Antitumor Activity of Orally Bioavailable Farnesyltransferase Inhibitor, ABT-100, Is Mediated by Antiproliferative, Proapoptotic, and Antiangiogenic Effects in Xenograft Models


Abstract

Purpose: To evaluate the preclinical pharmacokinetics, antitumor efficacy, and mechanism of action of a novel orally active farnesyltransferase inhibitor, ABT-100.

Experimental Design: *In vitro* sensitivity of a panel of human cell lines was determined using proliferation and clonogenic assays. *In vivo* efficacy of ABT-100 was evaluated in xenograft models (flank or orthotopic) by assessing angiogenesis, proliferation, and apoptosis in correlation with pharmacokinetics. Efficacy of the racemate of ABT-100 (A-367074) was also compared with R115777 (tipifarnib).

Results: ABT-100 inhibited proliferation of cells *in vitro* carrying oncogenic H-Ras (EJ-1 bladder; IC50 2.2 nmol/L), Ki-Ras (DLD-1 colon, MDA-MB-231 breast, HCT-116 colon, and MiaPaCa-2 pancreatic; IC50 range, 3.8-9.2 nmol/L), and wild-type Ras (PC-3 and DU-145; IC50 70 and 818 nmol/L, respectively) as well as clonogenic potential. ABT-100 shows 70% to 80% oral bioavailability in mice. ABT-100 regressed EJ-1 tumors (2-12.5 mg/kg/d s.c., every day for 21 days) and showed significant efficacy in DLD-1, LX-1, MiaPaCa-2, or PC-3 tumor-bearing mice (6.25-50 mg/kg/d s.c. once daily or twice daily orally). A-367074 showed equivalent efficacy to R115777 given at approximately one-fourth the total dose of R115777 for a shorter duration (EJ-1 and LX-1). Antitumor activity was associated with decreased cell proliferation (Ki-67), increased apoptosis (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling), and decreased angiogenesis. A reduction in tumor angiogenic cytokine levels (vascular endothelial growth factor, basic fibroblast growth factor, and interleukin-8) correlated with a reduction in tumor vascularity (CD31).

Conclusions: Overall, ABT-100 has an acceptable pharmacokinetic profile, is well tolerated, and possesses broad-spectrum antitumor activity against a series of xenograft models similar to farnesyltransferase inhibitors in clinical development; therefore, it is an attractive candidate for clinical evaluation.

Protein farnesylation is required for the membrane localization and function of several proteins involved in signal transduction (1). Farnesyltransferases catalyze the first step in the post-translational modification of several cellular proteins. These include Ras (2), cyclic GMP phosphodiesterase α (3), the centromere proteins CENP-E and CENT-F (4), the protein tyrosine phosphatases PRL-1, PRL-2, and PRL-3 (5), rhodopsin kinase (6), γ-subunit of the retinal protein transducin (7), peroxisomal protein PxF (8), cytoplasmic chaperone HDJ-2 (9), and nuclear intermediate filament proteins prelamin A (10) and lamin B (11). Ras proteins act as on/off switches that regulate signal transduction pathways controlling cell growth, differentiation, and survival (12). To become biologically active, Ras proteins have to undergo a series of post-translational modifications. These modifications are required for the proper subcellular localization of Ras to the plasma membrane (2) that ensures interaction with membrane receptors leading to the activation of downstream cytoplasmic and nuclear events. Mutations in Ras proteins result in constitutive activation of downstream signaling pathways that can lead to uncontrolled cell proliferation and inhibition of apoptosis (13).

Oncogenic Ras mutations have been identified in ~30% of all types of human malignancies (14). The highest incidence is detected in pancreatic (90%) and colon (50%) cancers. Because processing and function of oncogenic Ras protein was believed to play a key role in malignant transformation, farnesyltransferase inhibitors were developed to block this event (15, 16). However, in recent years, it has become apparent that farnesyltransferase inhibitors have shown antitumor activity against a variety of human tumor cell lines and xenografts that do not harbor Ras mutations (17, 18). It is now widely accepted...
that inhibition of Ras processing is not the most important downstream consequence of farnesyltransferase inhibitor action. Several oncoproteins other than Ras (e.g., RhoB and CENPs) have been shown to undergo farnesylation, suggesting that the mechanism of action of farnesyltransferase inhibitors is much more complex (4, 19–21). Other studies propose that farnesylated proteins associated with the phosphatidylinositol 3-kinase/Akt2–mediated cell survival pathway act as targets for farnesyltransferase inhibitors as well (22, 23). Further, oncogenic Ras mutations have also been implicated in tumor metastasis and angiogenesis. For instance, the expression of the angiogenic growth factor vascular endothelial growth factor (VEGF) is increased in K-Ras- and H-Ras-transformed epithelial cells through the Raf pathway, and genetic disruption of the mutant K-Ras allele in human cancer cell lines was associated with a reduction in VEGF activity (24). Therefore, another proposed mechanism of farnesyltransferase inhibitor activity is suppression of angiogenesis (25).

Several farnesyltransferase inhibitors have been evaluated recently in clinical trials (26–28). These include R115777 (tipifarnib), SCH66336, and BMS-214662 (26–33). Clinical trials of two farnesyltransferase inhibitors, the methylquinoline R115777 and the tricyclic SCH66336, as single agents have shown disease stabilization or objective responses in 10% to 15% of patients with refractory malignancies. In phase I trials, evidence of activity was observed with R115777 in a variety of solid tumors, including colon cancer and non–small cell lung cancer, with an intermittent dosing schedule demonstrating improved therapeutic index compared with a continuous dosing regimen (32, 34). In phase II trials, R115777 has shown clinical activity in patients with metastatic breast cancer, acute myelogenous leukemia, and myeloproliferative disorders (35–37). SCH66336 has shown no objective response in a phase II trial in patients with metastatic colorectal cancer and was also associated with significant gastrointestinal toxicity (38). BMS-214662, a benzodiazepine derivative that was given as a single 1-hour i.v. infusion every 21 days in phase I/II studies, has shown no objective responses, although hints of antitumor activity in patients with pancreatic cancer were observed (39). In addition, BMS-214662 showed evidence consistent with pharmacokinetic drug interaction resulting from both the induction and the inhibition of the hepatic metabolism of the drug (39). These findings emphasize the need for farnesyltransferase inhibitors with better efficacy and tolerability.

ABT-100 (Fig. 1) is a novel imidazole farnesyltransferase inhibitor that is orally active, potent, and selective. It has >100,000-fold selectivity for farnesyltransferase over the closely related enzyme geranylgeranyltransferase I (40, 41). In this article, we show that ABT-100 has ~80% oral bioavailability in mice. It is a potent cell growth inhibitor of various human tumor cell lines of different tissue origin and was observed (39). In addition, BMS-214662 showed evidence consistent with pharmacokinetic drug interaction resulting from both the induction and the inhibition of the hepatic metabolism of the drug (39). These findings emphasize the need for farnesyltransferase inhibitors with better efficacy and tolerability.

ABT-100 was given at 25, 12.5, 6.25, and 3.125 mg/kg/d to HCT-116 mice (ages 5-6 weeks, Charles River Laboratories, Wilmington, MA). ABT-100 was prepared in DMSO at a stock concentration of 10 mM/L. The cells were treated with varying concentrations of ABT-100 ranging from 0.1 to 100 mM/L in complete medium and changed twice weekly with fresh medium containing compound or vehicle. After 7 days, cell proliferation was quantitated by trypsinization and subsequent analysis using a hemacytometer. Cell viability was assessed by trypan blue exclusion method (42).

Clonogenic assays were done to determine cell viability as well as the ability of surviving cells to proliferate. The cells were allowed to adhere in complete medium for 24 hours and then exposed to varying concentrations of ABT-100 as described above for 72 hours. The cells were trypsinized and plated in drug-free medium at 100 to 400 cells in 60 mm culture plates. Cells were incubated for an additional 10 days until visible colonies appeared. The plates were then stained with Coomassie blue, and colonies were counted. Mean IC_{50} values for the inhibition of proliferation by the drug under different experimental conditions were determined by linear regression analysis of the logit-transformed data.

Pharmacokinetic studies. ABT-100 efficacy and pharmacokinetic studies were done in both CD1 nude and scid male tumor-bearing mice (ages 5-6 weeks, Charles River Laboratories, Wilmington, MA). ABT-100 was given at 25, 12.5, 6.25, and 3.125 mg/kg/d to HCT-116 tumor-bearing or non-tumor-bearing mice either orally or subcutaneously on every day or twice daily schedules (four mice per dose group
caudal spleen tip and attached pancreas tail were exteriorized and a small skin and muscle incision was made over the left renal area. The orthotopic pancreas was removed into the exposed prostate. For the orthotopic pancreatic model, a small skin and muscle incision was made over the left renal area. For the orthotopic xenograft model, a small skin and muscle incision was made over the left renal area. The orthotopic pancreas was removed into the exposed prostate. A ScieX API 2000 bioluminescence mass analyzer (Applied Biosystems/ MDS Scieix, Foster City, CA). The high-performance liquid chromatography system was coupled to the mass spectrometry using the TurbolonSpray interface. Analytes were ionized in the positive ion mode. Source temperature was ~400°C. Nitrogen was used as nebulizer gas. Detection was in the multiple reaction monitoring mode at m/z 505.1 → 226.1 for ABT-100. Peak areas for ABT-100 and the internal standard were determined using the ScieX MacQuan software. Calibration curves were derived using least-squares linear regression analysis of the peak area ratio (parent/internal standard) of the spiked plasma or tumor homogenate standards versus concentration. The four mice with highest concentrations were averaged to provide the peak plasma concentration (Cmax); the time for these four samples was averaged to provide the time to peak plasma concentration (Tmax). The ABT-100 mean plasma concentration data were submitted to log-linear regression to determine the plasma elimination rate constant (β). The area under the mean plasma concentration-time curve from 0 to t hours (time of the last measurable plasma concentration) after dosing (AUC0→t) was calculated using the linear trapezoidal rule for the plasma concentration-time profiles. The residual area extrapolated to infinity, determined as the final measured plasma concentration (Ct→∞) divided by the terminal elimination rate constant (β), was added to AUC0→t to produce the total AUC (AUCt→∞). The bioavailability was calculated as the dose-normalized AUCt→∞ from the oral or s.c. dose divided by the corresponding value derived from an i.v. dose.

In vivo efficacy studies using xenograft models. CD1 nude and C57 scid male mice were housed in a barrier facility of Abbott Laboratories. Food and water were provided ad libitum. All animal studies were conducted in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines. Tumor cells (range, 2 × 105-3 × 105) in 0.2 mL containing 1:1 mixture of medium and Matrigel (BD Biosciences, Bedford, MA) were injected subcutaneously into the flank of the animal. For LX-1, a tumor brei was prepared from several stock tumors and a 1:1 mixture with Matrigel (BD Biosciences, Bedford, MA) were injected subcutaneously into the flank of the animal. For LX-1, a tumor brei was prepared from several stock tumors and a 1:1 mixture with Matrigel (BD Biosciences, Bedford, MA). Working solutions were prepared accordingly. For proliferation, apoptosis, and angiogenesis were assessed by immunohistochemistry of zinc-fixed (Streck tissue fixative, Streck Laboratories, Omaha, NE), paraffin-embedded 5 μm sections from EJ-1 bladder orthotopic xenografts treated with vehicle or ABT-100 (12.5, 6.25, or 3.125 mg/kg/d s.c., every day) and harvested 11 days following treatment as described above. Proliferation was assessed by Ki-67 immunostaining at room temperature for 60 minutes (mouse monoclonal Ki-67, clone MBI, 1:50, DAKO, Carpinteria, CA).

Endogenous peroxidase was blocked with 3% H2O2/methanol for 10 minutes and then rinsed twice with distilled water followed by two rinses with PBS. Sections were then incubated with secondary antibody (biotinylated goat anti-mouse, DAKO) at 1:250 for 30 minutes at room temperature. For CD31 staining, endogenous peroxidase was blocked with 3% H2O2/methanol for 10 minutes and stained with CD31 PECAM (1:30, BD Biosciences, San Jose, CA) overnight at 4°C. After rinsing twice with PBS, sections were incubated with secondary antibody (biotinylated polyclonal rat IgG, 1:200, BD Biosciences) for 30 minutes at room temperature. Both Ki-67- and CD31-stained slides were rinsed twice with PBS and streptavidin-biotin horseradish peroxidase was applied for 30 minutes at room temperature. Sections rinsed twice with PBS and incubated in peroxide/diaminobenzidine as substrate/chromogen (3,3′-diaminobenzidine) for 5 minutes. Slides were washed well with distilled water, counterstained with methyl green for 10 minutes, dehydrated through graded alcohols, cleared in xylene, and mounted. Apoptosis was determined by the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay using the ApopTag Detection kit (S7101, Serotemins, Norcross, GA). Working solutions were prepared according to manufacturer directions.

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Measurement of proliferation, apoptosis, and angiogenesis. Markers for proliferation, apoptosis, and angiogenesis were assessed by immunohistochemistry of zinc-fixed (Streck tissue fixative, Streck Laboratories, Omaha, NE), paraffin-embedded 5 μm sections from EJ-1 bladder orthotopic xenografts treated with vehicle or ABT-100 (12.5, 6.25, or 3.125 mg/kg/d s.c., every day) and harvested 11 days following treatment as described above. Proliferation was assessed by Ki-67 immunostaining at room temperature for 60 minutes (mouse monoclonal Ki-67, clone MBI, 1:50, DAKO, Carpinteria, CA).

Endogenous peroxidase was blocked with 3% H2O2/methanol for 10 minutes and then rinsed twice with distilled water followed by two rinses with PBS. Sections were then incubated with secondary antibody (biotinylated goat anti-mouse, DAKO) at 1:250 for 30 minutes at room temperature. For CD31 staining, endogenous peroxidase was blocked with 3% H2O2/methanol for 10 minutes and stained with CD31 PECAM (1:30, BD Biosciences, San Jose, CA) overnight at 4°C. After rinsing twice with PBS, sections were incubated with secondary antibody (biotinylated polyclonal rat IgG, 1:200, BD Biosciences) for 30 minutes at room temperature. Both Ki-67- and CD31-stained slides were rinsed twice with PBS and streptavidin-biotin horseradish peroxidase was applied for 30 minutes at room temperature. Sections rinsed twice with PBS and incubated in peroxide/diaminobenzidine as substrate/chromogen (3,3′-diaminobenzidine) for 5 minutes. Slides were washed well with distilled water, counterstained with methyl green for 10 minutes, dehydrated through graded alcohols, cleared in xylene, and mounted. Apoptosis was determined by the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay using the ApopTag Detection kit (S7101, Serotemins, Norcross, GA). Working solutions were prepared according to manufacturer directions.

Determination of tumor angiogenic cytokines. Orthotopic EJ-1 bladder tumor tissues were harvested from vehicle-treated or ABT-100 (12.5, 6.25, and 3.125 mg/kg/d s.c., every day for 11 days)–treated mice were homogenized in buffer (50 mmol/L Tris-HCl, 1 mmol/L EDTA, 1 mmol/L EGTA), and levels of VEGF, basic fibroblast growth factor (bFGF), and interleukin-8 (IL-8) were determined by ELISA (R&D Systems, Minneapolis, MN) following the manufacturer’s instructions. The cytokine levels were expressed as picogram or nanogram per gram of tissue. The statistical significance of differences between treatment groups was assessed using ANOVA, and P values were obtained following application of Fisher’s least significant difference multiple comparison test. P < 0.05 was considered significant.

Results

ABT-100 demonstrates dose-dependent growth inhibition of human cancer cell lines. To determine the growth inhibitory effect of ABT-100, a panel of cell lines from various epithelial tissues with different Ras and p53 status was chosen (Table 1). Five different human carcinoma cell lines showed wide variation in sensitivity to ABT-100, with IC50 concentrations daily or every two day schedule for 21 days. Evidence of weight loss was negligible during treatment with ABT-100 and its racemate, A-367074. Tumor volumes were calculated according to the following equation: tumor volume (mm3) = (length × width) / 2. Antitumor activity was evaluated using two criteria, the % treated versus control (%T/C) and the time to reach a mean tumor volume of 1 mL as an estimate of survival. The median day to reach this end point was used as the median survival time estimate to compute the percentage of increase in life span (%ILS). Therapeutic comparisons were made between control and treatment groups by determining the median survival times and calculating the %ILS: %ILS = [(median survival time of treatment / median survival time of control) - 1] × 100. Statistical significance between the various groups was determined by log-rank analysis using a significance criterion of P < 0.05.
ranging from 2 to 818 nmol/L (Table 1). EJ-1 was the most sensitive followed by DLD-1, MDA-MB-231, HCT-116, MiaPaCa-2, PC-3, and DU-145. To assess the ability of ABT-100 to inhibit clonogenicity, the same cell lines were used to perform clonogenic assays. These assays showed that ABT-100 also inhibited colony formation at concentrations comparable with which it inhibited anchorage-dependent growth (Fig. 2). However, the potency range was lower (IC50 range, 2.6-84 nmol/L) compared with the 7-day growth inhibition results. The clonogenic sensitivity in descending order was HCT-116 > DLD-1 > DU-145 > PC-3 = EJ-1.

Pharmacokinetic studies. The pharmacokinetic properties of ABT-100 were evaluated in nude and scid mice (Tables 2 and 3). Peak plasma concentrations and AUC values following daily or twice daily s.c. administration of ABT-100 in both nude and scid mice were proportional to the dose (Table 3). Plasma concentrations declined with an elimination half-life of 2 to 3.1 hours. Bioavailability from all of the s.c. doses was quantitative (Table 2). Oral bioavailability was ~70% to 80% for all dose groups (Table 3). The tumor and plasma concentrations were similar with tumor/plasma ratios of 1:1.

ABT-100 inhibits in vivo growth of a series of xenografts grown subcutaneously in the flank and in orthotopic sites. ABT-100 was given to EJ-1 flank tumor-bearing mice at 12.5 and 6.25 mg/kg/d s.c., every day for 21 days, when the mean tumor volume was 265 mm3. ABT-100 induced regression of these tumors, which was maintained as long as the treatment was continued (Fig. 3A). For the 12.5 and 6.25 mg/kg/d groups, the %ILS values were 223 and 239, respectively (P < 0.001 compared with vehicle-treated groups). In a separate experiment, ABT-100 given at 6.25, 3.125, and 2 mg/kg/d orally twice daily produced similar antitumor activity (data not shown). In addition, ABT-100 given at 12.5, 6.25, and 3.125 s.c., every day for 11 days to scid mice bearing orthotopic EJ-1 bladder xenografts showed dose-dependent reduction in bladder tumor weights. The %T/C values were 37, 34, and 60 for the 12.5, 6.25, and 3.125 dose groups, respectively (Table 4). This inhibition was statistically significant at P < 0.001 for the two high-dose groups and at P < 0.05 for the low-dose group. In a separate study, efficacy of R115777 delivered at 100 mg/kg/d (total dose of 2,100 mg) s.c., twice daily for 21 days was compared with the racemate (A-367074) of ABT-100 delivered at 50 mg/kg/d (total dose 550 mg) on an every two days schedule for 11 doses in EJ-1 bladder flank tumor-bearing mice (Fig. 3B). The %ILS values were 250 and 300 for R115777.

Table 1. Antiproliferative effects of ABT-100 on human carcinoma cell lines of different tissue origin

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue</th>
<th>Ras status</th>
<th>p53 status</th>
<th>IC50 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EJ-1</td>
<td>Bladder</td>
<td>Ha</td>
<td>Mutant</td>
<td>2.2</td>
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<tr>
<td>HCT-116</td>
<td>Colon</td>
<td>Ki</td>
<td>WT</td>
<td>6.9</td>
</tr>
<tr>
<td>DLD-1</td>
<td>Colon</td>
<td>Ki</td>
<td>Mutant</td>
<td>3.8</td>
</tr>
<tr>
<td>MiaPaCa-2</td>
<td>Pancreas</td>
<td>Ki</td>
<td>Mutant</td>
<td>9.2</td>
</tr>
<tr>
<td>PC-3</td>
<td>Prostate</td>
<td>WT</td>
<td>WT</td>
<td>70.0</td>
</tr>
<tr>
<td>DU-145</td>
<td>Prostate</td>
<td>WT</td>
<td>Mutant</td>
<td>818.0</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast</td>
<td>Ki</td>
<td>Mutant</td>
<td>5.9</td>
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</table>
Table 2. Plasma concentrations of ABT-100 following multiple s.c., daily dosing in nude and scid mice

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Dose (mg/kg/d)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-12&lt;/sub&gt; (µg·h/mL)</th>
<th>F (%)</th>
</tr>
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<tbody>
<tr>
<td>Nude</td>
<td>6.25</td>
<td>0.60 ± 0.05*</td>
<td>2.3 ± 0.7</td>
<td>2.7</td>
<td>5.56</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>1.32 ± 0.07</td>
<td>2.3 ± 0.7</td>
<td>2.6</td>
<td>10.54</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>2.18 ± 0.01</td>
<td>4.2 ± 2.5</td>
<td>2.8</td>
<td>27.33</td>
<td>124</td>
</tr>
<tr>
<td>Scid</td>
<td>3.125</td>
<td>0.77 ± 0.06</td>
<td>2.7 ± 1.7</td>
<td>2.8</td>
<td>5.75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>1.39 ± 0.13</td>
<td>1.0 ± 0.0</td>
<td>2.6</td>
<td>9.73</td>
<td>100</td>
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<tr>
<td></td>
<td>12.5</td>
<td>2.77 ± 0.24</td>
<td>1.0 ± 1</td>
<td>2.7</td>
<td>19.07</td>
<td>100</td>
</tr>
</tbody>
</table>

*Mean ± SEM (n = 4). Animals were dosed once daily; samples were taken after dose 5.

and A-367074, respectively, suggesting that ABT-100 is at least equivalent to R115777 in efficacy given at approximately one-fourth of the total dose of R115777 and for shorter duration. Altogether, these findings indicate that ABT-100 has significant antitumor activity in the EJ-1 bladder xenografts grown both in the flank and in the orthotopic site and its efficacy is at least equivalent to that of R115777 in this model.

Efficacy of ABT-100 was evaluated in LX-1 tumor-bearing nude mice with different doses, routes, and schedules. ABT-100 given orally to LX-1 tumor-bearing mice on a twice daily schedule for 21 days at 25, 12.5, and 6.25 mg/kg/d starting 9 days after tumor inoculation (mean tumor volume, 125 mm<sup>3</sup>) showed dose-dependent statistically significant efficacy (P < 0.001 for the high-dose groups and P < 0.05 for the low-dose group; Fig. 3C). The %T/C values at day 31 were 28, 31, and 50 for the 25, 12.5, and 6.25 mg/kg/d doses, respectively. Similar to the EJ-1 bladder xenograft study described above, efficacy of R115777 delivered at 100 mg/kg/d s.c., twice daily for 21 days (total dose, 2,100 mg) was compared with the racemate (A-367074) of ABT-100 at 100 mg/kg/d s.c., twice daily for 21 days (total dose, 2,100 mg) was compared with the racemate (A-367074) of ABT-100 at 100 mg/kg/d s.c., twice daily for 21 days, respectively. Similar to the EJ-1 bladder xenograft study described above, efficacy of R115777 delivered at 100 mg/kg/d s.c., twice daily for 21 days (total dose, 2,100 mg) was compared with the racemate (A-367074) of ABT-100 at 100 mg/kg/d s.c., twice daily for 21 days, respectively.

Nude mice bearing DLD-1 s.c. tumors were treated for 21 days with ABT-100 at 50, 25, and 12.5 mg/kg/d s.c., every day when the average tumor volume reached 260 mm<sup>3</sup>. ABT-100 showed significant antitumor activity (Fig. 3D) with the %T/C ratios of 46, 49, and 61 (day 41) and %ILS values of 93, 93, and 36 for the 50, 25, and 12.5 mg/kg/d groups, respectively. The %T/C and %ILS values were significantly different compared with vehicle-treated controls at P < 0.001 for the two high-dose groups and P < 0.01 for the lowest-dose group.

ABT-100 at 25, 12.5, and 6.25 mg/kg/d given on the same schedule to PC-3 tumor-bearing mice showed significant efficacy (Fig. 3E). The %T/C values on day 35 were 43, 54, and 62 and the %ILS values were 47, 47, and 32 for 25, 12.5, and 6.125 doses, respectively. In a separate study, administration of ABT-100 at 25 mg/kg/d once daily for 21 days s.c. to orthotopic PC-3 tumor-bearing mice showed significant reduction (P < 0.01) in tumor weights with a %T/C ratio of 43 (on day 35, tumor weights were 433 ± 56 and 186 ± 26 mg for the vehicle- and ABT-100-treated groups, respectively).

Statistically significant (P < 0.05) antitumor activity was also shown in MiaPaCa-2 pancreatic tumor-bearing scid mice with %T/C ratios of 42, 59, and 69 on day 25 and %ILS values of 58, 21, and 21 for the 50, 25, and 12.5 mg/kg/d doses (orally, twice daily for 21 days), respectively (Fig. 3F). In a separate study, ABT-100 at 50 mg/kg/d was given s.c., every day for 14 days and

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Table 3. Plasma and tumor concentrations of ABT-100 following multiple oral dosing twice daily in HCT-116 tumor-bearing nude mice

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-12&lt;/sub&gt; (µg·h/mL)</th>
<th>F (%)</th>
<th>AUC tumor/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>0.27 ± 0.01*</td>
<td>1.4 ± 0.6</td>
<td>1.83</td>
<td>66.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.24 ± 0.05</td>
<td>7.5 ± 0.9</td>
<td>1.60</td>
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<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>0.67 ± 0.07</td>
<td>2.0 ± 0.6</td>
<td>3.96</td>
<td>71.8</td>
<td>1.0</td>
</tr>
<tr>
<td>12.5</td>
<td>0.50 ± 0.03</td>
<td>8.3 ± 0.8</td>
<td>4.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>1.39 ± 0.04</td>
<td>2.0 ± 0.6</td>
<td>8.71</td>
<td>79.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Tumor</td>
<td>1.45 ± 0.15</td>
<td>2.0 ± 1.3</td>
<td>7.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SEM (n = 4). C<sub>max</sub> (µg/mL or µg/g) and AUC (µg·h/mL or µg·h/g). Treatment began 11 days after tumor inoculation when tumors were ~ 200 mm<sup>3</sup>. Animals were dosed twice daily for 5 days and samples were taken after dose 9.
for an additional four doses every other day to orthotopic MiaPaCa-2 tumor-bearing mice, which resulted in significant reduction in tumor weights (on day 28, %T/C was 41 and the tumor weights were 442 ± 49 and 218 ± 26 mg for the vehicle- and ABT-100-treated groups, respectively).

**ABT-100 affects proliferation of tumor cells** in vivo. To assess whether inhibition of tumor growth by ABT-100 was mediated by a reduction in cell proliferation and an increase in apoptosis, markers for proliferation (Ki-67 staining) and apoptosis (TUNEL staining) were investigated by immunohistochemistry. The EJ-1 orthotopic bladder xenografts treated for 11 days with vehicle or three doses of ABT-100 showed that ABT-100 induced significant dose-dependent reduction in proliferation as indicated by a significant reduction in Ki-67 staining...
(Fig. 4A) with a concomitant increase in apoptosis as indicated by an increase the number of TUNEL-positive cells (Fig. 4B). These results suggest that ABT-100 inhibits tumor growth and clonogenicity and increasing apoptosis.

**ABT-100-induced inhibition of angiogenesis correlates with antitumor effect in vivo.** To determine whether the antitumor effect of ABT-100 is mediated by inhibition of tumor angiogenesis, EJ-1 orthotopic bladder tumors treated for 11 days with ABT-100 at 3.125, 6.25, and 12.5 mg/kg/d s.c., every day were harvested and bFGF, VEGF, and IL-8 levels were determined. Tumor VEGF and IL-8 concentrations were significantly inhibited (4- to 5-fold; \( P < 0.001 \) compared with vehicle-treated controls) by all three doses of ABT-100, although bFGF levels were inhibited (3- to 4-fold; \( P < 0.001 \) compared with vehicle-treated controls) only at the two highest doses of ABT-100 (Table 4). This reduction in tumor angiogenic cytokine levels was associated with a dose-dependent reduction in tumor blood vessel density as indicated by CD31 staining (Fig. 5). The 3- to 5-fold reduction in tumor cytokine levels observed following ABT-100 treatment in the EJ-1 orthotopic xenografts was also confirmed with EJ-1 bladder tumors grown in the flank. The tumor VEGF levels following a 11-day treatment with ABT-100 were 13.5, 6.2, and 4.3 \( \times 10^{-12} \) ng/g of tissue for vehicle, 3.125, 6.25, and 12.5 mg/kg/d dose groups, respectively, with low plasma clearance, higher volume of distribution, and long elimination half-lives (40). ABT-100 reaches significant levels in tumors with a tumor/plasma ratio of 1:1 across a wide dose range that correlates to significant antitumor activity *in vivo*. In efficacy studies, ABT-100 was given at doses ranging from 3.125 to 25 mg/kg/d. In this dose range, ABT-100 displayed linear pharmacokinetics and significantly antitumor activity. Efficacy studies with the racemate of ABT-100 showed that it can be given once daily on an every two days schedule or on a 4 days on, 3 days off schedule for three cycles.

ABT-100 shows considerable antiproliferative effects against a series of transplantable human cancers regardless of their Ras status. However, H-Ras mutant tumor lines (EJ-1) seem to be more sensitive to farnesyltransferase inhibitors than tumors bearing K-Ras mutations. This has been attributed not only to the high affinity of K-Ras to farnesyltransferase but also due to K-Ras in the presence of farnesyltransferase inhibitors to be a substrate for alternate prenylation by the related enzyme geranylgeranyltransferase I (41). In addition, it has been shown that geranylgeranylated proteins are critical in H-Ras oncogenesis (43) and that K-Ras and H-Ras-transformed cells differ in their utilization and/or dependence on signaling pathways for the apoptotic response (44).

Plasma pharmacokinetic variables for *scid* and *nude* mice showed that bioavailability from all s.c. doses were 100% for both strains, whereas peak plasma concentrations and drug clearance were higher in *scid* mice compared with *nude* mice. The AUC values were proportional to the dose. The oral bioavailability was ~70% to 80% in both strains of mice. Dog and monkey oral bioavailability was 35% and 42%, respectively, with low plasma clearance, higher volume of distribution, and long elimination half-lives (40). ABT-100 reaches significant levels in tumors with a tumor/plasma ratio of 1:1 across a wide dose range that correlates to significant antitumor activity *in vivo*. In efficacy studies, ABT-100 was given at doses ranging from 3.125 to 25 mg/kg/d. In this dose range, ABT-100 displayed linear pharmacokinetics and significant antitumor activity. Efficacy studies with the racemate of ABT-100 showed that it can be given once daily on an every two days schedule or on a 4 days on, 3 days off schedule for three cycles.

### Table 4. ABT-100 inhibits the growth and angiogenic cytokine levels in the EJ-1 bladder xenografts grown in the orthotopic site of *scid* mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg/d s.c., daily)</th>
<th>Bladder weight (mg)</th>
<th>%T/C</th>
<th>VEGF (ng/g tissue)</th>
<th>bFGF (ng/g tissue)</th>
<th>IL-8 (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>210 ± 18†</td>
<td>60†</td>
<td>3.3 ± 0.3</td>
<td>36 ± 0.8</td>
<td>4.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>ABT-100</td>
<td>3.125</td>
<td>126 ± 24†</td>
<td>0.6 ± 0.1†</td>
<td>32 ± 1.2</td>
<td>12 ± 0.8†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>72 ± 23†</td>
<td>0.7 ± 0.1†</td>
<td>9 ± 1.3†</td>
<td>ND†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>77 ± 19†</td>
<td>0.7 ± 0.2†</td>
<td>13 ± 4.3†</td>
<td>ND†</td>
<td></td>
</tr>
</tbody>
</table>

*Ratio of tumor weight for treated versus control (vehicle).
†Mean ± SEM (\( n = 6 \)).
‡Treatment began 4 days after tumor inoculation for 11 days.
\( \* \) \( P \) values versus vehicle < 0.05.
\( \ddagger \) \( P \) values versus vehicle < 0.001.
\( \# \) ND, below the level of detection.
with *in vitro* growth inhibition results presented here as well as reported observations made with other farnesyltransferase inhibitors (45–48). Overall, these studies confirm that overexpression, mutations, or functional activation of Ras is not predictive of the antitumor activity of farnesyltransferase inhibitors, including ABT-100. The lack of regression in many established tumors tested suggests that ABT-100 is a cytostatic drug. The apparent requirement for the drug to be continuously present for significant antitumor effect also supports this notion. This is illustrated by growth curves demonstrating tumor growth rates equal to that of the control vehicle-treated tumors on cessation of ABT-100 treatment (Fig. 3). However, when tumor-bearing mice are treated with ABT-100, tumor VEGF level is significantly reduced and cells are driven to

![Fig. 4. ABT-100 affects tumor cell proliferation (A) and apoptosis (B) in EJ-1 orthotopic bladder xenografts. Immunohistochemical staining for proliferation (Ki-67 staining) or apoptosis (TUNEL staining) in EJ-1 orthotopic bladder xenografts harvested from vehicle-treated or ABT-100-treated (12.5, 6.25, or 3.125 mg/kg/d s.c. once daily for 11 consecutive days). Treatment started 4 days after tumor implantation. Arrows, dark stained cells representing apoptotic cells. Several samples were analyzed. Representative tumors are shown. Tumors from animals treated with ABT-100 show a significant reduction in tumor cell proliferation and a concomitant increase in the incidence of apoptosis compared with vehicle-treated controls.](attachment://fig4.jpg)
apoptosis as shown by an abundance of TUNEL-positive tumor cells compared with vehicle-treated mice (Table 4; Fig. 4B). Thus, ABT-100 may indirectly induce tumor cells to undergo apoptosis by inhibiting a known tumor survival factor, VEGF (49).

It has been shown that the tumor microenvironment plays a critical role in the response to anticancer therapy. The current studies show that ABT-100 not only is effective in s.c. flank tumors but also shows equivalent efficacy when the same tumor types are grown in the orthotopic site with a microenvironment similar to the sites of the tumor origin. Histologic examination of the treated orthotopic tumors indicates that the antitumor response to ABT-100 goes beyond its antiproliferative effects. For instance, the EJ-1 bladder antitumor activity was associated not only with a reduction in tumor cell proliferation by Ki-67 staining but also with a concomitant increase in apoptosis (TUNEL staining). Therefore, the ability of ABT-100 to inhibit tumor growth in the flank and in the orthotopic tumors may be attributed to a reduction in proliferation and an increase in apoptosis.

One of the mechanisms of antitumor efficacy of farnesyltransferase inhibitors has been proposed to be through inhibition of angiogenesis. Ras is activated by FGF-2 and VEGF in endothelial cells and has been shown to regulate proliferation, migration, differentiation, and survival, which are cellular processes required for angiogenesis (50–53). Ras activation up-regulates VEGF expression and inhibition of Ras by farnesyltransferase inhibitors has led to a significant decrease in the expression and secretion of VEGF, thereby affecting angiogenesis (24, 25). Several studies have reported that different farnesyltransferase inhibitors inhibit angiogenesis in a variety of tumor models (25, 54, 55). In the current study, we have also shown that ABT-100 not only induces a reduction of tumor VEGF and bFGF but also similarly induces a reduction in another angiogenic cytokine, IL-8, in the bladder tumors. This inhibition is associated with a reduction in tumor blood vessel density as shown by CD31 staining. Interestingly, plasma concentrations of VEGF, bFGF, and HGF have been reported to be elevated in patients with chronic myeloid leukemia, myelofibrosis, and multiple myeloma (56). This increase may be correlated with proangiogenic factors contributing to tumor growth. However, this increase in plasma concentrations of VEGF in patients with chronic myeloid leukemia has been associated with an adverse outcome independent of other prognostic factors. This may be because VEGF functions as a tumor survival factor in addition to being an angiogenic cytokine. In a recent phase I study, it was also shown that multiple myeloma and chronic myeloid leukemia patients who had higher plasma concentrations of VEGF before treatment showed better clinical response to the farnesyltransferase inhibitor, R115777, lending credence to the role of VEGF functioning as tumor survival factor. However, the exact mechanism of R115777 or farnesyltransferase inhibitors in general and how it affects angiogenic factors still remains to be determined.

In summary, ABT-100 has an acceptable pharmacokinetic profile and improved antitumor efficacy in preclinical models similar to the farnesyltransferase inhibitors currently in clinical development. Thus far, R115777 has shown good clinical response in phase II trials in patients with metastatic breast cancer, acute myelogenous leukemia, and myeloproliferative disorders. Therefore, compounds like ABT-100 would be expected to be effective in these patient populations and merits further investigation in clinical trials.
References


Antitumor Activity of Orally Bioavailable Farnesyltransferase Inhibitor, ABT-100, Is Mediated by Antiproliferative, Proapoptotic, and Antiangiogenic Effects in Xenograft Models


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