

Phase II Study of Recombinant Human Endostatin in Patients With Advanced Neuroendocrine Tumors

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A B S T R A C T

Purpose

Endostatin is a 20-kd proteolytic fragment of collagen XVIII that, in preclinical studies, has been shown to have antiangiogenic and antitumor activity. Both preclinical and human phase I studies of recombinant human endostatin (rhEndostatin) suggested activity in neuroendocrine tumors, which are known to be hypervascular. We therefore performed a multicenter phase II study of rhEndostatin in patients with carcinoid or pancreatic neuroendocrine tumors.

Patients and Methods

Forty-two patients with advanced pancreatic endocrine tumors or carcinoid tumors were treated with rhEndostatin administered as a bid subcutaneous injection at a starting dose of 60 mg/m²/d. Steady-state trough levels were obtained after 6 weeks of therapy; patients who did not achieve a target therapeutic level of 300 ng/mL underwent dose escalation to 90 mg/m²/d. Patients were observed for evidence of toxicity, response, and survival.

Results

rhEndostatin was associated with minimal toxicity. However, among 40 patients assessable for radiologic response, none experienced partial response to therapy, as defined by WHO criteria. The median steady-state trough level achieved after dose escalation was 331 ng/mL, within the postulated therapeutic range.

Conclusion

Treatment with rhEndostatin did not result in significant tumor regression in patients with advanced neuroendocrine tumors.

J Clin Oncol 24:3555-3561. © 2006 by American Society of Clinical Oncology

INTRODUCTION

Endostatin is one of a number of endogenous peptides that inhibit the migration and proliferation of vascular endothelial cells.¹ Structural studies have demonstrated that endostatin is a 20-kd fragment derived from the carboxy-terminal region of collagen XVIII, a proteoglycan that is a major constituent of blood vessels throughout the body.² Endostatin initially was isolated from a murine hemangioendothelioma cell line, and was subsequently shown to inhibit tumor angiogenesis and cause tumor regression in carcinogen-induced mammary tumors, mouse xenografts, and transgenic mice.^{1,3-5}

Initially, three separate phase I studies of recombinant human endostatin (rhEndostatin) in humans were undertaken, in which patients with advanced cancer were treated at doses ranging from 15 to 600 mg/m²/d without evidence of dose-limiting toxicity.⁶⁻⁸ In these studies, rhEndostatin

was administered as a daily intravenous bolus injection, after which serum concentrations of rhEndostatin declined rapidly. Preclinical studies in mouse xenografts suggested that the administration of endostatin as a continuous infusion is associated with higher rates of tumor growth inhibition, at lower overall doses, than required with bolus injections.⁹ Therefore, in a fourth phase I human study, both continuous intravenous infusion and bid subcutaneous administration of rhEndostatin were evaluated at doses ranging from 3.75 to 120 mg/m²/d.¹⁰ This study demonstrated that both the intravenous and subcutaneous modes of administration resulted in steady-state serum concentrations in the range (200 to 300 ng/mL) predicted to induce antitumor effects.

A total of 94 patients received treatment in the first four phase I endostatin studies.^{6-8,10} Although no formal radiologic partial responses were observed in these studies using standard response

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Submitted January 11, 2006; accepted May 18, 2006.

Supported by Entremed Inc. Supported in part by National Institutes of Health Grants No. K23 CA 093401 and K30 HL04095, and gifts from Raymond and Beverly Sackler, the Caring for Carcinoid Foundation, and the Stephen and Caroline Kaufer Fund for Neuroendocrine Tumor Research (M.H.K.).

Presented in part at the 39th Annual Meeting of the American Society of Clinical Oncology, May 31-June 3, 2003, Chicago, IL.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/06/2422-3555/\$20.00

DOI: 10.1200/JCO.2006.05.6762

criteria, minor tumor responses were observed in a patient with melanoma, a patient with synovial cell carcinoma, and one patient with advanced, metastatic pancreatic neuroendocrine tumor. The pancreatic neuroendocrine tumor patient experienced maximum tumor reduction of 17%, which lasted for more than 11 months. The fact that neuroendocrine tumors are highly vascular further supported the continued evaluation of rhEndostatin in this patient population, as did the observation that endostatin treatment was associated with 60% tumor shrinkage in a transgenic mouse pancreatic endocrine tumor model.^{4,11}

We performed a multicenter phase II study of rhEndostatin in patients with advanced neuroendocrine tumors. Because of their similar histologic and clinical characteristics, patients with both pancreatic neuroendocrine and carcinoid tumors were enrolled. rhEndostatin was administered subcutaneously, using a bid dosing schedule, at a starting dose of 60 mg/m² daily without scheduled treatment breaks. Steady-state trough levels of rhEndostatin were monitored, and patients who had not achieved a target therapeutic steady-state trough level of 300 ng/mL underwent dose escalation to 90 mg/m². Patients were observed for evidence of toxicity, biochemical response, radiologic response, and survival.

PATIENTS AND METHODS

Patient Population

All patients had histologically documented, metastatic neuroendocrine tumors, excluding small-cell carcinoma. Measurable disease, as defined by WHO criteria was required. Prior treatment with systemic chemotherapy or an investigational agent (except rhEndostatin) was allowed if patients had discontinued prior therapy at least 4 weeks before study enrollment. Patients receiving octreotide were allowed to continue therapy at stable doses throughout study treatment. Additional inclusion criteria included: Eastern Cooperative Oncology Group status of 0 or 1, AST and ALT less than 5× the upper limit of normal, total bilirubin less than 2.0 mg/dL, serum creatinine ≤ 2.0 mg/dL, WBC ≥ 3,000/mL, absolute neutrophil count ≥ 1,000/ μ L, and platelets ≥ 100,000/ μ L. Exclusion criteria included clinically apparent CNS metastases or carcinomatous meningitis, myocardial infarction or angina pectoris in the 6 months before study treatment, major surgery within 2 weeks, concurrent treatment with heparin, pregnancy, or lactation. Patients from Dana-Farber Cancer Institute (Boston, MA), Massachusetts General Hospital (Boston, MA), the Beth Israel Deaconess Medical Center (Boston, MA), or the University of California, San Francisco Comprehensive Cancer Center (San Francisco, CA) were eligible for enrollment. All patients provided informed consent as required by the institutional review boards of the respective institutions.

Treatment Program

rhEndostatin was self-administered at a dose of 30 mg/m² as a bid subcutaneous injection (total daily dose, 60 mg/m²) at 12-hour intervals, except on day 1 of the initial treatment period. Completion of 4 weeks (28 days) of treatment was considered one cycle of therapy. Serum rhEndostatin concentrations were measured using the Accucyte enzyme immunoassay kit for human endostatin (CytImmune Sciences, Rockville, MD). rhEndostatin concentrations were determined in five study patients at baseline and at 0.5, 1, 1.5, 2, 4, 6, 8, 16, and 24 hours after initial administration. Concentrations were measured in all patients at baseline, during cycle 2 on day 15 (before dosing), and after subsequent cycles of therapy were completed.

Dose adjustments were made after completion of the second 4-week treatment cycle, based on tumor response and serum pharmacokinetics in each patient. If the tumor was stable or had not responded, and if the cycle 2, day 15 blood sample indicated that the serum levels of rhEndostatin protein had not achieved a steady-state trough level of 300 ng/mL, then the dose for

that patient was escalated to 45 mg/m² bid (90 mg/m²/d). No additional dose escalations or reductions were performed over the course of the study. Patients discontinued therapy in the event of grade 3 or greater nonhematologic toxicity, if they experienced a grade 4 hematologic toxicity that did not resolve in 14 days, if they missed more than 14 consecutive days of rhEndostatin treatment, if they became pregnant, if they chose to terminate study participation, or at the discretion of the investigator.

Radiologic assessments of response with computed tomography or magnetic resonance scan were made at baseline and after every two cycles (8 weeks) of study therapy. Radiologic response was classified according to the WHO criteria. Complete response required total resolution of all detectable disease lasting for at least 4 weeks. Partial response required a decrease of more than 50% in the sum of the products of the largest perpendicular diameters of all measurable lesions, persisting for at least 4 weeks, without progression of any nonmeasurable sites and without the appearance of new sites of disease. Progressive disease included an increase of 25% or more in the sum of the products of the largest perpendicular diameters of one or more measurable lesions, the development of new lesions, or progression of nonmeasurable but assessable sites of disease. Stable disease was defined as having neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.

Serum chromogranin A (CGA), 24-hour collections of urinary 5-hydroxyindoleacetic acid (5-HIAA), and urinary levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were measured at baseline and after each cycle of therapy. CGA and 5-HIAA were measured using standard clinical assays. VEGF and bFGF were measured using the Quantikine human VEGF and Quantikine high-sensitivity human bFGF enzyme-linked immunosorbent assay kits (R&D Systems Inc, Minneapolis, MN). CGA or 5-HIAA responses were defined as a decrease from baseline of at least 50% on two consecutive measurements in patients who had elevated baseline levels (defined as CGA > 36.4 ng/mL or 5-HIAA > 6 mg/24 hours).

Statistical Considerations

This phase II study was designed with a primary end point of response. The study used a two-stage design to test the null hypothesis that the rate of response or stable disease would be 20% or less. After the first 21 patients were enrolled, an interim analysis was performed and at least three patients were required to have response or stable disease to proceed to the full accrual of 42 patients. With a sample size of 42 patients, the study had 80% power to reject the null hypothesis at $\alpha = .05$. Progression-free survival and overall survival estimates were calculated using Kaplan-Meier methodology. Toxicity and complications of treatment were based on reports of adverse events, physical examinations, and laboratory measurements. For pharmacokinetic analysis, serum concentration-time profiles were analyzed.

RESULTS

Patient Characteristics

A total of 42 patients were entered onto the study at the four participating centers. Baseline characteristics of the patients are shown in Table 1. Patients had a median age of 55 years, 55% were male, and the median time from their original diagnosis was 49.5 months. Fifty-three percent of the cohort had metastatic carcinoid tumors, and 47% had metastatic pancreatic endocrine tumors. In nearly all patients (90%) the tumor was histologically described as well differentiated; only 10% had moderately or poorly differentiated neuroendocrine tumors. The majority (64%) had received some form of prior therapy other than surgery; prior treatments included systemic chemotherapy, interferon alfa, and embolization of hepatic metastases.

Pharmacokinetic Data and Dose Escalation

Forty-two patients received treatment for a median of 6.4 months (range, 10 days to 45 months). All patients initiated therapy at

Table 1. Baseline Patient Characteristics

Characteristic	No. of Patients	%
Total	42	
Age, years		
Median	55	
Range	30-80	
Time from diagnosis, months		
Median	49.5	
Range	1.9-261	
Sex		
Male	23	55
Female	19	45
Race/ethnicity		
White	39	93
African American	1	2
Asian	2	5
Prior treatment		
Surgery	32	76
Other treatment	27	64
Systemic chemotherapy*	18	43
Interferon†	5	12
Hepatic embolization‡	12	29
Baseline symptoms		
Diarrhea	21	50
Flushing	17	40
Fatigue	18	43
Tumor grade		
Well differentiated	38	90
Moderately or poorly differentiated	4	10
Tumor origin		
Pancreatic endocrine	20	47
Carcinoid	22	53
Small bowel	11	26
Duodenum	1	2
Lung	3	7
Rectum	1	2
Unknown primary	6	14
Sites of measurable disease		
Liver metastases	36	86
Lymph node metastases	22	53
Mesenteric mass	8	19
Pancreatic mass	6	14
Other	10	24

*Best response from prior chemotherapy regimens: partial response (n = 3), stable disease (n = 8), progressive disease (n = 2), unknown response (n = 5).
†Best response from prior interferon: stable disease (n = 3); progressive disease (n = 2).
‡Best response among prior embolizations: partial response (n = 3), stable disease (n = 7), unknown response (n = 2).

a dose of 60 mg/m² daily. Serial endostatin levels were measured in five study patients at baseline and during the first 24 hours after administration of the first dose of 30 mg/m² on day 1, and after administration of the morning dose of 30 mg/m² on day 28. The median baseline level of endostatin protein in these five patients was 33 ng/mL, reflecting endogenous endostatin levels. Median endostatin levels increased to a peak of 66 ng/mL 12 hours after initial administration (Fig 1A) and decreased back to baseline levels (37 ng/mL) at 24 hours. The median endostatin concentration before dosing on day 28 was 179 ng/mL. The endostatin concentration in-

creased to a maximum level of 241 ng/mL before decreasing to 123 ng/mL 12 hours after administration (Fig 1B).

The median endostatin level for the entire patient cohort during cycle 2, before dose escalation, was 259 ng/mL, which was below the target endostatin steady-state trough level of 300 ng/mL (Fig 1C). Twenty-two (52%) patients were found to have subtherapeutic trough levels, and underwent subsequent dose escalation to the 90 mg/m²/d dose level. Following this dose escalation, the median monthly endostatin concentrations for cycles 3 to 12 in the study cohort ranged from 241 to 583 ng/mL, with a median level of 331 ng/mL.

Toxicity

All 42 patients were assessable for toxicity; treatment-emergent adverse events are shown in Table 2. The majority of patients (64%) developed local injection site reactions, which usually were mild and did not result in treatment discontinuation or dose modification. Most of these reactions resolved during the first 2 months of treatment, despite continued administration of the drug. Other treatment-emergent adverse events included fatigue (30%), abdominal pain (29%), and diarrhea (26%). Hypertension, GI hemorrhage, myocardial infarction, which comprise toxicities reported with other angiogenesis inhibitors, were uncommon, and developed in 7%, 2%, and 2% of patients, respectively. All three cases of hypertension were grade 1; no occurrences of grade 2 or higher hypertension were observed. The GI hemorrhage developed from a presumed ileal primary tumor and resolved spontaneously. The patient who experienced the myocardial infarction on study had a prior history of coronary artery disease and myocardial infarction; he recovered after treatment discontinuation and placement of a coronary stent.

Biochemical and Symptomatic Response to Therapy

Thirty-one patients had elevated CGA levels at baseline (> 36.4 ng/mL) and were assessable for CGA response, defined as a more than 50% decrease in serum levels from baseline on two consecutive measurements. Two patients (6%) experienced CGA responses; both patients had stable disease as their best radiologic response to therapy. Twenty patients had elevated baseline levels of urinary 5-HIAA (> 6 mg/24 hours) and were assessable for 5-HIAA responses; none responded. Consistent with the rarity of biochemical responses, resolution of hormonally mediated symptoms was uncommon during treatment. Before initiating treatment with rhEndostatin, 17 patients reported symptoms of flushing, 21 reported diarrhea, and 18 reported fatigue. After treatment initiation, no patients experienced resolution of flushing symptoms, two patients (9.5%) experienced resolution of their diarrhea, and one patient (5%) experienced resolution of fatigue.

Urinary VEGF and bFGF levels were measured at baseline and after each cycle of therapy in 26 of the 28 patients treated at the Dana-Farber Cancer Institute and Massachusetts General Hospital sites. The median baseline urinary bFGF level was 1,792.5 pg/L (range, < 1,000 to 11,653 pg/L), and the median baseline urinary VEGF level was 86.5 ng/mL (range, 35 to 581 ng/mL). No consistent changes in urinary VEGF or bFGF concentrations were observed in these patients during study therapy.

Efficacy

Of the 42 patients treated on the study, 40 completed at least one cycle of therapy and were assessable for radiologic response. One of the inassessable patients experienced a myocardial infarction after 2 weeks of therapy, and the second patient withdrew consent before

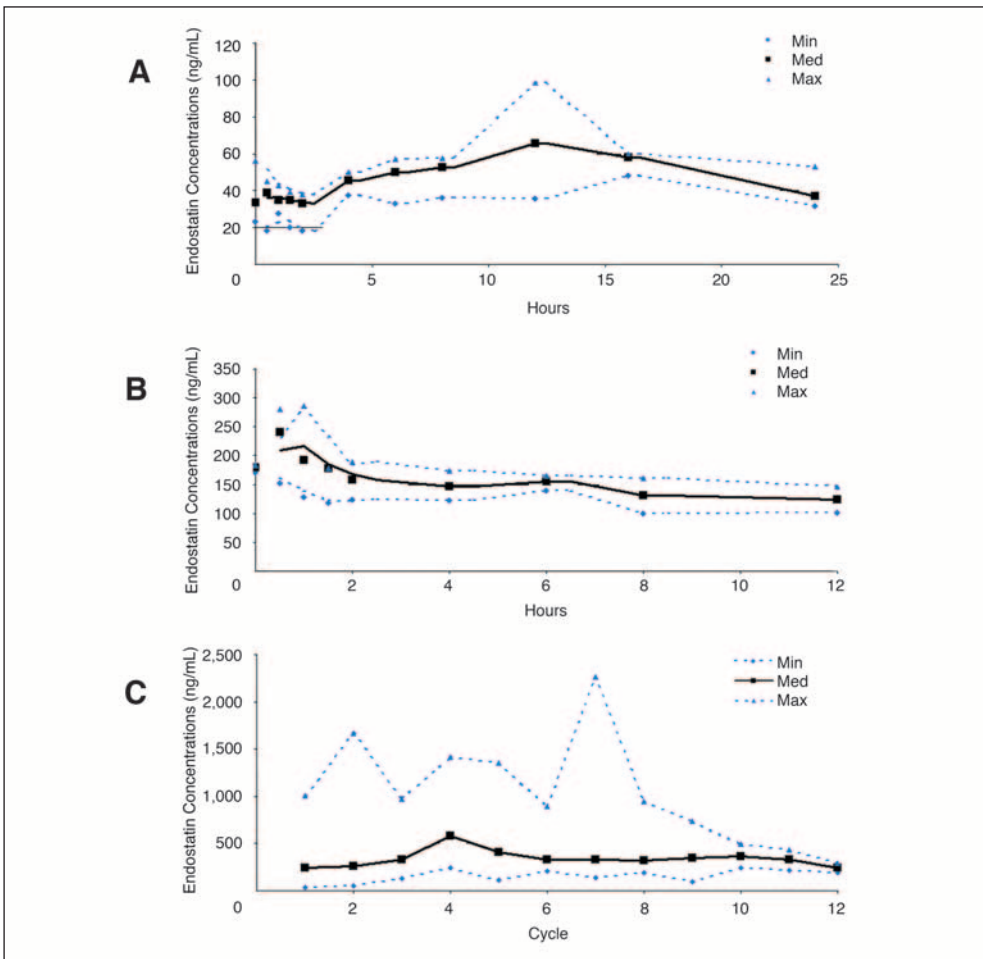


Fig 1. rhEndostatin pharmacokinetics. Endostatin concentrations were obtained at indicated time intervals in five patients after administration of 30 mg/m² dose on (A) day 1 and (B) day 28. (C) Steady-state trough rhEndostatin concentrations for the patient cohort, months 1 to 12 (obtained before scheduled subcutaneous dosing at the indicated time intervals). Min, minimum; med, median; max, maximum.

completing the first cycle of therapy. No patients experienced a partial or complete radiologic response to therapy, 32 (80%) had stable disease as their best response to therapy, and eight patients (20%) experienced disease progression (Table 3). Among the 32 patients with radiologically stable disease, the median duration of stable disease was 10.8 months.

To better assess whether the high incidence of stable disease in the study reflected treatment effect or the natural tumor growth rate of the enrolled patients, patients were evaluated retrospectively for reported disease progression before study enrollment. Twenty-one (95%) of 22 assessable patients were reported to have stable disease for at least 2 months before study enrollment, and 11 (48%) of 23 were reported to have stable disease for at least 6 months before study enrollment.

The most common reason for discontinuation of study treatment was disease progression (20 patients; 48%). Other reasons for treatment discontinuation included adverse events (four patients; 10%) and death (one patient; 2%). Seventeen patients (41%) either withdrew consent for additional treatment or discontinued for other, unspecified reasons. The median progression-free survival time was 5.8 months (range, 1.9 to 13.5 months) for patients with pancreatic endocrine tumors and 7.6 months (range, 5.3 to 19.2 months) for patients with carcinoid tumors. The median overall survival was 17.2 months (range, 8.1 to 27.2 months) for patients with pancreatic endocrine tumors and 22.6 months (range, 17.8 to > 27.4 months) for patients with carcinoid tumors (Figs 2 and 3).

DISCUSSION

Our study demonstrates that the administration of rhEndostatin is associated with minimal toxicity when administered during a prolonged period to patients with advanced neuroendocrine tumors. However, treatment with rhEndostatin was not associated with significant antitumor activity as measured by traditional response criteria. No patients achieved a partial or complete radiologic response, biochemical (CGA) responses were observed in only 6% of assessable patients, and less than 10% of patients reported resolution of hormonally mediated tumor-related symptoms.

One limitation of our study is the lack of firm data regarding the optimal therapeutic dose of rhEndostatin. The target steady-state level of rhEndostatin for this study was based primarily on studies using tumor xenografts, suggesting that steady-state levels of 200 to 300 ng/mL are required for tumor regression.⁹ Subsequent studies have suggested that the dose-response curve of rhEndostatin is biphasic, and that administration of higher doses of endostatin is not associated with further enhancement of antitumor activity.¹² Steady-state trough levels after the administration of the initial 60 mg/m²/d starting dose in our study were below the target therapeutic range of 300 ng/mL. After dose escalation to 90 mg/m²/d, the median steady-state trough level was 331 ng/mL, which closely approximated the target therapeutic dose. Notably, the pancreatic neuroendocrine tumor patient who

Table 2. Treatment-Emergent AEs

Adverse Event	Maximum AE Grade							
	1		2		3		4	
	No.	%	No.	%	No.	%	No.	%
Nonhematologic								
Injection site reaction	24	57	3	7	—	—	—	—
Fatigue	6	14	6	14	1	2	—	—
Abdominal pain	5	12	4	10	1	2	2	5
Diarrhea	3	7	7	17	1	2	—	—
Vomiting	7	17	1	2	3	7	—	—
Nausea	7	17	2	5	1	2	—	—
Dyspnea	5	18	5	18	—	—	—	—
Pyrexia	5	12	2	5	1	2	—	—
Anorexia	4	10	3	7	—	—	1	4
Peripheral edema	3	7	2	5	1	2	—	—
Arthralgia	2	5	2	5	2	5	—	—
Hypertension	3	7	—	—	—	—	—	—
Bowel obstruction	—	—	—	1 (%)	2	2	5	—
Flushing	1	2	—	—	1	2	—	—
Dehydration	—	—	1	2	1	2	—	—
GI hemorrhage	—	—	—	—	1	2	—	—
Myocardial infarction	—	—	—	—	—	—	1	2
Hematologic								
Anemia	2	5	4	10	1	2	—	—
Leukopenia	1	2	—	—	—	—	—	—
Thrombocytopenia	1	2	—	—	—	—	—	—

Abbreviation: AE, adverse event.

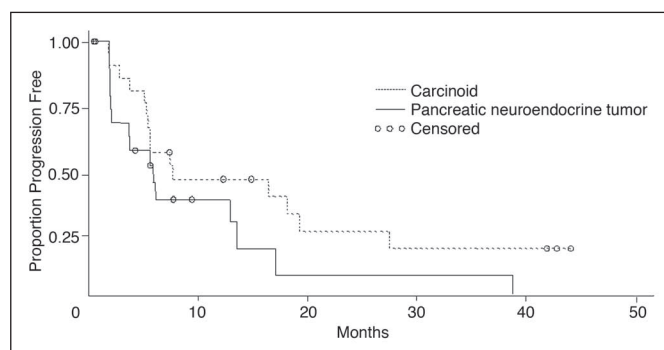
experienced a minor tumor response in an earlier phase I study had received intravenous bolus doses of 30 mg/m² daily, which were well below the dosing levels used in the present study.⁷

The precise mechanisms of the reported antiangiogenic and antitumor activity of endostatin have not yet been characterized fully. Endostatin contains a heparin-binding motif, and may exert some of its antiangiogenic effects through interactions with the heparan sulfate proteoglycans glypican-1 and -4.¹³ Binding of endostatin to $\alpha_5\beta_1$ integrin on the cell surface also seems to be critical in mediating a number of downstream antiangiogenic signaling pathways.¹⁴⁻¹⁶ A gene profiling and protein phosphorylation study of human endothelial cells demonstrated that endostatin treatment was associated with

Table 3. Best Response to Treatment With rhEndostatin

Response	No.	%
Radiologic (n = 40 assessable)		
Partial or complete response*	0	0
Stable disease	32	80
Progressive disease	8	20
Chromogranin A† (n = 31 assessable)		
Partial response	2	6
5-HIAA† (n = 20 assessable)		
Partial response	0	0

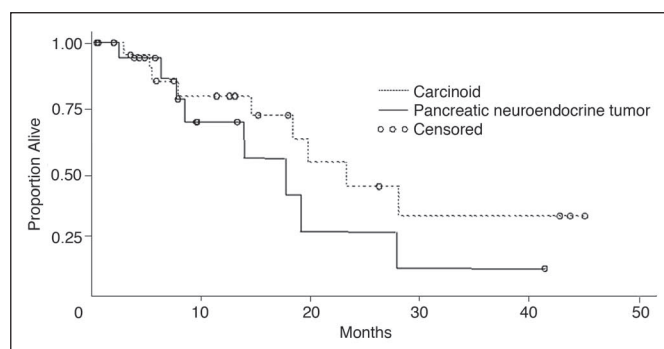
Abbreviation: rhEndostatin, recombinant human endostatin; 5-HIAA, 5-hydroxyindoleacetic acid.
*Confirmed response, WHO Criteria.
†Biochemical responses were defined as > 50% decrease from baseline on two consecutive measurements in patients with elevated baseline levels.

**Fig 2.** Progression-free survival.

upregulation of a large cluster of antiangiogenic genes and downregulation of genes involved in proangiogenic signaling pathways; taken together, 12% of the studied genes demonstrated more than two-fold alteration in expression levels in response to endostatin treatment.¹⁷

Several investigators have suggested that angiogenesis inhibitors may be cytostatic rather than cytotoxic, and that strategies other than evaluation of radiologic tumor regression may be necessary to assess their potential efficacy.¹⁸ The majority (80%) of patients in this study experienced stable disease as their best response to therapy. However, this observation may reflect the naturally indolent nature of their disease. In 22 study patients for whom prior progression could be assessed, 95% had stable disease for at least 2 months before study enrollment. The incidence of stable disease for at least 6 months before study enrollment in a similar subgroup of 23 patients was 48%, a figure that may explain the relatively long time to tumor progression observed in patients with either carcinoid (7.6 months) or pancreatic neuroendocrine tumors (5.6 months) during study treatment.

In contrast to the results observed in this study, modest antitumor activity has been observed recently after the treatment of neuroendocrine tumors with specific VEGF pathway inhibitors. In a preliminary report, the tyrosine kinase inhibitor sunitinib, which inhibits phosphorylation of VEGF receptor, platelet-derived growth factor receptor, c-Kit, and the product of *RET* proto-oncogene, was associated with an overall radiologic response rate of 9% in a 102-patient phase II study of carcinoid and pancreatic endocrine tumors.¹⁹ In a randomized phase II study, treatment with the monoclonal anti-VEGF antibody bevacizumab was associated with radiologic tumor responses in three of 18 (16%) patients with carcinoid tumors.²⁰

**Fig 3.** Overall survival.

A number of cytotoxic agents have also been shown previously to have activity in neuroendocrine tumors. The combination of streptozocin and fluorouracil has been associated with overall response rates of 16% to 21% in carcinoid tumors, and in pancreatic neuroendocrine tumors, the three-drug combination of streptozocin, fluorouracil, and doxorubicin has been associated with an overall response rate of 39%.²¹⁻²³ Regimens containing dacarbazine or its oral analog, temozolomide, have been associated with objective response rates of 8% to 16% in patients with advanced

carcinoid tumors and 33% to 45% in patients with pancreatic neuroendocrine tumors.^{24,25} Whether the addition of angiogenesis inhibitors to currently available cytotoxic regimens will enhance their efficacy is not yet known.

In conclusion, treatment with rhEndostatin did not result in significant tumor or biochemical regression in patients with advanced neuroendocrine tumors, nor was it associated with clear evidence of a cytostatic effect. Other angiogenesis inhibitors seem to have more activity in this disease.

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Acknowledgment

We thank Taylor S. Spear for assistance in manuscript preparation.

Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Authors	Employment	Leadership	Consultant	Stock	Honoraria	Research Funds	Testimony	Other
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William E. Fogler	Entremed Inc (N/R)							
Carolyn Sidor	Entremed Inc (N/R)							

Dollar Amount Codes (A) < \$10,000 (B) \$10,000-99,999 (C) ≥ \$100,000 (N/R) Not Required

Author Contributions

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