Antitumor Activity of CEP-751 (KT-6587) on Human Neuroblastoma and Medulloblastoma Xenografts

Audrey E. Evans, Kristin D. Kisselbach, Darrell J. Yamashiro, Naohiko Ikegaki, Anna Marie Camoratto, Craig A. Dionne, and Garrett M. Brodeur


INTRODUCTION

Neuroblastoma (NBL) and medulloblastoma (MBL) are tumors of the neuroectoderm that occur in children. NBL and MBL express Trk family tyrosine kinase receptors, which regulate growth, differentiation, and cell death. CEP-751 (KT-6587), an indolocarbazole derivative, is an inhibitor of Trk family tyrosine kinases at nanomolar concentrations. This study was designed to determine the effect of CEP-751 on the growth of NBL and MBL cell lines as xenografts. In vivo studies were conducted on four NBL cell lines (IMR-5, CHP-134, NBL-S, and SY5Y) and three MBL cell lines (D283, D341, and DAOY) using two treatment schedules: (a) treatment was started after the tumors were measurable (therapeutic study); or (b) 4–6 days after inoculation, before tumors were palpable (prevention study). CEP-751 was given at 21 mg/kg/dose administered twice a day, 7 days a week; the carrier vehicle was used as a control. In therapeutic studies, a significant difference in tumor size was seen between treated and control animals with IMR-5 on day 8 ($p = 0.01$), NBL-S on day 17 ($p = 0.016$), and CHP-134 on day 15 ($p = 0.034$). CEP-751 also had a significant growth-inhibitory effect on the MBL line D283 (on day 39, $p = 0.031$). Inhibition of tumor growth of D341 did not reach statistical significance, and no inhibition was apparent with DAOY. In prevention studies, CEP-751 showed a modest growth-inhibitory effect on IMR5 ($p = 0.062$) and CHP-134 ($p = 0.049$). Furthermore, inhibition of growth was greater in the SY5Y cell line transfected with TrkB compared with the untransfected parent cell line expressing no detectable TrkB. Terminal deoxynucleotidyl transferase-mediated nick end labeling studies showed CEP-751 induced apoptosis in the treated CHP-134 tumors, whereas no evidence of apoptosis was seen in the control tumors. Finally, there was no apparent toxicity identified in any of the treated mice. These results suggest that CEP-751 may be a useful therapeutic agent for NBL or MBL.

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3 The abbreviations used are: NBL, neuroblastoma; MBL, medulloblas-
toma; CHOP, Children’s Hospital of Philadelphia; b.i.d., twice a day; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; DAPI, 4,6-diamidino-2-phenylindole.
determine the effect of CEP-751 on human NBL and MBL cell lines growing as xenografts in nude (nu/nu) mice.

MATERIALS AND METHODS

Drug. CEP-751 (KT-6587), a derivative of K252a (8), was synthesized in the laboratories of Kyowa Hakko Kogyo (Tokyo, Japan) and was provided for these studies by Cephalon, Inc. CEP-751 was solubilized for in vivo experimentation, as described (10). CEP-751 was dissolved in a vehicle consisting of 40% polyethylene glycol 100 (Spectrum, Los Angeles, CA), 10% povidone C30 (ISP, Bound Brook, NJ), and 2% benzoyl alcohol (Spectrum) in distilled water. Initial in vivo studies demonstrated that CEP-751 could be given s.c. in nude mice two times a day at a dose of up to 48 mg/kg/day for at least 4 weeks, with no apparent morbidity or mortality (11).

Cell Lines. The NBL lines were available from the CHOP cell bank, and the MBL lines D283-Med and D341-Med were obtained from Drs. Henry Friedman (Duke) and Peter Phillips (CHOP). The DAOY MBL line was obtained from the American Type Culture Collection. The cell lines were grown in...
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Table 1  In vivo studies of NBL

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Day of analysis</th>
<th>Tumor size (cm³)</th>
<th>Analysis of slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Compound</td>
<td>Control</td>
</tr>
<tr>
<td>A. Neuroblastoma therapeutic studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMR-5</td>
<td>12</td>
<td>1.96 ± 0.35 (n = 5)</td>
<td>3.91 ± 1.22 (n = 5)</td>
</tr>
<tr>
<td>CHP 134</td>
<td>15</td>
<td>1.9 ± 0.75 (n = 4)</td>
<td>4.5 ± 0.98 (n = 3)</td>
</tr>
<tr>
<td>NBL-S</td>
<td>17</td>
<td>0.94 ± 0.24 (n = 5)</td>
<td>5.2 ± 2.46 (n = 4)</td>
</tr>
<tr>
<td>B. Neuroblastoma prevention studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMR-5</td>
<td>27</td>
<td>1.3 ± 1.63 (n = 7)</td>
<td>2.7 ± 1.55 (n = 6)</td>
</tr>
<tr>
<td>CHP 134 (combined)</td>
<td></td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>CHP 134 (early)</td>
<td>29</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>CHP 134 (late)</td>
<td>58</td>
<td>See text</td>
<td></td>
</tr>
</tbody>
</table>

* Figures given for the average daily growth rate.
* Treatment started after tumors measured >0.24 g.
* Treatment commenced prior to tumor appearance.

RPMI 1640 containing 10% fetal bovine serum maintained in 75-cm² Costar flasks in a humidified atmosphere of 5% CO₂ and air. For in vivo studies, cells were grown in 150-cm² culture flasks to ~75% confluence, split to insure they were growing in log phase, and harvested in 24 h. Cells were harvested using 0.2% tetrasodium EDTA in PBS.

**Animals.** Four-week-old female athymic NCR (nu/nu) mice were obtained from the National Cancer Institute (Frederick, Maryland). The Institutional Animal Care and Use Committee of the Joseph Stokes, Jr. Research Institute at CHOP approved all animal studies. Mice were maintained at four per cage under humidity- and temperature-controlled conditions and a light/dark cycle that was set at 12-h intervals. All animals were fed autoclaved Purina mouse chow and water ad libitum.

**In Vivo Antitumor Experiments.** Tumors were measured in unaesthetized animals two times a week using a Vernier caliper. Tumors were measured in three dimensions d₁ × d₂ × d₃, and calculations of tumor volume were made by multiplying the product of three dimensions by π/6. Body weights were obtained, and the dose of compound was modified as the weight of the animal changed. For NBL cell lines, 2.5 × 10⁶ cells/animal were injected subcutaneously in RPMI 1640 with equal volume of Matrigel (Becton Dickinson; Refs. 12 and 13). In the MBL lines D341 and D283, 1 × 10⁷ cells were injected in a similar fashion. The success rate of tumor development after injection with tumor cells was generally from 75 to 100%. However, DAOY cells required passage as the xenograft through mice before their growth was sufficiently rapid to be used in the in vivo study. Tumor tissue was minced, homogenized in a tissue homogenizer, and then transferred to 10-ml syringes and forced through hypodermic needles of decreasing size down to 22 gauge, prior to injection. Subsequently, the time required for tumors to appear decreased from 80 to 24 days.

Two study approaches were used: (a) the treatment was started after the s.c. tumor was palpable at ~0.2 cm³ (therapeutic study). Animals were assigned to treatment or control groups by matching tumor sizes in pairs, usually when at least six animals had measurable tumors; and (b) the treatment was started 4–6 days after the implantation, before tumors were palpable (prevention study). The day of initiating treatment varied with the growth rate of the cell line; D341 had the shortest time to tumor appearance (7 days), and treatment was started day 4 from inoculation, whereas CHP-134 tumors appeared in 15–17 days, therefore treatment was started day 6. Treatment with CEP-751 or vehicle control was administered b.i.d., 7 days/week.

**Trk Studies.** The level of Trk expression in the cell lines used was measured by a semiquantitative reverse transcription-PCR method. All NBL and MBL cell lines (except DAOY) expressed low levels of TrkA and/or TrkB. To assess the importance of Trk expression for responsiveness to CEP-751, the SY5Y cell line was transfected with TrkB (14) in a constitutive expression vector (15).

**Apoptosis Detection by the TUNEL Method.** The apoptosis detection system measures the fragmented DNA of apoptotic cells by catalytically incorporating fluorescein-12 dUTP at the 3'-OH DNA ends using the enzyme terminal deoxynucleotidyl transferase (TUNEL assay; Ref. 16). Using tumor touch preparations, the cells harvested from tumors on days 2, 4, and 6 after treatment were fixed with formaldehyde (4% in PBS), permeabilized with Triton X-100, and incubated with equilibration buffer, nucleotide mix, and terminal deoxynucleotidyl transferase enzyme. After incubation, DAPI was added to stain the cells. Slides were analyzed at 460 nm to see the blue DAPI-stained cells and at 350 nm to visualize the green apoptotic cells. The proportion of the

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4 N. Ikegaki et al., unpublished observations.
green cells in the blue background was calculated, and the results were compared between treated and control tumors.

**Statistical Analysis.** The exact Wilcoxon-Mann-Whitney test of significance (17) was used to compare the tumor size at a particular test day between CEP-751 treated and control groups.5 Also, each subject’s individual slope was calculated assuming that tumor size is a linear function of time, and the slopes in the two groups (CEP-751 versus vehicle) were compared using Student’s t test (18).

**RESULTS**

**NBL Therapeutic Studies.** After injection with $2.5 \times 10^7$ IMR-5 cells/animal, five mice were treated with CEP-751 at 9.3 mg/kg b.i.d., and five control animals were treated with vehicle alone. Tumors appeared between 9 and 18 days from inoculation and ranged from 0.43 to 0.8 cm$^3$ (median, 0.47 cm$^3$) in control animals and 0.36 to 0.55 cm$^3$ (median, 0.47 cm$^3$) in animals treated with CEP-751. By day 8 from the start of treatment, tumors in the treated animals averaged 1.21 cm$^3 \pm 0.43$, which was significantly smaller than the tumors in control animals (2.60 cm$^3 \pm 0.53$; $P = 0.008$; Fig. 1A). The analysis of slope (average daily tumor growth rate) for the treated animals was $0.17 \text{cm}^3/\text{day} \pm 0.051$ compared with the control rate of $0.29 \text{cm}^3/\text{day} \pm 0.11$ ($P = 0.032$). Similar differences were also apparent at day 12 (Table 1; Fig. 1A).

Twelve animals injected with CHP-134 developed tumors within 24–34 days. The control tumors ranged in size from 0.29 to 1.3 cm$^3$ (median, 0.64 cm$^3$), and the treated tumors ranged in size from 0.35 to 0.78 cm$^3$ (median, 0.41 cm$^3$) when treatment was started with 21 mg/kg b.i.d. (this dose was used for all subsequent studies). Six animals were treated with CEP-751 and six with vehicle. However, two animals in each group died of accidental death early in the study, and one animal in the control group had to be sacrificed on day 9 because of rapid tumor growth. Thus, for the analysis of tumor size on day 15, there were four treated animals and three controls remaining. The treated tumors were significantly smaller than tumors in control animals (1.9 cm$^3$ versus 4.5 cm$^3$; $P = 0.034$; Table 1; Fig. 1B). The analysis of slope was applied to the animals that died early, because there was more than one tumor measurement to document early growth rate. For the CEP-751-treated animals, the slope was $0.151 \pm 0.055 \text{cm}^3/\text{day}$ versus $0.299 \pm 0.068$ in controls, but this difference did not reach significance ($P = 0.12$).

Twenty animals were injected with NBL-S, and by day 13, 15 of 20 had developed small tumors that grew slowly. Treatment was started on day 38 after injection, when most of the animals had measurable tumors. Two animals with internal tumors were not included. Seven were assigned to the treatment group (average tumor size, 0.23 cm$^3$; range, 0.19–0.34 cm$^3$), and six were assigned to the control group (average, 0.31 cm$^3$; range, 0.23–0.37). One animal in each arm died of accidental death. The remainder were analyzed at day 17 of treatment. The treated tumors were significantly smaller than controls (0.94 cm$^3$ versus 0.79 cm$^3$), but this difference did not reach statistical significance ($P = 0.041$). The analysis of slope (average daily tumor growth rate) for the treated animals was $0.072 \text{cm}^3/\text{day} \pm 0.069$ compared with $0.186 \text{cm}^3/\text{day} \pm 0.039$ in controls ($P = 0.016$).

**NBL Prevention Studies.** Fourteen animals were injected with $2.5 \times 10^7$ IMR-5 cells/animal. One control animal died of accidental death early in the study. Treatment with 21 mg/kg b.i.d. was started on day 5 from injection of IMR-5 cells, before the tumors appeared. In six control animals, the tumors appeared by day 13, and in the seven treated animals, the tumors appeared by day 17. However, the overall delay in the median growth of tumors was not significant ($P = 0.27$). On day 27, the average tumor size in seven treated animals was smaller at 1.3 cm$^3$ versus 2.7 cm$^3$ in the six-control group (Table 1; Fig. 1D), but this difference did not reach statistical significance ($P = 0.062$). Also, the analysis of slope showed a clear trend ($0.118 \pm 0.036$ treated versus $0.188 \pm 0.041$ control), but this also did not reach significance ($P = 0.073$).

Animals were injected with $2.5 \times 10^7$ CHP-134 cells, and treatment (21 mg/kg b.i.d.) was started on day 6 prior to the appearance of tumor. A total of 12 tumors developed (6 in each group). In the CEP-751-treated animals, they appeared between 2.14 and 3.4 days (median, 2.7 days) after treatment started with 21 mg/kg b.i.d. (this dose was used for all subsequent studies). Six animals were treated with CEP-751 and six with vehicle. However, two animals in each group died of accidental death early in the study, and one animal in the control group had to be sacrificed on day 9 because of rapid tumor growth. Thus, for the analysis of tumor size on day 15, there

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**Table 2** In vivo studies of MBL

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Day of analysis</th>
<th>Tumor size (cm$^3$)</th>
<th>Analysis of slope$^a$</th>
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<tbody>
<tr>
<td>A. Medulloblastoma therapeutic studies$^b$</td>
<td></td>
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<td></td>
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<tr>
<td>D341</td>
<td>15</td>
<td>$1.98 \pm 1.86 (n = 5)$</td>
<td>$2.24 \pm 0.790 (n = 5)$</td>
</tr>
<tr>
<td>DAOY</td>
<td>16</td>
<td>$1.51 \pm 0.689 (n = 5)$</td>
<td>$1.97 \pm 0.603 (n = 3)$</td>
</tr>
<tr>
<td>D283</td>
<td>39</td>
<td>$1.044 \pm 0.297 (n = 8)$</td>
<td>$1.54 \pm 0.51 (n = 8)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P = 0.39$</td>
<td>$P = 0.031$</td>
</tr>
<tr>
<td>B. Medulloblastoma prevention study$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D341</td>
<td>16</td>
<td>$0.91 \pm 0.92 (n = 5)$</td>
<td>$2.17 \pm 1.42 (n = 6)$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P = 0.10$</td>
<td>$P = 0.126$</td>
</tr>
</tbody>
</table>

$^a$ Figures given for the average daily growth rate.

$^b$ Treatment started after tumors measured $>0.24$ g.

$^c$ Treatment commenced prior to tumor appearance.
days 15 and 46 (median, day 29) and in the control group between days 9 and 25 (median, day 15). However, because the treated tumors grew more slowly, it was not possible to compare tumor size in treated versus control on a specific day of treatment. Furthermore, tumors developed at two substantially different rates; six developed early (days 9–21), and six developed later (days 22–46). By analyzing the slopes of the total group, the daily growth of treated tumors was $0.06 \pm 0.05$ versus $0.122 \pm 0.02$ in the controls ($P = 0.058$). Little effect of treatment was seen on the tumor growth slopes in the early group ($0.148$ versus $0.169$ cm$^3$/day; $P = 0.28$), but in the later group, treated tumors grew at a significantly slower rate ($0.022$ versus $0.140$ cm$^3$/day; $P = 0.049$; Table 1; Fig. 1, E and F).

**MBL Therapeutic Studies.** Sixteen animals were injected with D341 MBL cells, and 12 developed tumors by day 10. These animals started treatment on day 14; the six tumors in the control group ranged from 0.03 to 0.29 cm$^3$ (median, 0.14 cm$^3$), and the treated group ranged from 0.07 to 0.31 cm$^3$ (median, 0.15 cm$^3$). Two tumors developed internally (one in each group) and grew very rapidly at parallel rates, reaching 6.8 cm$^3$ in 10 days of treatment. Excluding these two, on day 15 the average tumor size in the five treated animals was 1.98 cm$^3$, whereas the average in the five control animals was 2.24 cm$^3$ (Table 2 and Fig. 2A), but this difference was not statistically significant ($P = 0.60$). The analysis of slope ($0.199$ versus $0.238$ cm$^3$/day) also did not show a significant difference ($P = 0.75$).

Fourteen mice were injected with 0.3 cm$^3$ of DAOY minced tumor tissue. The first tumor started to grow by day 3, and nine tumors were measurable by day 13. Treatment was started on day 16 from injection, by which time control tumors ranged from 0.51 to 1 cm$^3$ (median, 0.62 cm$^3$) and treated tumors ranged from 0.61 to 1 cm$^3$ (median, 0.72 cm$^3$). By day 16 of treatment, tumors in the treated animals measured 1.51 cm$^3$ versus 1.97 cm$^3$ in the controls, which was not significant ($P = 0.039$; Fig. 2B), and analysis of slope (0.15 versus 0.17 cm$^3$/day) was not significant ($P = 0.67$; Table 2).

Sixteen animals were inoculated with D283, and all grew tumors; 8 control tumors size ranged from 0.3 to 0.5 cm$^3$ (median, 0.35 cm$^3$), and 8 treated tumors measured 0.3–0.45 cm$^3$ (median, 0.34 cm$^3$). Tumors appeared by day 11, and treatment was started 6 days later. The growth rate of the control tumors was slow, and by day 39, the largest tumor measured 2.4 cm$^3$ (range, 1.0–2.4 cm$^3$). The difference between the treated versus control tumors (1.04 and 1.54 cm$^3$) was significant ($P = 0.031$; Table 2 and Fig. 2B), and the analysis of slope (0.017 versus 0.031 cm$^3$/day) was also significant ($P = 0.021$).

**MBL Prevention Studies.** Eighteen animals were injected with $1 \times 10^7$ D341 cells/animal, and tumors appeared between day 3 and 13. Treatment was started on day 4 from injection. On day 16, there was a difference between the average tumor size in the treated versus control animals (0.9 cm$^3$ and 2.17 cm$^3$, respectively) with marginal significance ($P = 0.10$).
The analysis of slope (0.092 versus 0.186 cm³/day) did not reach statistical significance (P = 0.126; Table 2; Fig. 2C).

**Mechanism of Antitumor Activity.** SY5Y, an NBL line that expresses low levels of TrkB, was transfected with TrkB. Two of the resulting clones, G8 and G12 expressing low and high levels of TrkB, respectively, were inoculated into nude mice (15). Interestingly, the SY5Y-G12 clone expressing the highest level of TrkB had the fastest growth rate in untreated or vehicle-treated tumors, and the effect of CEP-751 was the greatest. The daily growth rates of SY5Y, SY5Y-G8, and SY5Y-G12 were 0.16, 0.20, and 0.21 cm³/day, and the significant difference between treated and control tumor size was 0.4, 0.1, and 0.01 cm³, respectively (Fig. 3).

The effect of treatment on the tumors was studied by the TUNEL method to determine whether there was evidence of apoptosis (16). The CEP-751-treated tumors showed marked evidence of apoptosis during the course of treatment (Fig. 4). A rare cell was seen with fluorescent staining on day 2 (0–3% of cells per high power field) and increasing numbers on days 4 (42%) and 6 (92%). No evidence of apoptosis was seen in the control group. The tumors prepared for TUNEL staining were also studied by light microscopy. In the treated tumors, there was no evidence of necrosis or maturation of the tumor.

**Toxicity.** Mice were observed twice daily and weighed every 2 weeks; no toxicity was observed in terms of progressive weight loss, rashes, or unusual behavior. Unexplained acute deaths occurred usually during injection (presumably from cervical cord injury due to resistance to restraint), or from skin, lymph node, or other infection, but there were no differences noted between the treated and control animals.

**DISCUSSION**

NBLs can be classified in at least two or three different categories (19). A favorable subset has a peak incidence around 6 months of age and is uncommon after age two. These tumors are generally responsive to treatment. Moreover, they have a propensity to regress spontaneously in infants or to mature to a benign ganglioneuroma in older patients. The commitment to regress, as well as the propensity to differentiate, may both be mediated by the nerve growth factor/TrkA pathway (19). In contrast, the unfavorable MBLs have a peak age of 2–4 years. These tumors are generally metastatic at the time of diagnosis and have a very poor outcome. Interestingly, these tumors frequently express both TrkB and its ligand, brain-derived neurotrophic factor, which may represent an autocrine growth and survival pathway (21). Thus, agents that target the Trk family receptors may be useful therapeutically for patients with both favorable and unfavorable diagnoses.
CEP-751 is a relatively specific inhibitor of the Trk family of tyrosine kinases (TrkA, TrkB, and TrkC) at low concentrations. Indeed, this agent was shown to have antitumor activity both in vitro and in vivo in prostate cancer, ovarian cancer, and melanoma (10, 11, 22). Given the important roles that members of the Trk family play in NBLs, this tumor appeared to be a very attractive candidate for treatment with CEP-751.

We tested the effect of this compound on four NBL cell lines growing as xenografts in nude mice, either by treating palpable tumors or by trying to prevent tumor development after inoculation. In the therapeutic studies, treatment with CEP-751 resulted in a statistically significant decrease in tumor growth in three lines (IMR-5, CHP-134, and NBL-S) in animals. In both models, the drug had no apparent effect for the first 7 days; then...
a substantial difference in the slope of tumor growth was observed. In the prevention studies, there was a noticeable delay in the development of tumors in the treated animals versus the controls. In the CHP-134 animals, the delay in appearance of tumors was so pronounced that many of the control animals had died from their tumor burden before the treated animals developed palpable tumors.

To determine to what extent the level of Trk receptors played a role in the degree of response, SY5Y cells expressing very low levels of TrkB were transfected with TrkB. Cells from two of the resulting clones were grown in nude mice, which were then treated with CEP-751. The SY5Y-G12 clone with the highest expression of TrkB was the most sensitive to treatment; therefore, the effect of CEP-751 may be mediated at least in part through its inhibition of TrkB.

It has been suggested that Trk expression supports not only the growth and differentiation of neuronal cells but is also important for cell survival. If Trk receptors and/or their ligands are involved in a survival pathway for NBL cells, treatment with CEP-751 would be expected to result in increased cell death. To examine this hypothesis, tumors were harvested at several times posttreatment and were examined for evidence of apoptotic cells using the TUNEL assay. There was no apparent difference after 1 day of treatment; however, there were more apoptotic cells in the treated tumors after 4 and 6 days compared with the corresponding controls. These results suggest that CEP-751 may induce apoptosis in these tumors, presumably as a consequence of blocking the autophosphorylation and signaling of the Trk receptor(s) being expressed.

MBLs are neuroectodermal tumors of the central nervous system. Recent studies have suggested that these tumors also express members of the Trk family, and high expression of TrkC was correlated with improved survival (8, 9). Therefore, we tested the effect of CEP-751 on three MBL cell lines growing as xenografts in nude mice in vivo. The tumors treated with CEP-751 were generally smaller than those in the control group. Studies of D283, which grew more slowly, did show a therapeutic effect of the compound. The difference in D341 was not statistically significant, and a difference in DAOY was not seen.

In summary, CEP-751 reduced in vivo growth of NBL cells growing as xenografts using two different treatment paradigms. It also resulted in a decrease in growth of MBL xenografts, with borderline significance. Evidence of in vivo antitumor efficacy using established NBL cell lines suggests that this compound may be even more effective in primary tumors from untreated patients, which express higher levels of Trk family receptors than cell lines. Furthermore, the apparent greater efficacy in slower growing tumors and in retarding tumor appearance suggests that its therapeutic efficacy may be greater in states of minimal residual disease. Thus, CEP-751, or potential derivatives of this compound, appear promising as therapeutic agents for tumors such as NBLs and possibly MBLs, either alone or in combination with other agents.

ACKNOWLEDGMENTS

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REFERENCES


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