The Trk Tyrosine Kinase Inhibitor CEP-701 (KT-5555) Exhibits Significant Antitumor Efficacy in Preclinical Xenograft Models of Human Pancreatic Ductal Adenocarcinoma[1]

Sheila J. Miknyoczki, Hong Chang, Andres Klein-Szanto, Craig A. Dionne, and Bruce A. Ruggeri[2]

Department of Oncology, Cephalon, Inc., West Chester, Pennsylvania 19380 [S. J. M., H. C., C. A. D., B. A. R.], and Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 [A. K-S.]

ABSTRACT

The aggressive behavior and poor prognosis of pancreatic ductal adenocarcinoma (PDAC) is associated with an increased expression of many growth factors and their cognate receptors. We have previously demonstrated the aberrant expression of the Trk receptors (Trks A, B, and C), enhanced tumor stromal expression of neurotrophins in primary PDAC specimens and human PDAC-derived cell lines, and a dose-dependent biological response of PDAC cells (*in vitro* invasiveness) to selective neurotrophins (Miknyoczki, S. J., *et al.*, Int. J. Cancer, *81:* 417–427, 1999). On the basis of these data, we have evaluated the therapeutic potential of inhibiting neurotrophin-Trk interactions using a selective and potent Trk tyrosine kinase inhibitor (CEP-701) in several preclinical models of human PDAC. CEP-701 is currently approved for clinical trials within the United States We demonstrate that CEP-701 administration at 10 mg/kg s.c. b.i.d. 5 days a week for 21–28 days inhibited tumor growth in a statistically significant manner in Panc-1, AsPc-1, BxPc-3, Colo 357, and MiaPaCa2 s.c. xenografts in athymic nude mice compared with vehicle-treated controls. Reductions in tumor growth volume of 50–70% relative to vehicle-treated controls were observed in xenografts responsive to CEP-701 administration. Significant reductions of *in vivo* PDAC tumor invasiveness were likewise observed in four of six CEP-701-treated rat tracheal xenografts implanted s.c. in athymic nude mice. The antitumor efficacy of CEP-701 was observed in the absence of pronounced morbidity or toxicity *in vivo*. Taken together, these data suggest that CEP-701 may be effective as a potential therapeutic agent in the treatment or management of PDAC.

INTRODUCTION

PDAC[3] is the fifth most common cause of cancer deaths in the United States with an estimated 28,100 deaths in 1997 (1). The survival rate for patients with PDAC remains among the poorest for all cancers because of the fact that by the time the cancer is diagnosed, the tumor is surgically unresectable in the majority of cases, or has metastasized to distant organ sites such as the liver, lung, and duodenum (1, 2). The median survival time after diagnosis is approximately 4–6 months with a 5-year survival rate of 1–4% (1).

Treatment options available for patients with PDAC have proven to be largely ineffective. The most commonly used drugs for chemotherapy, 5-fluorouracil and mitomycin, are mainly used for palliative and adjuvant therapy. These drugs, used either alone or in combination, cause tumor regression only in 10–30% of patients. In addition, the drugs have not produced any remissions and do not significantly increase survival time (1–3). Gemcitabine, the first chemotherapeutic agent approved for use in patients with pancreatic cancer in 35 years, has been shown to improve quality of life (clinical benefit response) in Phase II trials of stage III and IV PDAC patients (4, 5). In clinical trials evaluating the effectiveness of gemcitabine and 5-fluorouracil, objective response rates were determined to be 5.4% and 0, respectively. The median survival time was 5.7 months for gemcitabine and 4.4 months for 5-fluorouracil, with 1-year survival rates of 18 and 2%, respectively (4). Relief from symptoms occurred within 7 weeks of starting therapy with gemcitabine and lasted approximately 18 weeks, and gemcitabine was generally better tolerated than 5-fluorouracil (1–4). Other treatment methods, such as external beam radiation therapy, aid in local control of the cancer but offer no significant benefit for increasing patient survival (2, 3, 5). In addition, radiation and chemotherapy cause toxic side effects such as nausea, vomiting, mucositis, neutropenia, thrombocytopenia, and anorexia (2–5). Finally, only 5–15% of patients with PDAC are candidates for surgical resection, with long-term survival rate of only 10% (1, 3, 5). Thus, a treatment that would produce tumor regression or increase patient survival without the toxic side effects associated with current chemo- and radiotherapeutic treatment regimens would be of great benefit in the treatment and management of this disease.

The aggressive behavior and poor survival rate of PDAC has been associated with genetic mutations, such as mutations in the *p53* tumor suppressor gene and the *K-RAS* oncogene, as well

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[1] This work was funded in part by TAP Holdings, Inc.
[2] To whom requests for reprints should be addressed, at Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380. Phone: (610) 738-6637; Fax: (610) 738-6643.

[3] The abbreviations used are: PDAC, pancreatic ductal adenocarcinoma; NT, neurotrophin; ATCC, American Type Culture Collection, FBS, fetal bovine serum; VEGF, vascular endothelial growth factor; VEGF-R, VEGF receptor; PDGF, platelet-derived growth factor.

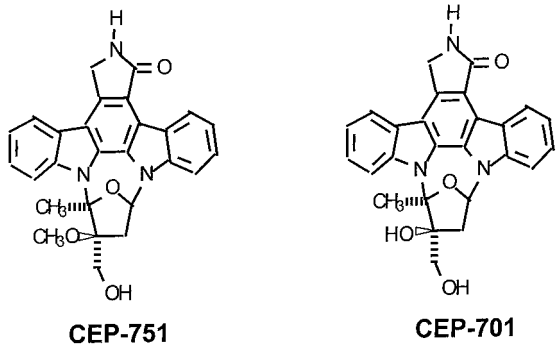

Fig. 1 Structures of CEP-751 and CEP-701

as aberrant overexpression of various growth factors and their corresponding receptors (6–14). Recently, we have demonstrated the expression of the NT growth factor family and aberrant overexpression of their corresponding Trk receptors immunohistochemically and by *in situ* hybridization in a majority of human PDAC specimens relative to normal pancreata. Further, we demonstrated in a series of Boyden chamber invasion assays that at low nanomolar concentrations of specific NTs (BDNF, NT-3), there was a significant increase in the *in vitro* invasiveness of human PDAC-derived cell lines through growth factor-reduced Matrigel (15). On the basis of our data demonstrating aberrant Trk expression in PDAC and a Trk-mediated biological response of PDAC cells to specific NTs, we have examined the antitumor efficacy of the potent and selective Trk receptor tyrosine kinase inhibitor, CEP-701 (16, 17) on the development and progression of PDAC. This compound is currently in Phase I clinical trials in the United States as an orally administered therapeutic agent.

MATERIALS AND METHODS

Tissue Culture and Cell Lines. Cell lines MiaPaCa2 and Panc 1 (ATCC, Rockville, MD) and Colo 357 (Dr. Batra, Eppley Institute, Omaha, NB) were cultured in MEM with 10% FBS. BxPc3 and AsPc1 cell lines (ATCC) were cultured in RPMI medium with 20% FBS. The CFPAC cell line (ATCC) was cultured in DMEM with 10% FBS. All of the cell lines were maintained at 37°C in 5% CO_2.

Reagents. The Trk receptor tyrosine kinase inhibitor CEP-701 (provided by Cephalon, Inc., West Chester, PA) was formulated in a vehicle of 40% polyethylene glycol 1000 (Spectrum, Los Angeles, CA), 10% povidone C30 (ISP, Boudbrook, NJ), and 2% benzyl alcohol (Spectrum) in distilled water (Fig. 1).

Animals. Female athymic nu/nu mice (8–10 weeks old; Harlan Sprague Dawley, Inc. Indianapolis, IN) were maintained at five per cage in microisolator units. Animals were given a commercial diet and water *ad libitum*, housed at 48 ± 2% humidity and 22 ± 2°C, and light-dark cycle was set at 12 h intervals. Mice were quarantined for at least 1 week before experimental manipulation. Mice weighed between 22 and 25 g on the day of tumor or trachea implantation. All of the animal experiments were performed at Allegheny University of the Health Sciences (Philadelphia, PA) under protocol 60762-92P approved by the Institutional Animal Care and Use Committee of the University.

Preparation and Evaluation of s.c. Xenografts. Cultures of human PDAC-derived cell lines were grown to subconfluency in 10% FBS in their respective growth media. On counting viable cells using trypan blue (Fisher Scientific, Malvern, PA), 1×10^6 cells in serum-free media were injected s.c. in the left flank of nude mice. When tumors reached 150–200 mm^3, mice were randomized into two groups of 10 mice each, and the administration of CEP 701 (10 mg/kg in 100 μl s.c. b.i.d., 5 days a week) or vehicle (100 μl s.c. b.i.d., 5 days a week) was begun. This dose of CEP-701 was determined on the basis of pharmacokinetic data for s.c. administration of this compound in mice and rats as well as on prior experience evaluating the antitumor efficacy of CEP-701 against human prostatic carcinoma xenografts in athymic mice.[4] The poor oral bioavailability of CEP-701 in mice (approximately 3–5%) was the basis for s.c. administration of this compound on a rotating injection pattern on the dorsal surface of tumor-bearing mice.

Mice were weighed, and tumor volumes in mm^3 [$V = l \times w\,(l + w \div 2) \times 0.526$, where l = length and w = width] were calculated using a vernier caliper every 3–4 days (16–18). Tumor volume doubling times were determined at varying days of CEP-701 treatment compared with vehicle-treated controls. Animals were treated for 21–28 days and killed by CO_2 asphyxiation; tumors were removed and either fixed in 10% neutral buffered formalin or frozen. Tumor tissues obtained at the completion of the study were evaluated histologically by one of us (A. K-S.) to confirm the pathology and differentiation grade of the PDAC xenografts as described previously (8, 19). Trk immunostaining was evaluated in these lesions as described in Table 1. The end points for assessing antitumor activity were tumor volume (calculated as above) and tumor growth inhibition (T/C%):

$$T/C\% = \frac{\text{mean } (V_t/V_o) \text{ CEP-701 group}}{\text{mean } (V_t/V_o) \text{ vehicle group}} \times 100 \qquad (1)$$

where V_t refers to tumor volume on a given day and V_o is the volume of the same tumor at the start of CEP-701 treatment.

Preparation and Evaluation of Tracheal Xenografts. Rat tracheas (Zivic Miller, Pittsburgh, PA) were cleaned, mounted on polyethylene tubing, and de-epithelialized nonenzymatically by repetitive freezing (−80°C) and thawing as detailed previously (20, 21). Cultures of human PDAC-derived cell lines were grown to subconfluency in 10% FBS in their respective growth media. On counting viable cells using trypan blue (Fisher Scientific, Malvern, PA), 5×10^5 cells in serum-free media were inoculated into each trachea, and the tracheas were sealed with hemoclips. Female nu/nu mice were lightly anesthetized with Metafane (Halocarbon Lab., River Edge, N.J.) and tracheas were implanted s.c. (one trachea/mouse). One week after implantation, dosing was initiated with CEP 701 (10 mg/kg in 100 μl volume s.c. b.i.d., 5 days a week) or vehicle (100 μl s.c. b.i.d., 5 days a week) and continued for 21–28 days. Mice

[4] Unpublished data.

Table 1 Summary of antitumor efficacy of CEP-701-treated PADC-derived xenografts in athymic nude mice

Cell line	Source	Differentiation	*K-RAS* status	*p53* status[a–c]	*T/C*[d] %	*In vivo* invasiveness[e]		Trk expression[f] (IHC)
						Vehicle	CEP-701	
Panc-1	Primary	Poorly differentiated	Codon 12	Codon 273	25[g]	3	1	+/++
BxPc3	Primary	Moderately well differentiated	Wild-type	Codon 220 and 265	45[g]	3	0	+/−
AsPc1	Ascites	Moderately well differentiated	Codon 12	Codon 273	27[g]	3	1	+++
Colo 357	Metastases to colon	Moderately differentiated	Codon 12	Wild-type	38[g]	2	2	+++
MiaPaCa2	Primary	Poorly differentiated	Codon 12	Codon 248	60[h]	2	1	++
CFPAC	Metastases to liver	Well differentiated	Codon 12	Codon 242	241	3	3	+++

[a] Redston, M. S., *et al.* (32).
[b] Kalthoff, H., *et al.* (33).
[c] Ruggeri, B., *et al.* (7).
[d] T:C ratios (22) for s.c. xenografts were calculated using the following formula:

$$T/C\% = \frac{\text{mean } (V_t/V_o) \text{ drug group}}{\text{mean } (V_t/V_o) \text{ vehicle group}} \times 100$$

where V_t refers to tumor volume on a given day (day 21) and V_o refers to the same tumor volume at the start of treatment.
[e] Momiki, S., *et al.* (21).
[f] Miknyoczki, S. J. *et al.* (15). For Trk IHC staining, −, negative, immunostaining intensity equal to tissue control; (+/−, immunostaining intensity greater than control but limited to <25% of the cells; +, moderate immunostaining intensity relative to tissue control but limited to <50% of the cells; ++ (+++), strong-intense immunostaining relative to tissue control and present in >50% of the cells.
[g] CEP-701-treated tumors showed a statistically significant reduction in tumor volume as compared with vehicle-treated controls. $P < 0.01$ using Student-Newman-Keuls method or Mann-Whitney rank-sum test.
[h] CEP-701-treated tumors showed a statistically significant reduction in tumor volume as compared with vehicle-treated controls. $P < 0.05$ using Student-Newman-Keuls method or Mann-Whitney rank-sum test.

were weighed, and tumor volumes were calculated as above using a vernier caliper every 3–4 days. At the end of dosing, mice were killed by CO_2 asphyxiation, and tracheas were removed, fixed in 10% neutral buffered formalin, paraffin-embedded, and stained with H&E. The level of invasiveness through the tracheal wall was based on the following criteria: (*a*) level 0: no invasion of the tracheal wall, cells are confined to the lumen or lining the lumenal surface; (*b*) level 1: tumor cells are found in the mucosa and superficial lamina propria; (*c*) level 2: the lamina propria is completely infiltrated by tumor cells and the pars membranacea and trachealis muscle are invaded. The adventitia is not invaded by tumor cells; and (*d*) level 3: the malignant cells have reached the adventitia, and the whole tracheal wall is invaded (21). All of the tumor xenograft studies were repeated as described above for experimental confirmation and confirmed histologically by one of us (A.K-S.) using the above criteria.

Statistical Analysis. The effect of CEP-701 treatment on tumor and trachea xenograft volumes and T/C values were analyzed using one-way ANOVA and the Student-Newman-Keuls Method. All of the analyses were performed using Sigma Stat for Windows (Jandel Scientific, San Rafael, CA). Values for T/C indices were calculated as described previously (22).

RESULTS

The Effects of CEP-701 Administration on s.c. Xenografts in Nude Mice. The antitumor efficacy of CEP-701 was determined using six different models of human PDAC. Animals were injected with 1×10^6 cells, and dosing with CEP-701 (10 mg/kg body weight s.c. b.i.d. 5 days a week for 21–28 days) or vehicle (s.c. b.i.d. 5 days a week for 21–28 days) began when the tumors reached between 150 and 200 mm^3. CEP-701 inhibited significantly the growth ($P < 0.05$ to $P < 0.01$) of five of six of the PDAC xenografts tested over the 21-to-28-day dosing period (Fig. 2; Table 1) regardless of their state of differentiation, level of Trk expression (as determined by immunohistochemistry), or *K-RAS* and/or *p53* mutation status (Table 1). The exception to these observations was the CFPAC xenografts, which did not respond to CEP-701 treatment in two independent experiments (Table 1). The most pronounced response to CEP-701 administration was observed in the Panc1 and AsPc1 xenografts, which showed significant decreases ($P < 0.01$) in tumor growth volumes at days 14 and 18, respectively, and throughout the remainder of the study (Fig. 2). The remaining tumor xenografts that demonstrated a significant response to CEP-701 (Colo 357, BxPc3, and MiaPaCa2) showed decreases in tumor volume between days 5 and 14, with a statistically significant reduction in tumor volume ($P < 0.05$) of 60, 59, and 50%, respectively, by day 21 (Fig. 2). All of the vehicle-treated tumors grew throughout the dosing period (Fig. 2), which confirmed that inhibitory effects seen in these xenograft models were specific to CEP-701 treatment.

The Effects of CEP-701 on Tracheal Xenografts in Nude Mice. The rat tracheal s.c. xenograft model (20, 21) was used to further assess the inhibitory effects of CEP-701 on both the growth and *in vivo* invasiveness of human PDAC-derived cell lines. Tracheal s.c. xenografts require less cells, have a higher take rate, and a shorter latency than direct s.c. xenografts. Furthermore, it is possible to evaluate histologically the *in vivo* invasiveness of the PDAC cells through the tracheal wall (see "Materials and Methods"; Refs. 20 and 21).

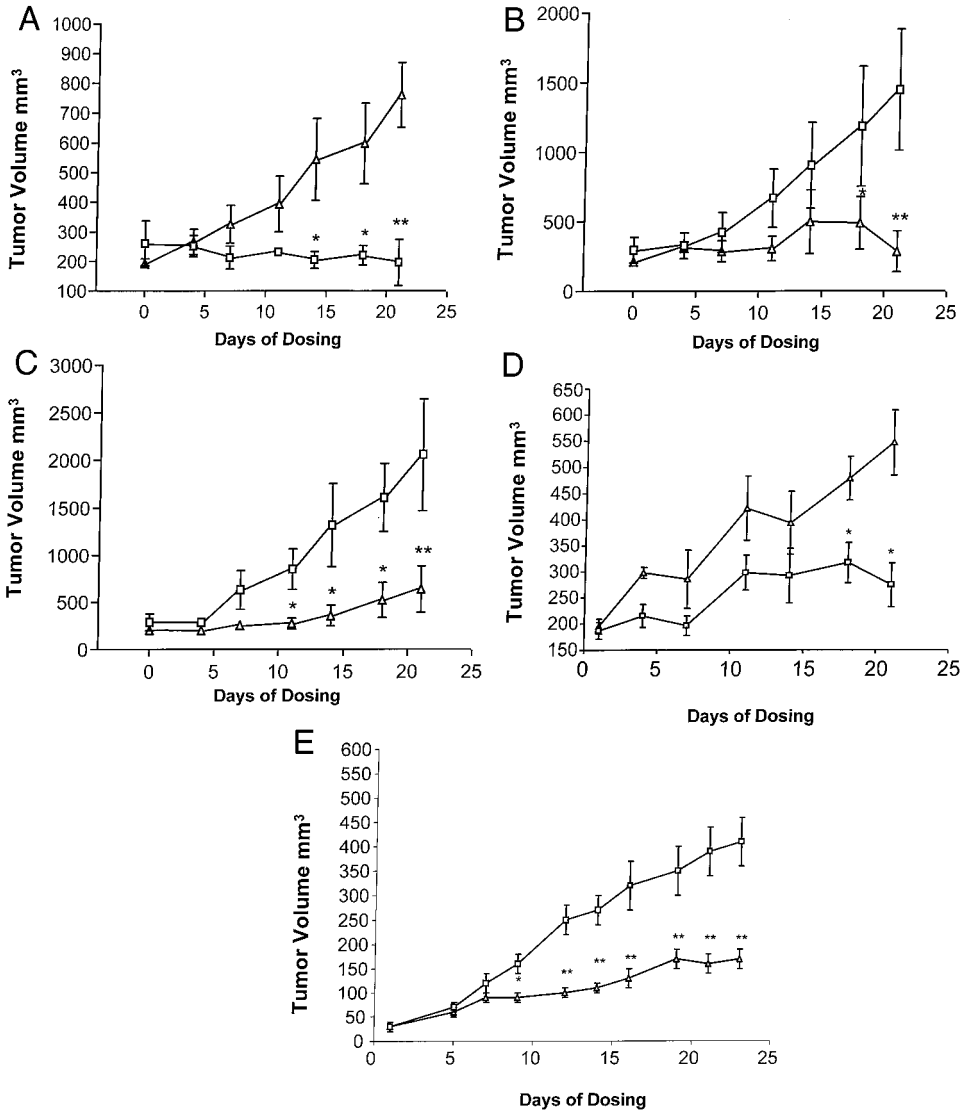

Fig. 2 The effects of CEP-701 on s.c. xenografts in athymic nude mice. Athymic nude mice were injected with 1×10^6 cells, and dosing with CEP-701 [at 10 mg/kg body weight s.c. b.i.d., 5 days a week for 21–28 days (□)] or with vehicle [s.c. b.i.d., 5 days a week (△)] began when the tumors reached between 150 and 200 mm^3 in size. Values shown are mean ± SE for tumor volumes (mm^3); n = 10 mice/group unless noted otherwise. Panc1 (*A*) and AsPc1 (*B*) s.c. xenografts showed the most significant responses to CEP-701 treatment as compared with vehicle-treated controls. The remaining xenografts—BxPc3 (*C*); MiaPaCa2 (n = 6 mice/group; *D*); and Colo 357 (*E*)—also showed significant responses to CEP-701 treatment as compared with vehicle-treated controls. *, $P < 0.05$; **, $P < 0.01$ by Student-Newman-Keuls analysis (for parametrically distributed data) or Mann-Whitney rank-sum test (for nonparametrically distributed data).

Tracheas inoculated with 5×10^5 cells were implanted s.c. in the backs of athymic nude mice. One week after implantation, treatment began using the dosing schedule detailed above. At the end of dosing, the tracheas were removed, fixed in 10% formalin, stained with H&E, and evaluated histologically for *in vivo* invasiveness through the tracheal wall (20, 21). Histological evaluation of the levels of invasiveness through the tracheal wall for vehicle-and CEP-701-treated xenografts are summarized in Table 1. CEP-701 treatment had the most pronounced effects on the invasiveness of BxPc3 tracheal xenografts, with an invasive level of 0, whereas the corresponding BxPc3 vehicle-treated tracheal xenografts had an invasive level of 3 (Fig. 3; Table 1). Panc1 and AsPc1 tracheal xenografts had invasive levels for CEP-701 and vehicle-treated xenografts of 1 and 3, respectively (Fig. 3; Table 1). Moreover, tumor growth of Panc1 (Fig. 4) and AsPc1 s.c. tracheal xenografts (data not shown) were significantly inhibited in CEP-701-treated mice ($P < 0.05$) relative to vehicle-treated controls in agreement with our direct s.c. xenograft data. Modest effects on tumor invasiveness were observed in MiaPaCa2 xenografts, with an invasive level of 1 for CEP-701-treated tracheal xenografts and a level of 2 for vehicle-treated xenografts (Fig. 3; Table 1). The CFPAC s.c. tracheal xenografts did not show a reduction in growth or invasiveness in response to treatment with CEP 701 (Fig. 4), which corroborated the absence of antitumor efficacy observed on direct s.c. xenograft growth. These data suggest that in this model, CEP-701 treatment influences both the growth and invasive phenotype of select human PDAC-derived xenografts.

Body weights of CEP-701-treated animals bearing both direct s.c. and tracheal s.c. tumor xenografts were similar to those of the vehicle-treated controls throughout the experiment, and no acute signs of CEP-701-associated morbidity (lethargy, abnormal behavior) or mortality were observed. In the tumor xenograft studies presented in Fig. 2 and 4, the mean +/− SE

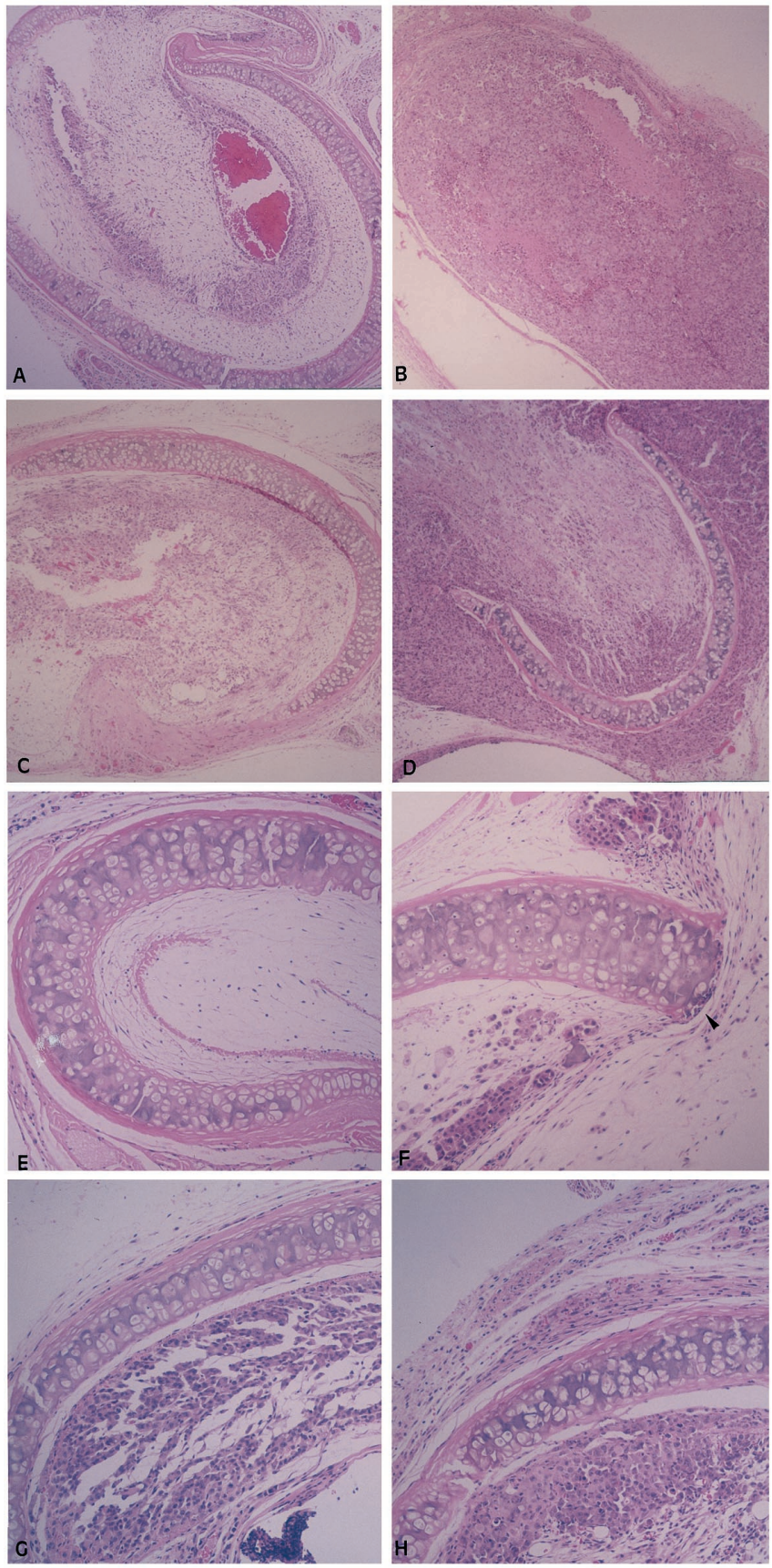

Fig. 3 Histological sections of CEP-701 and vehicle-treated tracheal xenografts in athymic nude mice. Tracheas inoculated with 5×10^5 cells were implanted s.c. in the backs of athymic nude mice. One week after implantation, dosing began with CEP-701 (10 mg/kg body weight s.c. b.i.d., 5 days a week for 21–28 days) or vehicle (s.c. b.i.d., 5 days a week for 21–28 days). Panc1 xenografts from CEP 701-treated mice (*A*) were confined within the trachea to the mucosal and submucosal tissues (level 1), whereas the corresponding vehicle-treated xenografts (*B*) totally obscured the lumen and invaded the pars membranacea of the trachea and tracheal wall (level 3). AsPc1 tracheal xenografts from CEP 701-treated mice (*C*) were confined within the trachea to the mucosal and submucosal tissues (level 1); the vehicle-treated AsPc1 xenografts (*D*) are invading through the tracheal wall (level 3). CEP-701-treated BxPc3 xenografts (*E*) are confined in the tracheal lumen lining the lumenal surface (level 0), whereas vehicle- treated BxPc3 xenografts (*F*) are invading through the entire tracheal wall (level 3); *arrows*, sites of tumor cell penetration and or invasion through the tracheal wall. Weaker effects on invasiveness were observed in the CEP-701-treated MiaPaCa2 xenografts (*G*). Cells were able to partially occlude the tracheal lumen (level 1) with cells of the vehicle-treated xenografts (*H*) invading through the pars membranacea (level 2).

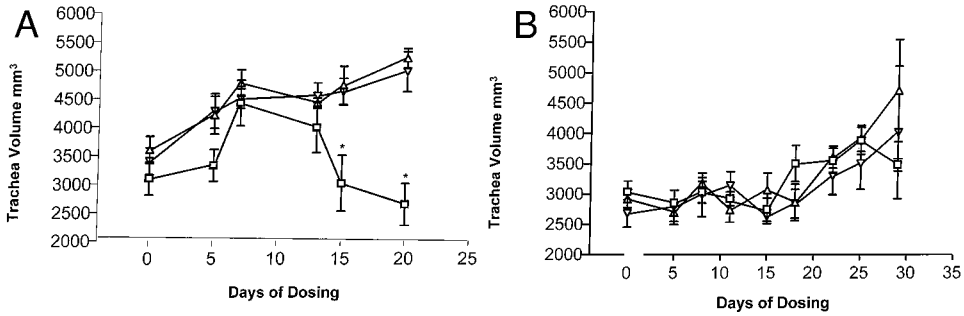

Fig. 4 The effects of CEP-701 on tracheal xenografts volumes in athymic nude mice. Tracheas inoculated with 5×10^5 cells were implanted s.c. in the backs of athymic nude mice. One week after implantation, dosing began with CEP-701 at 10 mg/kg body weight s.c. b.i.d. 5 days a week for 21–28 days (□), or vehicle s.c. b.i.d. 5 days a week for 21–28 days (△). Tumor growth in untreated xenografts is also illustrated (▽). Values shown are mean ± SE for tumor volumes (mm^3); $n = 10$ mice/treatment group. Panc1 xenografts (*A*) showed a significant decrease in trachea xenograft volume throughout the dosing period, whereas the CFPAC xenografts (*B*) did not respond to treatment. *, $P < 0.05$; **, $P < 0.01$ by Student-Newman-Keuls analysis (for parametrically distributed data) or Mann-Whitney rank-sum test (for nonparametrically distributed data).

body weights (g) of vehicle-treated mice after 21 days of dosing were 23.9 +/− 1.8 g ($n = 60$). For the various PDAC xenografts examined, the mean +/− SE of body weights of CEP-701-treated mice after 21 days of dosing were: 23.5 +/− 0.52 g (Panc-1); 24.0 +/− 1.1 g (AsPc-1); 23.5 +/− 0.63 g (BxPc-3); 24.0 +/− 0.68 g (MiaPaCa2); 26.1 +/− 0.59 g (Colo357); and 22.9 +/− 0.74 g (CFPAC). The data indicate that CEP-701 administration had no pronounced effects on the growth of the athymic mice used in these studies. Furthermore, the absence of gross toxicity (adverse events) in the animals given CEP-701 indicates that the compound is well tolerated at doses efficacious at inhibiting PDAC tumor growth.

DISCUSSION

The available treatment options for patients with PDAC have little or no effect on patient survival and are mainly used for symptom palliation (1, 5). In addition, many of the treatments often have adverse side effects such as nausea, vomiting, and alopecia (2). To date, surgery remains the only curative treatment; however, only 5–15% of PDAC patients are surgical candidates. In addition, postoperative complications such as sepsis and hemorrhaging reduces the surgical survival rate (23). In the past 35 years, only one new drug (gemcitabine) has been approved for use in pancreatic cancer treatment (1, 4). Although this drug shows some promising results, the 5-year survival rate still remains low (1, 4).

CEP-701, and its parent compound, CEP-751 (Fig. 1) are members of a family of synthetic derivatives of the indolocabozole K252a. These drugs inhibit the kinase activity of the Trk family of tyrosine kinase receptors by competing for the ATP-binding site of the kinase domain (16, 17). In addition to its inhibition of the Trk tyrosine kinases, CEP-701 is a selective inhibitor of the VEGF receptor kinase VEGF-R2/flk-1 (IC_{50} = 65 nM) and, to a lesser extent, the PDGF receptor β kinase (IC_{50} = 216 nM) activities (16, 18).

Experiments using CEP-751, the parent compound of CEP-701, show that CEP-751 was able to exert antitumor effects against human and rat androgen-dependent and androgen-independent prostatic carcinomas in athymic mice and syngeneic rats, respectively, as well as *in vivo* models of human neuroblastoma and medulloblastoma in athymic mice (18). Similarly, in animal models of human and rat prostatic carcinoma, CEP-701 administration inhibited significantly the growth of androgen-dependent and -independent tumors in athymic nude mice and caused tumor regression in the androgen-dependent Dunning H model in Copenhagen rats. Furthermore, potentiation of antitumor efficacy in the rat Dunning H prostate model was observed when CEP-701 administration was combined with surgical or chemical (leuprolide) androgen ablation compared with either monotherapy alone (24). Currently, the lysinyl-β-alanyl ester of CEP-751 is in Phase I clinical trails as an i.v. administered agent, and CEP-701 is in Phase I trials as an orally administered agent.

In this study, we report the first evidence for the significant antitumor efficacy of CEP-701 administration in six preclinical models of PDAC using two independent xenograft approaches in athymic nude mice. Treatment of human PDAC-derived direct s.c. xenografts in athymic nude mice with 10 mg/kg s.c. b.i.d. 5 days a week for 21–28 days demonstrated that five of the six xenografts exhibited statistically significant reductions in tumor growth as compared with vehicle-treated controls, with maximum reductions of 50–70% observed. In addition, no CEP-701-associated effects on morbidity or mortality were noted, which indicates that CEP-701 was well tolerated in these animals.

Previous studies (25) have demonstrated that low-molecular-weight protein tyrosine kinase inhibitors (tyrphostins) inhibited reversibly the *in vitro* mitogenesis and growth of selective PDAC-derived cell lines. The lack of selectivity of these agents and their associated toxicity *in vivo* has largely precluded their use therapeutically.

In comparison with these and other drugs in similar preclinical models of PDAC, CEP-701 had comparable or more pronounced antitumor efficacy in the absence of toxicity. Previous studies (26) examining the efficacy of adriamycin, cisplatin, and 5-fluorouracil treatment on trocar-implanted MiaPaCa2 and Panc1 cell lines in CD1 nu/nu mice demonstrated no effect on tumor growth. Conversely, gemcitabine produced a 69 and 76% inhibition on MiaPaCa2 and Panc1 tumor growth, respectively (26). In another study examining the

efficacy of a novel drug delivery system, BxPc3 xenografts showed a 72–79% inhibitory response when treated with 5-fluorouracil, cisplatin, or doxorubicin (27). The preclinical data reported in these studies suggest by comparison that CEP-701 may be of clinical value for the treatment of PDAC, used either alone or in combination with other available chemotherapeutic agents. Preliminary studies in our laboratory have assessed the efficacy of CEP-701 administration to that of gemcitabine, alone and in combination, on the growth of Colo357 xenografts. In these studies, gemcitabine (2.5 mg/kg i.p. every day for 5 days a week) had an antitumor efficacy that was virtually identical to that of CEP-701 administration at 10 mg/kg s.c. b.i.d. for 5 days a week. Throughout the 65-day dosing period, the combination of CEP-701 and gemcitabine treatment had comparable or greater antitumor efficacy in the absence of overlapping toxicities (data not shown). These studies and additional CEP-701/gemcitabine combination trials in AsPc-1 and BxPc-3 xenografts are currently in progress.

The mechanism(s) by which CEP-701 and CEP-751 exert their antitumor effects have yet to be determined. CEP-751 has been demonstrated to selectively induce cell death of prostatic carcinoma cells *versus* normal prostatic epithelial cells *in vivo*, independent of effects on the cell cycle (18). Inhibition of select ovarian carcinoma (OVCAR-3) and melanoma (SK-Mel-5) s.c. tumor xenograft growth has likewise been demonstrated in CEP-751-treated animals (18). Similarly, CEP-701 has been shown to induce apoptosis of neuroblastoma and medulloblastoma cells *in vivo*.[5] It remains to be established in additional *in vivo* studies whether CEP-701 administration is inducing apoptosis in pancreatic ductal carcinoma cells but not in normal pancreatic ductal epithelial cells in a cell-cycle independent fashion as observed for CEP-751 in prostatic carcinoma (Dunning H) models (18). In addition, it is possible that the antitumor efficacy observed in our studies on preclinical models of PDAC using CEP-701 are not solely due to the inhibition of Trk tyrosine kinase activity because of the fact that, along with the potent inhibition of Trk kinase activity (IC_{50} of <25 nM), CEP-701 possess inhibitory effects against the VEGF receptor flk1/KDR/VEGF-R2 kinase (IC_{50} of 65 nM), platelet derived growth factor (PDGF) receptor kinase (IC_{50} of 216 nM), and protein kinase C (IC_{50} of 226 nM; Ref. 17). Paracrine and/or autocrine influences of aberrantly expressed growth factors and their receptors play a key role in the development and progression of PDAC (11, 12, 14, 28, 29). For example, the expression of VEGF has been demonstrated to be up-regulated in PDAC specimens, with expression of the flk1/KDR/VEGF-R2 receptor localized to the endothelial cells of the vascular bed within the PDAC tumor stroma (30, 31). Similarly, the overexpression of the PDGF receptor in PDAC cells—concurrent with the increased expression of PDGF—results in an autocrine influence on PDAC development (12). Therefore, it remains to be determined whether the antitumor effects demonstrated by CEP-701 in human PDAC xenografts are due solely to the inhibition of Trk receptor tyrosine kinase activity or whether they are the result of a multimodal mechanism inhibiting other receptor tyrosine kinase targets (VEGF-R2, PDGF receptor β) implicated in the growth and progression of PDAC.

In conclusion, we have demonstrated that the novel Trk tyrosine kinase inhibitor CEP-701 significantly inhibited the *in vivo* growth and/or invasiveness of five of six human PDAC-derived xenografts in two independent xenograft model systems. Taken together, these results provide preliminary evidence to support additional studies investigating the effects of CEP-701 in additional models of human PDAC either alone or in combination with other chemotherapeutic treatment regimens, *e.g.*, gemcitabine and 5-fluorouracil. In addition, preclinical studies evaluating the antitumor efficacy of CEP-701 in orthotopic models of PDAC are planned to assess the effects of CEP-701 on local dissemination and metastatic spread of PDAC in these models.

[5] A. E. Evans, D. J. Yamashiro, K. D. Kisselbach, N. Ikegaki, A. M. Camoratto, C. A. Dionne, and G. M. Brodeur. The antitumor activity of CEP-751 on human neuroblastoma and medulloblastoma cells *in vitro* and *in vivo*, submitted for publication.

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Sheila J. Miknyoczki, Hong Chang, Andres Klein-Szanto, et al.

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