Vandetanib Inhibits Growth of Adenoid Cystic Carcinoma in an Orthotopic Nude Mouse Model

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Abstract

Purpose: Adenoid cystic carcinoma (ACC) can often be controlled with surgery and postoperative adjuvant radiotherapy but is also characterized by late local recurrence and distant metastasis. No effective systemic therapeutic agents have been found to alter the natural history of ACC. Therefore, new therapeutic approaches are needed. In this study, we evaluated whether vandetanib (Zactima), a potent inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) and epidermal growth factor receptor (EGFR) tyrosine kinases, had antitumor efficacy in vitro and in an orthotopic nude mouse model of human ACC.

Experimental Design: The in vitro effects of vandetanib were assessed in three ACC cell lines on cell growth, apoptosis, and VEGFR-2 and EGFR phosphorylation levels. The in vivo antitumor activity of vandetanib was examined in nude mice bearing parotid gland ACC tumors. The mice were treated for 4 weeks with vandetanib (50 mg/kg/d) or placebo (control). Tumors were resected at necropsy, and immunohistochemical and immunofluorescence staining were done.

Results: In vitro, vandetanib caused dose-dependent inhibition of VEGFR-2 and EGFR phosphorylation in ACC cells. Vandetanib also inhibited the cell proliferation and induced their dose-dependent apoptosis. In vivo, mice in the vandetanib group had tumor volumes significantly lower than those in the control group (P < 0.01). In addition, immunohistochemical staining showed a decrease in microvessel density and an increase in apoptosis of both tumor cells and endothelial cells within the tumor xenografts.

Conclusion: These results suggest that vandetanib inhibits the growth of ACC in vitro and in vivo, making it a promising novel agent for the treatment of ACC.
migration, survival, and capillary tube formation (9, 10). A recent study reported that VEGF is a major factor in angiogenesis and that microvessel density (MVD) is considered to be a prognostic factor for distant metastasis in ACC (11). In a previous study, we observed that VEGF/VEGFR-2 is overexpressed in human ACC tissues (12). Similarly, it has been reported in numerous studies that EGFR, a tyrosine kinase receptor, plays a critical role in tumor cell proliferation, invasion, metastasis, and survival (13, 14). In addition, EGFR is overexpressed in a variety of epithelial tumors (15). In ACC tissues, EGFR expression has been reported to range from 10% to 85% (13, 16).

The VEGF and EGFR signaling pathways seem to be closely related, particularly with respect to angiogenesis, in many tumors. The EGFR pathway increases angiogenesis by up-regulating VEGF or other key mediators in the angiogenic process (17). Activation of VEGF expression is considered to be one mechanism used by tumors to become resistant to anti-EGFR therapy (18). Moreover, preclinical studies have shown that EGFR blockade with monoclonal antibodies or small-molecule tyrosine kinase inhibitors results in down-regulation of proangiogenic mediators, including VEGF, interleukin-8, and basic fibroblast growth factor (19). Therefore, dual inhibition of the VEGF and EGFR signaling pathways theoretically can result in greater antitumor effects than inhibition of either one alone. Several biological agents that target both the VEGF and EGFR signaling pathways have shown clinical benefit in several human cancers when administered either alone or in combination with standard cytotoxic therapies (19).

Vandetanib (Zactima, AstraZeneca Pharmaceuticals) is a small-molecule inhibitor of the VEGFR-2 and EGFR tyrosine kinase activities (20). Vandetanib has shown significant antitumor activity in various xenograft models of human cancer (21). Based on these favorable preclinical results, clinical trials of vandetanib are currently under way in several different tumor types; however, no studies have investigated its antitumor effects in ACC.

In this study, we hypothesized that VEGF-2 signaling pathways might be important for the progression and metastasis of human salivary ACC and that inhibition of these pathways might be a beneficial part of an integrated treatment strategy for ACC. To test our hypothesis, we investigated the preclinical efficacy of vandetanib in an orthotopic nude mouse model of human ACC.

Materials and Methods

Cell lines and culture conditions. Human salivary ACC cell lines, ACC2, ACC3, and ACCM, were provided by the Ninth People’s Hospital (Department of Oral and Maxillofacial Surgery, Shanghai Jiao Tong University School of Medicine, Shanghai, People’s Republic of China; refs. 22, 23). The cells were maintained as monolayer cultures in RPMI 1640 containing penicillin-streptomycin (Flow Laboratories), nonessential amino acids, sodium pyruvate, L-glutamine, and 10% fetal bovine serum (FBS). The cells were incubated in a humidified atmosphere of 5% CO2 and 95% air at 37°C. The cultures were obtained from frozen stocks, and all experiments were done within 12 wk after thawing.

Reagents. Vandetanib was provided by AstraZeneca Pharmaceuticals. For our in vitro study, stock solutions of vandetanib were prepared in DMSO (Sigma-Aldrich Corp.) and diluted with culture medium. For our in vivo study, vandetanib was dissolved in 1% Tween 80 with PBS just before its administration to mice at a dose of 50 mg/kg once daily. Propidium iodide (PI) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were both purchased from Sigma-Aldrich. Cell stocks were prepared by dissolving 1 mg each of PI and MTT in 1 mL of PBS. The solution was then filtered to remove particles, protected from light, stored at 4°C, and used within 1 mo.

The primary antibodies for immunohistochemical analysis were purchased as follows: rabbit polyclonal anti-EGFR and anti-VEGFR-2 (Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-phospho-VEGFR-2 (Oncogene), rabbit polyclonal anti-phospho-EGFR (Bio-source International), mouse anti-proliferating cell nuclear antigen clone PC-10 (DAKO A/S), and rat monoclonal anti-CD31/platelet/endothelial cell adhesion molecule 1 (PECAM-1; Pharmingen). The secondary antibodies used were as follows: peroxidase-conjugated rat anti-mouse immunoglobulin G2a (Serotec; Harlan Bioproducts for Science, Inc.), peroxidase-conjugated goat anti-rabbit immunoglobulin G1 (Jackson Research Laboratories), and Alexa Fluor 594–conjugated goat anti-rat immunoglobulin G and Alexa Fluor 488–conjugated anti-rabbit immunoglobulin G (Molecular Probes).

Animals and orthotopic implantation of tumor cells. Eight- to 12-wk-old athymic nude male mice were purchased from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD). The mice were housed and maintained in laminar flow cabinets under specific pathogen-free conditions approved by The American Association for Accreditation of Laboratory Animal Care and in accordance with the current regulations and standards of the U.S. Department of Agriculture, the U.S. Department of Health and Human Services, and the NIH. The mice were used in accordance with the M. D. Anderson Cancer Center Institutional Animal Care and Use Committee guidelines.

To produce tumors, ACC3 and ACCM cells were harvested from confluent cultures and a total of 1 × 106 cells were reseeded in 30 μL of serum-free RPMI 1640. The skin overlying the parotid gland of mice was cleaned with ethanol, and a 0.5-cm incision was made in the right preauricular area to expose the parotid gland in mice. A suspension of 1 × 106 cells was injected into the parotid gland using a 30-gauge hypodermic needle and a tuberculin syringe. Visual detection of a well-localized bleb confirmed a successful injection without leakage of the tumor cells. The skin and s.c. tissues were closed in one layer using a single metal clip. Animals were monitored for tumor size using calipers and weight loss and were sacrificed if they lost >20% of their initial body weight or if they became moribund.

Western blotting. To determine whether VEGF-2 and EGFR are overexpressed in salivary gland cancer cell lines, we studied VEGF-2, EGFR-2, mitogen-activated protein kinase (MAPK), and Akt expression levels in ACC cells. Cell lysates were obtained and subjected to Western blot analysis as previously described (24).

To evaluate the proposed mechanism of vandetanib in vitro, we studied its ability to inhibit EGF-induced tyrosine phosphorylation of EGFR, MAPK, Akt, and VEGF-induced tyrosine phosphorylation of VEGFR in the human ACC3 cell line. Under serum-free conditions, ACC3 cells showed a low level of autophosphorylation that was enhanced after exposure to recombinant human EGF and VEGF-1 for 15 min. Cells were plated onto a six-well plate at a concentration of 3.5 × 105 per well and incubated in 10% FBS medium overnight. The next day, cells were washed with PBS and incubated with serum-free medium for 24 h. Cells in the study wells were then treated with vandetanib at concentrations ranging from 0.1 to 10 μmol/L, and cells in the control wells with DMSO for 90 min. The cells were then activated with recombinant human EGF (40 ng/mL) for 15 min and human VEGF-1 (50 ng/mL) and washed with PBS. Cell lysates were obtained and subjected to Western blot analysis as previously described (24). The following primary antibodies were used in Western blot analysis: anti-EGFR (Upstate Biotechnology, Inc.), anti-phospho-EGFR (Tyr1068;
Effects of Vandetanib in Adenoid Cystic Carcinoma

Tumor volumes from the control group were significantly larger than those from the three treatment groups using the paired Student’s t-test to determine whether differences between them were significant at P < 0.01. The quantities of MVD, TUNEL, and phosphorylated-ERK (p-ERK) were measured as the average of the ratio of apoptotic endothelial cells to total number of endothelial cells. Quantification of apoptotic endothelial cells (CD31/TUNEL) was measured as the average of the ratio of apoptotic endothelial cells to total number of endothelial cells.

Statistical analysis. Tumor volumes from the control group were compared with those from the three treatment groups using the paired Student’s t-test to determine whether differences between them were significant at P < 0.01. The quantities of MVD, TUNEL, and phosphorylated-ERK were measured as the average of the ratio of apoptotic endothelial cells to total number of endothelial cells.

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Results

**Vandetanib inhibits phosphorylation of the EGFR and VEGFR-2 and downstream signaling kinases in ACC cell lines.** To investigate the expression levels of EGFR, VEGFR-2, and the downstream targets of VEGFR-2, Akt, and MAPK in human ACC cell lines, we did Western blot analysis of these receptors in the ACC2, ACC3, and ACCM cell lines in 10% FBS medium. We found that EGFR was expressed in all the ACC cells, with the highest level of EGFR expression observed in the ACC3 cell line. ACC3 cells also showed high levels of VEGFR-2 (Fig. 1A).

To confirm that vandetanib inhibits the phosphorylation of EGFR and VEGFR-2, Western blot analysis was done in ligand-stimulated ACC3 cells, which showed high phosphorylation levels for EGFR and VEGFR-2. Serum-starved ACC3 cells were treated with various concentrations of vandetanib for 90 min followed by stimulation with EGF for 15 min. We found that vandetanib inhibited the EGF-stimulated phosphorylation of EGFR, Akt, and MAPK in a dose-dependent manner (Fig. 1B). We also found that vandetanib inhibited the VEGF-stimulated phosphorylation of VEGFR-2 in ACC3 cells in a dose-dependent manner, with the level of inhibition attaining significance at a vandetanib concentration of 1 μmol/L (Fig. 1B).

**Vandetanib inhibits proliferation and induces apoptosis in ACC cells in vitro.** MTT assay revealed that ACC cell proliferation was inhibited in all cell lines in a dose-dependent fashion (Fig. 2A). The IC_{50} values of vandetanib were 4 μmol/L for ACC3, 6.7 μmol/L for ACC2, and 6.6 μmol/L for ACCM.

PI staining and flow cytometric analysis revealed that vandetanib induced apoptosis in the ACC cell lines in a dose-dependent manner. In addition, ACC3 cells were more sensitive to vandetanib than ACC2 and ACCM cells at all doses. In ACC3 cells, 50% induction of apoptosis occurred at 9.6 μmol/L (Fig. 2B), whereas in ACC2 and ACCM cells, 50% induction of apoptosis occurred at 12.7 and 17.2 μmol/L of vandetanib, respectively (data not shown).

**Vandetanib inhibits the growth of ACC in an orthotopic nude mouse model of ACC.** To determine the in vivo antitumor effects of vandetanib, we established ACC3 tumors in the parotid glands of mice and measured the tumor out to 4 weeks of treatment. As shown in Fig. 3A, the mean tumor volumes of mice in the vandetanib group were significantly lower than those of mice in the control group (90% decrease; \( P < 0.01 \)) on the last day of treatment. During the treatment period, vandetanib was well tolerated by the mice (i.e., we saw no adverse side effects or loss in body weight).

We confirmed the antitumor effects of vandetanib using an orthotopic nude mouse model of ACC generated by the ACCM cell line. Vandetanib significantly inhibited the tumor growth in orthotopically implanted ACCM (87% decrease; \( P < 0.01 \)) as shown in Fig. 3B.

**Vandetanib decreases MVD and induces apoptosis of tumor cells and tumor-associated endothelial cells.** To clarify the mechanism of the antitumor action of vandetanib, we stained tumor sections with CD31-specific antibodies and determined the MVD by measuring the number of CD31-positive cells (Fig. 4A). As shown in Table 1, the tumors of mice treated with vandetanib showed significantly lower MVD than those of mice in the control group (90% decrease; \( P < 0.01 \)) as compared with the controls, mice with ACC3 tumor and mice with ACCM treated with vandetanib had significantly lower tumor MVD values at 82% and 73%, respectively.

In the TUNEL staining assays (Fig. 4A), the percentages of TUNEL-positive cells in ACC3 tumors from mice in the vandetanib group and ACCM tumors from mice in the vandetanib group were 23.4 ± 2.2 and 20.4 ± 0.8, respectively (Table 1), versus 4.1% and 3.7% in placebo-treated tumors. Thus, vandetanib was responsible for a significant induction of apoptosis in these two groups relative to the control groups (\( P < 0.01 \)). CD31/TUNEL double staining in tumor specimens from the control mice did not show any endothelial cell apoptosis (Fig. 4B); however, the percentage of apoptotic endothelial cells was significantly higher in ACC3 tumor sections from mice in the vandetanib than in control tumors (\( P < 0.01 \); Table 1). The ACCM tumors from mice in the vandetanib group also had the
apoptotic endothelial cells; however, the difference did not reach statistical significance (Table 1).

To confirm that vandetanib inhibited tumor cell proliferation by acting on the EGFR and VEGFR-2 signaling pathway in vivo, we analyzed all tumor sections for proliferating cell nuclear antigen. There were no detectable changes in proliferating cell nuclear antigen staining in response to vandetanib treatment (data not shown).

**Vandetanib suppresses angiogenesis by inhibiting EGFR and VEGF phosphorylation in vivo.** Double staining for CD31/EGFR, CD31/phospho-EGFR, CD31/VEGFR-2, and CD31/phospho-VEGFR-2, which was carried out with CD31 (red staining) and EGFR, phospho-EGFR, VEGFR-2, and phospho-VEGFR-2 (green staining). Although the level of expression of total EGFR and VEGFR-2 did not vary significantly among tumors from mice in two groups, tumors from the mice with vandetanib had significantly decreased phosphorylation of these two receptor kinases (Fig. 5).

### Discussion

In the present study, we tested the antitumor effects of vandetanib in vitro and in an orthotopic xenograft model of ACC. Our study shows that vandetanib induces apoptosis and decreases the growth of human ACC cell lines in vitro by inhibiting the EGFR and VEGFR-2 signaling pathways. In addition, vandetanib inhibits tumor growth in an orthotopic nude mice model of parotid ACC by decreasing tumor-associated angiogenesis and inducing the apoptosis of tumor and endothelial cells.

ACC is characterized by local recurrence, late distant metastasis, and poor response to conventional chemotherapy. Therefore, new therapeutic approaches, possibly including molecular targeted therapies, are urgently needed to benefit patients with recurrent and/or metastatic ACC. Current molecular targets in ACC include c-KIT, EGFR, the human epidermal growth receptor-2, and estrogen and progesterone receptors (25). Based on those findings, trials of targeted therapy have been initiated in patients with advanced ACC.
Imatinib, which inhibits the c-KIT tyrosine kinase, has been evaluated in ACC, but no objective responses have been observed (1). The efficacy of gefitinib, trastuzumab, and lapatinib has also been investigated in salivary gland carcinomas including ACC (26–28). However, the effectiveness of these agents against ACC has also been disappointing. Therefore, other molecular targeted therapies based on additional and/or alternate molecular targets are needed, as there are currently no standard systemic therapies shown to have benefit for those afflicted with recurrent and/or metastatic ACC. Tumors require new vasculature to grow, and angiogenesis is crucial for the progression and metastasis of many types of human tumors (29). Most cancer deaths result from metastases, making metastatic lesions an important target of antiangiogenic agents.

Table 1. Quantitative analysis of immunohistochemical staining of ACC tumors in parotid glands of nude mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group (ACC3)</th>
<th>Treatment group (ACCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vandetanib</td>
</tr>
<tr>
<td>Tumor cells (mean ± SE)</td>
<td>4.1 ± 0.7</td>
<td>23.4 ± 2.2 †</td>
</tr>
<tr>
<td>TUNEL (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells (mean ± SE)</td>
<td>15.8 ± 0.5</td>
<td>2.8 ± 0.2 †</td>
</tr>
<tr>
<td>MVD †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD31/TUNEL (%) ‡</td>
<td>0</td>
<td>14.2 ± 1.1 †</td>
</tr>
</tbody>
</table>

NOTE: ACC3 and ACCM cells were injected into the parotid glands of nude mice. Ten days later, mice were randomized and treatment started with vehicle or 50 mg/kg vandetanib. Mice were sacrificed after 12 d of treatment. Primary tumors were harvested and stained with specific antibodies.

* TUNEL positivity was quantified as the ratio of positively stained cells/total cells × 100 per field in five random 0.039-mm² fields at ×200 magnification.

† P < 0.01, compared with controls.

‡ MVD was determined by counting the number of completely stained blood vessels in 10 random 0.159-mm² fields at ×100 magnification.

§ CD31/TUNEL positivity was quantified as the ratio of CD31/TUNEL-positive cells/total endothelial cells in each of five random 0.039-mm² fields at ×200 magnification.
therapy. In contrast to squamous cell carcinoma of the head and neck, ACC frequently leads to distant metastasis. Hence, antiangiogenic therapy may be an attractive strategy for the treatment of ACC. Many of the angiogenesis inhibitors currently available have been designed to block the VEGF/VEGFR-2 signaling pathway, which is well known as a key regulator of tumor angiogenesis (30). Bevacizumab, a humanized anti-VEGF antibody, and small-molecule inhibitors of VEGF-2 tyrosine kinase activity have been studied extensively in preclinical models and clinical trials (31). However, multiple signaling pathways are involved in tumor angiogenesis and progression. Therefore, single agents that can inhibit multiple targets, such as AEE788, vatalanib, and vandetanib, may have greater antitumor effects than agents targeting VEGF/VEGFR-2 signaling alone (12, 20, 32). By targeting two pathways (VEGFR-2 and EGFR), vandetanib offers the combined benefit of directly inhibiting tumor cell proliferation and survival like EGFR inhibitors and decreasing tumor angiogenesis by inhibiting VEGF signaling. Its good oral bioavailability supports once-daily oral therapy in vivo (33).

We showed that vandetanib (at concentrations <1 μmol/L) inhibited VEGFR-2 phosphorylation in the ACC3 cell line, which had high expression of VEGFR-2 in vitro. Although the functional significance of tumor-expressed VEGFRs is currently unclear, this finding suggests that these receptors may contribute to tumor progression by receiving growth and survival signals from the tumor cells themselves. In addition, we also showed that the treatment with vandetanib inhibited VEGFR-2 phosphorylation in the ACC tumors and their endothelial cells in vivo. Thus, anti-VEGF therapies may have both direct and indirect effects on tumor cells as well as effects on endothelial cells.

The primary antitumor effect of vandetanib is considered to be generated by its inhibition of VEGF signaling in tumor endothelial cells (20). However, our in vitro study showed that the agent directly inhibits tumor cell growth in ACC3, ACC2, and ACCM cells in a dose-dependent manner. The IC₅₀ of vandetanib in these ACC cell lines is similar to those previously reported for various other types of tumor cells (2.7-13.5 μmol/L; ref. 20). This direct antiproliferative effect on tumor cells is thought to be due to inhibition of EGFR signaling because the IC₅₀ of vandetanib in these assays is similar to that of EGFR tyrosine kinase inhibitors such as gefitinib (34). Vandetanib also has an antitumor effect by readily inducing apoptosis in the VEGFR-2-expressing ACC3 cell line, whereas ACC2 and ACCM cells were less sensitive to induction of apoptosis by vandetanib. These differences in sensitivities between these cell lines may be attributed to different expression levels of EGFR tyrosine kinase; however, we believe that these relatively high IC₅₀ values could also be due to nonspecific effects of the drug at these high concentrations.

Our in vivo findings that vandetanib reduced ACC3 tumor growth by 90% compared with control in the nude mice and that chronic administration of vandetanib was well tolerated are consistent with other reports of the agent in preclinical models of human lung, breast, prostate, ovarian, and colon cancers (20). We observed a similar inhibition of tumor growth in the mouse model with tumors grown from ACCM cells, which have a low level of VEGFR-2 and EGFR expression compared with ACC3 cells. These results are consistent with the concept that the antitumor effects of vandetanib in ACC may result primarily from inhibition of VEGF signaling and indirect antitumor effects rather than direct antiproliferative effects on the tumor.

Vandetanib may be used as part of a multimodality treatment scheme. Many preclinical studies suggest that vandetanib may provide synergistic effects when used in combination with radiation therapy, cytotoxic chemotherapy, and cyclooxygenase-2 inhibition (21, 35, 36). To extend these findings, we tested the in vivo effect of vandetanib + paclitaxel (Bristol-Myers Squibb). However, synergistic effects were not observed in these ACC models (data not shown). Given the poor response of ACC to conventional chemotherapy and radiation therapy, we

![Fig. 5. Immunohistochemical analyses of ACC3 xenografts. Tumors were harvested from control and treated mice after 12 d of treatment. Immunofluorescence double staining for CD31, an endothelial cell marker (red), plus EGFR, activated EGFR, VEGFR, or activated VEGFR (green) was done. Original magnification, ×200.](www.aacjournals.org)

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suggest that combined therapies that include vandetanib should be tested further to determine the maximal benefit that can be derived from these new antiangiogenic agents.

The antiangiogenic effect of vandetanib was evaluated by quantifying the tumor microvessels. We found that vandetanib significantly decreased the tumor microvessel number and size, which is consistent with the results of other studies done in lung, pancreatic, and gastric cancers (37–39).

To confirm whether the antitumor effects of vandetanib were due to its induction of tumor and tumor-associated endothelial cell death, we did a separate short-term study in which tumor tissues were taken from mice after 12 days of vandetanib treatment. Vandetanib induced significant apoptosis of tumor and endothelial cells, but the level of tumor-associated endothelial cell apoptosis was relatively low compared with that of tumor cell apoptosis.

Our findings of no difference in proliferating cell nuclear antigen staining between control mice and vandetanib-treated mice suggest that vandetanib-mediated inhibition of tumor cell proliferation is unlikely to be the primary mechanism of action of vandetanib in this model. Therefore, other mechanisms, such as antiangiogenesis and induction of apoptosis, likely are responsible for the observed vandetanib-mediated antitumor effects.

Based on promising preclinical results, clinical trials are under way with vandetanib in a range of solid tumors, including lung, breast, and prostate (40, 41). A phase I study in Japanese patients showed that once-daily oral vandetanib at ≤300 mg/d was generally well tolerated (41). A phase I to II study of vandetanib plus docetaxel in platinum-pretreated patients with non–small cell lung cancer was recently completed with 50% of patients showing stable disease (42) and four phase III studies are currently ongoing in patients with advanced non–small cell lung cancer. Clinical trials with vandetanib are also under way in head and neck squamous cell carcinoma. In patients with previously untreated, unresected, stage III to IV head and neck squamous cell carcinoma, a phase I/II study of vandetanib in combination with radiation therapy and vandetanib in combination with radiation therapy and cisplatin chemotherapy is ongoing.7 However, no previous clinical trials have been conducted in patients with ACC.

In summary, vandetanib, an orally available agent that inhibits both EGFR and VEGFR tyrosine kinase activities, has shown significant antitumor activity in an orthotopic nude mouse model of ACC. Because ACC has shown little responsiveness to conventional chemotherapy and the long-term chances of recurrent and/or metastatic disease are relatively high, vandetanib warrants clinical assessment against this cancer.

Disclosure of Potential Conflicts of Interest

J.N. Myers has a commercial research grant from AstraZeneca.

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