Research Campaign Phase I Committee. J. Natl. Cancer Inst. (Bethesda), *85:* 1812–1818, 1993.

19. Miller, A. B., Hoogstraten, B., Staquet, M., and Winkler, A. Reporting results of cancer treatment. Cancer, *47:* 207–214, 1981.

20. Babb, J., Rogatko, A., and Zacks, S. Cancer Phase I clinical trials: efficient dose escalation with overdose control. Stat. Med., *17:* 1103–1120, 1998.

21. Motzer, R. J., Mazumdar, M., Bacik, J., Berg, W., Amsterdam, A., and Ferrara, J. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. J. Clin. Oncol., *17:* 2530–2540, 1999.

22. Prendiville, J., Crowther, D., Thatcher, N., Woll, P. J., Fox, B. W., McGown, A., Testa, N., Stern, P., McDermott, R., and Potter, M. A Phase I study of intravenous bryostatin 1 in patients with advanced cancer. Br. J. Cancer, *68:* 418–424, 1993.

23. Jayson, G. C., Crowther, D., Prendiville, J., McGown, A. T., Scheid, C., Stern, P., Young, R., Brenchley, P., Chang, J., and Owens, S. A Phase I trial of bryostatin 1 in patients with advanced malignancy using a 24-hour intravenous infusion. Br. J. Cancer, *72:* 461–468, 1995.

24. Varterasian, M., Eilender, D., and Mohammad, R. Phase I trial of bryostatin 1 in relapsed lymphoma and CLL. Proc. Am. Soc. Clin. Oncol., *15:* 481, 1996.

25. Zhang, X., Zhang, R., Zhao, H., Cai, H., Gush, K. A., Kerr, R. G., Pettit, G. R., and Kraft, A. S. Preclinical pharmacology of the natural product anticancer agent bryostatin 1, an activator of protein kinase C. Cancer Res., *56:* 802–808, 1996.

26. Blay, J. Y., Negrier, S., Combaret, V., Attali, S., Goillot, E., Merrouche, Y., Mercatello, A., Ravault, A., Tourani, J. M., and Moskovtchenko, J. F. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res., *52:* 3317–3322, 1992.

27. Walther, M. M., Johnson, B., Culley, D., Shah, R., Weber, J., Venzon, D., Yang, J. C., Linehan, W. M., and Rosenberg, S. A. Serum interleukin-6 levels in metastatic renal cell carcinoma before treatment with interleukin 2 correlates with paraneoplastic syndromes but not patient survival. J. Urol., *159:* 718–722, 1998.

28. Pagliaro, L., Daliani, D., Amato, R., Tu, S. M., Jones, D., Smith, T., Logothetis, C., and Millikan, R. A Phase II trial of Bryostatin-1 for patients with metastatic renal cell carcinoma. Cancer, *89:* 615–618, 2000.

29. Elson, P. J., Witte, R. S., and Trump, D. L. Prognostic factors for survival in patients with recurrent or metastatic renal cell carcinoma. Cancer Res., *48:* 7310–7313, 1988.