The Antiangiogenic Activity in Xenograft Models of Brivanib, a Dual Inhibitor of Vascular Endothelial Growth Factor Receptor-2 and Fibroblast Growth Factor Receptor-1 Kinases


Abstract

Tumor angiogenesis is a complex and tightly regulated network mediated by various proangiogenic factors. The fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) family of growth factors, and associated tyrosine kinase receptors have a major influence in tumor growth and dissemination and may work synergistically to promote angiogenesis. Brivanib alaninate is the orally active prodrug of brivanib, a selective dual inhibitor of FGF and VEGF signaling. Here, we show that brivanib demonstrates antitumor activity in a broad range of xenograft models over multiple dose levels and that brivanib alaninate shows dose-dependent efficacy equivalent to brivanib in L2987 human tumor xenografts. Brivanib alaninate (107 mg/kg) reduced tumor cell proliferation as determined by a 76% reduction in Ki-67 staining and reduced tumor vascular density as determined by a 76% reduction in anti-CD34 endothelial cell staining. Furthermore, Matrigel plug assays in athymic mice showed that brivanib alaninate inhibited angiogenesis driven by VEGF or basic FGF alone, or combined. Dynamic contrast-enhanced magnetic resonance imaging, used to assess the effects of brivanib alaninate on tumor microcirculation, showed a marked decrease in gadopentetate dimeglumine contrast agent uptake at 107 mg/kg dose, with a reduction in area under the plasma concentration-time curve from time 0 to 60 minutes at 24 and 48 hours of 54% and 64%, respectively. These results show that brivanib alaninate is an effective antitumor agent in preclinical models across a range of doses, and that efficacy is accompanied by changes in cellular and vascular activities.

Introduction

Angiogenesis or neovascularization, the process by which new blood vessels are formed from preexisting vessels, is critical to the growth, survival, and dissemination of tumors. Initiation of tumor angiogenesis is generally mediated by secretion of a variety of growth factors in response to local environmental triggers, as yet not fully understood but thought to include hypoxia (1). Prominent among these mediators are vascular endothelial growth factor ligand A (VEGF-A) and fibroblast growth factor (FGF)-1 (acidic FGF) and FGF-2 [basic FGF (bFGF)].

The identification and characterization of tumorderived angiogenic factors, their corresponding endothelial cell receptors, and complex signaling networks provides novel opportunities for the therapeutic development of new antineoplastic agents (2). The VEGF family of growth factors and associated tyrosine kinase receptors (VEGF receptor, VEGFR) has been shown to have a major influence on tumor angiogenesis, and anti-VEGF agents, including bevacizumab, have been developed and shown to provide therapeutic advantage in some tumor types (3, 4). VEGF-A binds preferentially to VEGFR-1 and VEGFR-2, the latter being recognized as a primary mediator of vascular permeability and a promoter of tumor-related endothelial cell migration and proliferation. The implications for the survival and growth of tumor cells make the therapeutic targeting of VEGFR-2 especially attractive (2, 5); however, there is growing evidence of VEGFR-1 promoting angiogenesis (2).

The FGF family is also known to be influential in the stimulation of endothelial cell proliferation and migration (6, 7) and therefore has potential as an additional antineoplastic target (8). FGF receptor (FGFR)-1 is the predominant FGFR to be expressed on endothelial cells, with FGFR-2 also present to a lesser extent. FGF/FGFR signaling is thought to contribute to both the early and late stages of tumor angiogenesis and to affect both tumor cell invasion and migration (6, 7). Some evidence suggests that stimulation of the FGF signaling network can induce a proangiogenic effect creating a favorable environment for vasculature growth through the modulation of endothelial cell proliferation and migration, the
production of proteases, and the promotion of integrin and cadherin receptor expression (7).

Recent research has pointed to the importance of crosstalk between bFGF and VEGF, which may reflect temporal differences in their role. Synergistic vascularization activity is seen when the pair is coexpressed simultaneously in athymic mouse xenograft models, resulting in fast-growing tumors with high vessel density patency and permeability (9). In mouse models, the administration of VEGFR-2 antagonists has been found to block both VEGF- and bFGF-induced angiogenesis and endothelial cell proliferation. These findings led to the hypothesis that endogenous VEGF is required for the in vitro angiogenic effect induced by exogenous bFGF (10, 11). The expression of dominant-negative FGFR-1 and FGFR-2 in glioma cells has been shown to result in decreased tumor vascularization paralleled by VEGF downregulation (12). These findings indicate that tumors may be able to evade initial VEGF inhibition by altering the regulation of other growth factors involved in angiogenesis and tumor cell proliferation. The complexity of the regulation of tumor angiogenesis suggests that the targeting of more than one pathway may be beneficial (4).

Brivanib is a small-molecule VEGFR/FGFR tyrosine kinase inhibitor that is currently undergoing clinical investigation. Due to the low aqueous solubility of brivanib, a prodrug strategy was pursued that resulted in a prodrug with high aqueous solubility, brivanib alaninate (13). Brivanib, the parent compound, has previously been shown to be a selective inhibitor of FGFR-1 and FGFR-2, and VEGFR-1, VEGFR-2, and VEGFR-3 in vitro (14, 15). Additionally, brivanib has also shown antitumor activity in vivo against the H3396 human breast tumor xenograft model (14). Here, we report the in vivo antiangiogenic and tumor growth-inhibitory effects of brivanib and its prodrug, brivanib alaninate, in human xenograft mouse models across a range of tumor types.

Materials and Methods

Reagents

Brivanib alaninate is the L-alanine ester prodrug of brivanib to improve the low aqueous solubility of brivanib at equilibrium (14, 15). In this series of studies, brivanib alaninate was administered at a volume of 0.01 mL/g of mouse with sodium citrate buffer, with a final pH of 3.5 as vehicle for the dosing solution. Brivanib was dissolved in a 7:3 solution of PEG400/H2O and was administered at a volume of 0.01 mL/g. Both compounds were orally dosed using disposable 20-g oral gavage needles (Popper and Sons, Inc.) on a daily schedule with a general duration of 14 d.

Animals

Female BALB/c athymic mice (nu/nu) were purchased at 5 to 6 wk of age from Harlan Sprague-Dawley Co. Mice were maintained in an ammonia- and pathogen-free environment and were fed water and food ad libitum. Quarantine was ensured for 7 d before tumor implantation and efficacy testing. All animal studies were done under the approval of the Bristol-Myers Squibb Animal Care and Use Committee and in accordance with the American Association for Accreditation of Laboratory Animal Care.

Xenograft Tumor Models

Human tumor xenografts used included L2987 (non-small-cell lung carcinoma), H3396 (breast carcinoma), and HCT116/VM46 (paclitaxel-resistant colon carcinoma), which were established at Bristol-Myers Squibb.

Tumors were implanted s.c. as small fragments of ≤0.1 to 0.2 mm3 using a 13-g trocar. Tumors were allowed to grow to an approximate size of 100 mm3 before the initiation of treatment. Treatment and control groups consisted of 8 to 10 mice. Tumor size was measured twice weekly. Tumor volume was calculated by measuring perpendicular tumor diameters with Vernier scale calipers, and using the formula: 1/2 [length × (width)²]. The health of the mice was closely monitored and the mice were immediately euthanized if any signs of distress were observed.

Antitumor efficacy was expressed as percentage tumor growth inhibition (% TGI) and therefore represents the maximum effect. TGI was calculated as follows:

\[
\text{% TGI} = \left\{1 - \left[\frac{(C_t - T_0)}{(C_t - C_0)}\right]\right\} \times 100
\]

in which \(C_t\) is the median tumor volume (mm³) of vehicle control (C)-treated mice at time \(t\); \(T_t\) is the median tumor volume (mm³) of treated mice, \(t\), at time \(t\); and \(C_0\) is the median tumor volume (mm³) of vehicle control-treated mice at time 0.

Tumor volume doubling time was measured over the linear growth range of the tumor, generally between 250 and 1,000 mm³ tumor size. TGI of ≥50% over one tumor volume doubling time was considered an active antitumor response.

Immunohistochemistry

L2987 tumor xenografts were excised, fixed in 10% neutral buffered formalin, processed for paraffin embedding, and sectioned at 5 μm. Slides were allowed to dry and immunohistochemistry was done. Cell proliferation was evaluated using an anti-Ki67 antibody (Santa Cruz Biotechnology, Inc.). Vascular density was visualized through an anti-CD34 antibody (BD Pharmingen). The dilution of both antibodies was 1:100 in a serum-free blocking agent (DAKO Cytomation). Tissue was blocked for endogenous peroxidase by immersion in 3% H2O2 for 8 min. Antigen retrieval was done using Citra Plus in a pressure cooker (Biocare Medical decloaking chamber; Biocare Medical). Tissue was heated to 98°F for 3 min and then allowed to cool for a further 15 min before rinsing in immunohistochemistry wash buffer (DAKO). This buffer was used as a rinse between all subsequent steps. Primary antibodies were applied to tissue sections and
were allowed to incubate overnight at 4°C in a humidified chamber. For the anti–CD34-treated tissues, an anti-rat polymer probe (Biocare Medical) was applied and incubated for 20 min. After rinsing, horseradish peroxidase complex (Biocare Medical) was applied and incubated for 20 min. For the anti–Ki67-treated tissues, a secondary polymer probe conjugated to peroxidase (EnVision, DAKO Cytomation) was applied for 20 min. 3,3′-Diaminobenzidine (Biocare Medical) was applied for 2 min each slide. Following the developing step, tissues were counterstained with Mayer’s hematoxylin for 20 s, rinsed in tap water, put through a series of graded alcohols to xylene, and coverslipped. Quantification of proliferation and vascular density was done using an Olympus BX-10 microscope (Tokyo) and Image-Pro Plus software (Media Cybernetics, Inc.), without prior knowledge of groups. Proliferation was analyzed by a cell count of positively stained cells. All fields of view were quantified at ×100 magnification. Vascular density (defined as the number of vessels per unit area) was done in 25 fields of view at ×200 magnification. Statistical analysis was done using the SAS JMP software (SAS Institute).

**Matrigel In vivo Assays and Histology.** The effects of brivanib alaninate, bevacizumab (Genentech), and DC101 (BioXCell) on endothelial cells were assessed in Matrigel plugs in nude mice. Matrigel (BD, growth factor reduced), stored frozen at −80°C, was thawed on ice at 4°C overnight. To prepare the Matrigel for injection, either 100 ng/μL VEGF (PeproTech) alone, 250 ng/μL bFGF (PeproTech) alone, or both in combination (100 ng/μL VEGF and 250 ng/μL bFGF) were suspended in the gel and mixed by gentle inversion. Approximately 24 h after implant, mice were treated with vehicle (PBS), brivanib alaninate at 50 and 100 mg/kg, DC101 at 1.63 mg/dose and 3.25 mg/dose, or bevacizumab at 4 mg/kg over a period of 10 d. At the end of the dosing period, Matrigel plugs were excised and fixed in 10% neutral buffered formalin before processing for paraffin embedding. They were then sectioned at 5 μm and stained with H&E. Identification of the endothelial cell population using immunohistochemical approaches was based on positive staining of CD34, a well-characterized marker of endothelial cells (16). The Ariol image analysis system (Applied Imaging Corp.; Genetix) was used.
to analyze the Matrigel plugs. Endothelial cells were counted at a magnification of ×200 and 10 fields of view were used for each animal. Raw data were transferred to a statistical software package, SAS JMP, and the mean number of cells migrating was calculated for each group. A Student’s t test was used to show significance.

Dynamic Contrast Enhanced-Magnetic Resonance Imaging

The in vivo activity of brivanib alaninate was assessed pharmacodynamically in L2987 tumor-bearing mice using dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI). All DCE-MRI experiments were done in accordance with the Bristol-Myers Squibb Animal Care and Use Committee Guidelines.

Athymic mice (18–28 g) bearing s.c. implanted L2987 human lung tumor cells (5 × 10^6) on the lateral portion near the hind limb were included in the study when tumor size reached 100 mm^3. Mice were anesthetized with isoflurane (AERRANE, Baxter Healthcare) and the tail veins were catheterized for administration of MRI contrast agent. Mice were dosed orally with brivanib alaninate after baseline DCE-MRI measurements were made. All animals received three doses of brivanib alaninate: immediately after the baseline DCE-MRI assessment and then at 24 and 48 h. The second and third DCE-MRI imaging were done within 2 h of dosing.

DCE-MRI images were acquired on a Bruker PharmaScan 4.7 T horizontal magnet with a 16-cm bore (Bruker Biospin). After initial axial scout images, a precontrast T1 measurement was done by saturation recovery for the slices that were to be used for dynamic imaging. The images were acquired with a variable repetition time (TR; 3,000, 1,200, 900, 600, 300, and 152.18 ms) and an echo time of 15 ms. A series of 40 dynamic-contrast T1-weighted echoes were obtained with a temporal resolution of 11.8 s for three contiguous 2-mm slices through the tumor and one through the leg muscle (reference tissue) using a spin echo sequence with repetition time TR = 110 ms, echo time T = 10 ms, and 128 × 128 matrix. Five DCE-MRI sets were collected before the administration of contrast. The next 35 images were collected after bolus injection of 0.3 mmol/kg gadopentetate dimeglumine (Magnevist, Berlex Labs) through a syringe pump with an infusion rate of 2 mL/min. A manual 220-μL saline wash was done over 5 s to flush the catheter line (180 μL line volume) and to ensure the complete delivery of the contrast agent.

T1 and DCE were initially processed using zero-filling and two-dimensional Fourier transformation to yield 256 × 256 data points. Images were analyzed as a pixel average of the regions of interest (ROI) by image sequence analysis (Bruker Biospin). ROIs were drawn around the entire tumor area for each tumor slice excluding the surrounding skin and muscle using the T1 images. A separate ROI was drawn in the contralateral leg for muscle T1 values. These ROIs were transferred to the DCE images with the same geometry. Individual T1 values were calculated for each ROI for both tumor and muscle using the image sequence analysis T1sat function and were used to normalize DCE images with their corresponding T1 values. The first five preinjection images were averaged to increase signal-to-noise ratio and subtracted from the postinjection images to provide contrast enhancement images of the tumor and muscle ROIs. T1 time courses for the ROIs of tumor and muscle were then obtained from the respective enhancements and baseline T1 values and were translated to gadopentetate concentration using gadopentetate relaxivity. The initial area under the contrast uptake curve for the first 60 s (AUC_60) after the contrast agent administration was then integrated and the tumor values were normalized to their

Figure 2. Representative photographs of the gross morphology of excised L2987 tumors from athymic mice treated with brivanib alaninate at two different dose levels. Brivanib alaninate suppressed tumor growth and vascularization.
corresponding muscle values to eliminate the animal-to-
animal variability in the arterial input function (17, 18). 
All data were tested for variance using the Student’s 
t test two-tailed paired analysis, which is used for test-
ing the significance between different groups of treat-
ment. Because the same animal was used for baseline 
and posttreatment imaging, each served as its own 
control. Reproducibility experiments performed in the 
same mice 24 h apart showed that the 95% limit of 
change was −18% to +22% for a group of nine mice; 
normalization to muscle improved the reproducibility 
of results (19). The within-subject coefficient of varia-
tion was 24%.

Results

Antitumor Activity in Xenograft Tumor Models

In xenograft models, brivanib shows potent antitumor 
activity in vivo against human H3396 breast tumor xeno-
grafts. The inhibition of tumor growth relative to control 
as measured by the TGI was 30%, 37%, and 88% after 
14 days of treatment with brivanib at doses of 7.5, 15, 
and 30 mg/kg twice a day, respectively (Fig. 1A). The ac-
tivity of brivanib in chemoresistant HCT116/VM46 hu-
man colon carcinoma xenografts was also shown 
(Fig. 1B). Brivanib was shown to have greater inhibitory 
effects on tumor growth at doses of 15 to 45 mg/kg twice 
a day for 14 days than 36 mg/kg paclitaxel i.v. every 
2 days for 10 days (maximum tolerated dose), which 
was ineffective in inhibiting tumor growth.

Both brivanib and its ester prodrug brivanib alaninate 
showed potent antitumor activity against L2987 human 
lung carcinoma xenografts over a variety of doses 
(Fig. 1C and D). The inhibition of tumor growth relative 
to control was also seen with the administration of briva-
nib alaninate, with treated/control ratios of 0.88, 0.40, 
and 0.28 at doses of 53, 80, and 107 mg/kg once a day, 
respectively, after 14 days of treatment.

Gross assessment of removed tumors from mice 
treated with brivanib alaninate also clearly showed a 
dose-dependent reduction in tumor growth and vascu-
larization (Fig. 2).
Effects on Tumor Cell Proliferation in Xenograft Tumor Models

Treatment with brivanib alaninate significantly inhibited tumor cell proliferation in L2987 lung tumor xenografts, as shown by a dose-dependent decrease in the number of Ki-67-positive cells on staining with anti-Ki-67 (Fig. 3). A statistically significant reduction in Ki-67 staining was observed at a dose of 36 mg/kg (45% reduction; \( P < 0.001 \) versus control), but a much more dramatic reduction (76%; \( P < 0.001 \) versus control) was observed at a dose of 107 mg/kg, which is a highly efficacious dose in xenograft models when given daily.

Effects of Brivanib on both VEGF- and bFGF-Stimulated Angiogenesis In vivo

The Matrigel plug assay was used to assess the ability of brivanib alaninate and other known angiogenesis inhibitors to inhibit angiogenesis stimulated by VEGF or bFGF alone or in combination. Identification of the endothelial cell population using immunohistochemical approaches was based on positive staining of CD34 (data not shown).

As illustrated in Fig. 4A, a significant reduction in endothelial cell number was observed in plugs containing either cytokine alone or when both cytokines were combined followed by treatment with brivanib alaninate. In addition, as summarized in Fig. 4B, a lower dose of brivanib alaninate, which generally results in limited efficacy in tumor xenograft studies, was still effective in inhibiting angiogenesis in plugs containing VEGF, bFGF, or both cytokines. In contrast, treatment with the VEGFR-2-selective inhibitor DC101, a monoclonal antibody specifically directed at the murine host VEGFR-2, or bevacizumab, a commercially marketed monoclonal antibody directed at the human VEGF ligand with minimal to no neutralizing activity against murine VEGF (20), resulted in no inhibition of either bFGF- or VEGF/bFGF-stimulated angiogenesis. In plugs containing only VEGF, bevacizumab, when delivered at a dose level and schedule that results in maximal preclinical antitumor activity,
showed significant inhibition consistent with its direct effect on the VEGF ligand that is known to stimulate both VEGFR-1 and VEGFR-2 (21).

**Effects on Vascular Density in Xenograft Tumor Models**

The effects of brivanib alaninate on vascular density within the L2987 tumor xenografts are shown in Fig. 5. There was a dose-dependent reduction in CD34 staining for endothelial cells compared with vehicle control at doses of 36 mg/kg (21% reduction) and 107 mg/kg (76% reduction), with a statistically significant difference observed only at the 107 mg/kg dose ($P < 0.001$ versus control).

**Effects on Tumor Microcirculation Assessed by DCE-MRI**

DCE-MRI was used to assess the effect of brivanib alaninate (36 mg/kg, $n = 10$; and 107 mg/kg, $n = 9$) on tumor microcirculation in the L2987 lung xenograft model. DCE-MRI analysis of coronal single-slice images through the tumor and the muscle of the hind limb area before and 24 hours after the administration with brivanib alaninate showed a markedly reduced uptake of contrast agent with brivanib alaninate 107 mg/kg when compared with the 36 mg/kg dose (Fig. 6A) in the tumor. Brivanib alaninate at a dose of 107 mg/kg was shown to be more efficacious than the lower dose level of 36 mg/kg. There was a significant reduction in AUC$_{60}$ of 54% and 64% at 24 and 48 hours, respectively, at the 107 mg/kg dose ($n = 9$). In contrast, there was no significant difference at 36 mg/kg ($n = 10$; Fig. 6B and C).

**Discussion**

Brivanib, the parent compound of the prodrug brivanib alaninate, has previously been identified as a selective dual inhibitor of FGF and VEGF signaling, with *in vitro* activity encompassing FGFR-1, FGFR-2, VEGFR-1, VEGFR-2, and VEGFR-3 (14, 15). Brivanib demonstrates good cellular selectivity as shown by the specific inhibition of human umbilical vascular endothelial cell proliferation when these cells were stimulated with VEGF or bFGF, but not by epidermal growth factor (EGF) or platelet-derived growth factor-β (14). The dual inhibition of VEGFR and FGFR activity with brivanib alaninate has previously been shown in preclinical studies in the GEO- and bevacizumab-resistant HT-29 colon cancer models.
This distinctive inhibitory profile provides additional potential for enhanced antitumor activity, drawing on the suggested synergistic mechanisms of VEGF and bFGF on tumor vasculature.

This series of in vivo studies show that brivanib and its prodrug brivanib alaninate have a broad spectrum of antitumor activity over multiple dose levels and schedules. The studies showed that antitumor activity encompasses breast, colon, and lung human tumor xenografts as well as in a model of chemoresistant disease consistent with its antiangiogenic activity. Additionally, the prodrug brivanib alaninate was equally efficacious in vivo against the L2987 human lung tumor model when dose levels were adjusted for the active parent moiety. The robust antitumor effects in the L2987 lung tumor model, which does not express VEGFR in vivo but still showed a marked antitumor response to brivanib alaninate, strongly suggest activity against a host-driven angiogenic mechanism. Furthermore, the results obtained in vivo against multiple tumor models resulting in tumor stasis are consistent with literature reports in preclinical models with other agents targeting VEGFR-2 (23–25).

Brivanib alaninate also clearly has an effect on endothelial cells in a Matrigel plug assay when using VEGF and bFGF alone or in combination as stimulators of angiogenesis. Using this model, we show that brivanib alaninate not only effectively inhibits angiogenesis driven by either VEGF or bFGF alone but also robustly inhibits angiogenesis driven by a combination of these cytokines. As shown in Fig. 4B, the combination of VEGF and bFGF resulted in a doubling of the endothelial cell population compared with either of these cytokines alone, and both dose levels of brivanib significantly reduced angiogenesis driven by the combined growth factors. Importantly, although bevacizumab was effective in inhibiting angiogenesis driven by VEGF alone, it had no impact on angiogenesis when VEGF was supplemented with bFGF. Therefore, the inhibition of VEGF signaling seems to be

![Figure 6](image_url)
insufficient to inhibit angiogenesis in the presence of bFGF signaling in the Matrigel plug assay. Consequently, we propose that the inhibition of VEGF- and bFGF-driven angiogenesis requires targeting of both signaling pathways, as is the case with brivanib. Whether blockade of only the FGF pathway will lead to the inhibition of VEGF- and bFGF-driven angiogenesis will require the identification and testing of a highly specific compound targeting only bFGF signaling. Interestingly, DC101 did not seem to inhibit angiogenesis in plugs containing only VEGF despite use of a dose that is well above the maximum efficacious level determined in preclinical antitumor models (26). It is possible that a longer period of treatment might be required for revealing an effect based on the in vivo antitumor regimen; alternatively, the blockade of VEGFR-2 alone may be insufficient to obtain a response in this assay in light of the additional angiogenic activities that are mediated by VEGFR-1 and which would not be inhibited by DC101 as suggested by Ferrara et al. (27).

The robust antiangiogenic activity of brivanib alaninate is also shown by the significant reduction in tumor vascular density and in tumor blood flow (as measured by DCE-MRI). Pharmacodynamic measurements in tumor-bearing mice were used to assess in vivo activity using DCE-MRI to measure contrast agent uptake, which is dependent on a combination of blood flow, vessel surface area, and permeability. This technique has been used previously with a variety of compounds targeting the VEGF pathway in preclinical models (28, 29). Several other agents targeting the VEGFR pathway have also shown DCE-MRI changes, although the relationship of DCE-MRI changes to clinical benefit still awaits validation (30).

A significant reduction in AUC_{60} with brivanib alaninate as measured by DCE-MRI over 48 hours was observed using a dose of 107 mg/kg. This dose produces complete cytostasis in this tumor model in athymic mice when dosed from 10 to 30 days and was designated as the efficacious dose. At the subefficacious dose of 36 mg/kg, tumor growth was not significantly different as the efficacious dose. At the subefficacious dose of 10 mg/kg, the AUC_{60} was 74\% of the control (data not shown).

In vivo treatment of tumor microcirculation, expressed as AUC_{60}, was funded by Bristol-Myers Squibb.

The preclinical DCE-MRI changes described here seem to highly correlate with imaging findings from patients receiving brivanib alaninate in an ongoing phase 1 study in which DCE-MRI also showed a dose-dependent decrease in AUC_{60} in tumors (33). Brivanib has similar potency against VEGFR-2 as the platelet-derived growth factor β/VEGF inhibitor sunitinib and has higher potency against VEGFR-2 than the currently approved VEGFR-2/c-Kit/β3 integrin inhibitor sorafenib (IC_{50} values: 25 nmol/L with brivanib versus 10 nmol/L with sunitinib versus 90 nmol/L with sorafenib; refs. 14, 34, 35). However, unlike sunitinib and sorafenib, brivanib targets the FGFR signaling pathway, which is critical for angiogenesis. Together with the data reported in the current study, this provides a strong rationale for the clinical investigation of brivanib in solid tumors. Based on these promising findings, brivanib alaninate has now progressed to phase 2/3 clinical investigation.

**Disclosure of Potential Conflicts of Interest**

All authors are employees of Bristol-Myers Squibb Research & Development.

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**References**


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