# Broad Antitumor Activity in Breast Cancer Xenografts by Motesanib, a Highly Selective, Oral Inhibitor of Vascular Endothelial Growth Factor, Platelet-Derived Growth Factor, and Kit Receptors

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**Abstract**

**Purpose:** Angiogenesis plays a critical role in breast cancer development and progression. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that regulates endothelial cell proliferation and survival. We investigated the effects of motesanib, a novel, oral inhibitor of VEGF receptors 1, 2, and 3; platelet-derived growth factor receptor; and Kit receptor, on the growth of xenografts representing various human breast cancer subtypes.

**Experimental Design:** Athymic nude mice were implanted with MCF-7 (luminal) or MDA-MB-231 (mesenchymal) tumor fragments or Cal-51 (mixed/progenitor) tumor cells. Once tumors were established, animals were randomized to receive increasing doses of motesanib alone or motesanib plus cytotoxic chemotherapy (docetaxel, doxorubicin, or tamoxifen).

**Results:** Across all three xenograft models, motesanib treatment resulted in significant dose-dependent reductions in tumor growth, compared with vehicle-treated controls, and in marked reductions in viable tumor fraction and blood vessel density. No significant effect on body weight was observed with compound treatment compared with control-treated animals. Motesanib did not affect the proliferation of tumor cells *in vitro*. There was a significantly greater reduction in xenograft tumor growth when motesanib was combined with docetaxel (MDA-MB-231 tumors) or with the estrogen receptor modulator tamoxifen (MCF-7 tumors), compared with either treatment alone, but not when combined with doxorubicin (Cal-51 tumors).

**Conclusions:** Treatment with motesanib alone or in combination with chemotherapy inhibits tumor growth *in vivo* in various models of human breast cancer. These data suggest that motesanib may have broad utility in the treatment of human breast cancer.

Breast cancer is the most common malignancy in women. In 2007, there were an estimated 178,000 new breast cancer cases, with 40,000 deaths attributed to the disease in the United States alone (1). Adjuvant treatment strategies after initial surgery include cytotoxic chemotherapy (e.g., docetaxel, 5-fluorouracil, and doxorubicin), radiation therapy, and hormonal therapy (e.g., tamoxifen; ref. 2). However, primary and recurrent metastatic breast cancer remains incurable: only 3% of patients with metastatic disease achieve a complete response for more than 5 years after combination chemotherapy (3); the median survival time after therapy is ~2 years. Breast cancer treatment and patient prognosis are influenced by several factors, including tumor subtype. Breast tumors are genotypically heterogeneous and fall into several subtypes based on their genotypic and phenotypic characteristics (4, 5). At the highest level, they are classified into luminal or nonluminal (basal or "triple negative") cancers.

In recent years, novel approaches to the treatment of breast cancer have led to the successful development of targeted therapies. For example, the monoclonal antibody trastuzumab, which targets HER2/neu, has been shown to significantly improve clinical outcomes for patients with HER2/neu-positive cancers (6, 7). Similarly, targeted endocrine therapies (such as tamoxifen) have long been used in the treatment and prevention of breast cancer (8).

It has been shown that angiogenesis is important in breast cancer development, invasion, and metastasis (9) and antiangiogenic therapies are actively being investigated as potential new therapeutic strategies (10). Vascular endothelial growth factor (VEGF) signaling plays a critical regulatory role in neovascularization (11). The effects of VEGF are mediated by activation of the receptor tyrosine kinases VEGFR1 (12) and VEGFR2 (KDR; ref. 13), with most of the proangiogenic effects of VEGF likely being mediated by VEGFR2 (14). In particular, activation of VEGFR2 promotes the proliferation and survival

## Translational Relevance

For breast cancer patients, targeted therapies have emerged as important alternative treatment options, although response to such treatments can vary considerably. Tumor subtype, which influences choice of treatment and prognosis, may potentially also affect response to targeted therapy. Motesanib is a novel, highly specific inhibitor of the vascular endothelial growth factor receptors 1, 2, and 3, as well as platelet-derived growth factor and Kit receptors. The study described herein shows that motesanib significantly inhibits the growth of three different xenograft models of human breast cancer representing both luminal and nonluminal subtypes. The data suggest that motesanib may have broad clinical antitumor activity in breast cancer, including tumor subtypes that have proven resistant to traditional cytotoxic therapies. Furthermore, treatment of the three different breast cancer xenografts with motesanib in combination with either chemotherapy (docetaxel) or the estrogen receptor modulator tamoxifen resulted in significantly increased inhibition of xenograft growth compared with either treatment alone. The ability to augment the efficacy of standard-of-care breast cancer treatment with motesanib is currently being actively investigated in clinical trials.

of endothelial cells (15). VEGFR3 and its activation by VEGF-C seem to promote lymphangiogenesis and metastatic spread (16). In breast cancer patients, increased VEGF expression is associated with increased microvessel density, poor prognosis, resistance to treatment, relapse, and disease recurrence (17–20). In preclinical studies, overexpression of VEGF increased the growth rate of human breast cancer xenografts (particularly in the early stages of tumor development), whereas suppression of its overexpression was associated with reduced tumor growth (21).

Current efforts in the development of new targeted breast cancer treatments include agents that target either VEGF or its cognate receptors (22). Motesanib (formerly known as AMG 706) is a novel, highly selective inhibitor of VEGFR1, VEGFR2, VEGFR3, Kit, and platelet-derived growth factor receptor that is being evaluated for its potential broad antitumor activity (23, 24). Early preclinical (23) and clinical (24) studies have shown that motesanib inhibited the growth of solid tumors. The aim of this study was to investigate the antitumor effects of motesanib in mouse xenograft models of human breast cancer and to investigate the mechanisms that mediate these effects. Our results show that motesanib treatment caused a dose-dependent reduction in tumor volume in both luminal and nonluminal breast cancer xenografts, which was accompanied by a concomitant reduction in both viable tumor fraction and tumor blood vessel density. Further decreases in tumor volume were achieved when animals were treated with motesanib in combination with docetaxel or tamoxifen but not in combination with doxorubicin. Motesanib had no effect on the growth of tumor cells *in vitro*. Together, these results suggest that the antiangiogenic activity of motesanib is the primary mediator of its antitumor activity observed in this study. The data support

further broad development of motesanib for the treatment of breast cancer.

## Materials and Methods

*Cell lines and reagents.* MCF-7 and MDA-MB-231 cells were obtained from the American Type Culture Collection. Cal-51 cells were kindly provided by Dennis Slamon, M.D., Ph.D. (University of California, Los Angeles, CA). All cells were determined to be free of *Mycoplasma* and common viral murine pathogens before use and were cultured in RPMI 1640 containing 1% L-glutamine and 10% fetal bovine serum (Invitrogen Corp.). Unless otherwise specified, all reagents were obtained from Sigma-Aldrich Corp.

*In vitro cell proliferation.* MCF-7, MDA-MB-231, and Cal-51 breast cancer cells were grown to confluence, trypsinized, and seeded in 96-well tissue culture plates at a concentration of 3,000 per well in complete medium plus 10% fetal bovine serum. The next day, cells were treated, in duplicate, with a 10-point serial dilution of motesanib (0.5 nmol/L to 10 μmol/L; Amgen, Inc.). Seventy-two hours after treatment, the number of viable cells was quantified using the ATPlite 1step Luminescence Assay (Perkin-Elmer). Luminescence was assessed using a Victor 1420 workstation (Perkin-Elmer).

*Breast cancer xenograft models.* Female athymic nude mice between 4 and 6 wk of age were obtained from Harlan Sprague Dawley, Inc. and housed in sterilized cages. All mice were fed Sterilizable Rodent Diet 8656 (Harlan Teklad) and provided reverse osmosis water *ad libitum*. Animals were maintained on a 12-h/12-h light/dark cycle; relative humidity was maintained between 34% and 73%. All procedures were approved by the Amgen Animal Care and Use Committee and met the standards of the Association for Assessment and Accreditation of Laboratory Animal Care.

MDA-MB-231 or MCF-7 tumor pieces ($\sim 2$ mm$^3$) were harvested from donor mice and implanted into the right mammary fat pad of recipient mice using a 10-gauge trochar under isoflurane/O$_2$ anesthesia. Mice implanted with MCF-7 tumor pieces were also implanted with 90-d-release 0.25 mg 17-β-estradiol pellets (Innovative Research of America). Cal-51 tumor cells [$5 \times 10^6$ in a 50% solution of Matrigel (BD Biosciences) in improved MEM] were injected s.c. into the right flank. Tumors were allowed to establish and grow to $\sim 200$ mm$^3$, at which time the mice were randomized into experimental groups. Mice were then treated with either vehicle (water, pH 2.5) or 7.5, 25, or 75 mg/kg motesanib by oral gavage twice daily. In experiments investigating the effects of combination treatments on tumor growth, mice were administered motesanib by oral gavage; or either 20 mg/kg docetaxel once per week i.p., 2.5 mg/kg doxorubicin once per week i.p., or 30 mg/kg tamoxifen i.p. five times per week for $\sim 1$ mo; or motesanib in combination with either docetaxel, doxorubicin, or tamoxifen at the indicated doses.

Tumor volume, measured with a Pro-Max electronic digital caliper (Rutland Tool and Supply Company, Inc.), and body weight were assessed twice per week. All tumor studies were done in a blinded fashion where the person measuring tumor volume had no knowledge of the treatment groups. Tumor volume was calculated as (width$^2$ × length)/2 for tumors derived from MCF-7 and MDA-MB-231 implants, and as length × width × height for tumors derived from Cal-51 implants. All tumor volumes are reported in cubic millimeters.

*Histologic tumor analyses.* Histologic analyses of tumor microvasculature and viability were done as previously described (23). Briefly, at the end of each experiment, tumors were removed and bisected along their longest axis. Paired half-tumors were fixed in either zinc-formalin or IHC-zinc (BD Biosciences) and embedded in paraffin. Tumor viability was determined from hematoxylin-stained tumor sections. Sections were photographed in their entirety at 1× objective magnification, and the total tumor cross-sectional area and the viable area for each were determined by RGB thresholding using MetaMorph software (MDS, Inc.). The viable fraction was expressed as a percentage of total

area. Sections were also immunostained for the vascular endothelium marker CD31 (BD Biosciences), using 3,3′-diaminobenzidine as the chromogen, and were lightly counterstained with hematoxylin. CD31-stained area (i.e., blood vessel area) was assessed as a percentage of the viable tumor area. The image fields used for the blood vessel area analyses were captured using a Nikon Microphot-FXA compound microscope (Nikon, Inc.) and a Nikon 10× Plan-APO objective lens. The cross-sectional area images used for the viable fraction analyses were captured using a Nikon SMZ-U stereozoom microscope equipped with a Nikon ED PLAN 0.5× objective lens. In both instances, standard brightfield transillumination was used. All images were acquired using a Nikon DXM1200C digital camera, controlled via Nikon's ACT-1 software. No significant postcapture image processing was used before blood vessel area or viable fraction analyses. All image analyses were conducted in a fully blinded fashion.

*Statistical analyses.* The effects of motesanib and/or docetaxel/doxorubicin/tamoxifen treatment on xenograft growth and body weight were assessed by repeated measures ANOVA followed by a Scheffé's post hoc test using StatView 5.0.1 software (SAS Institute). The effects of motesanib treatment on tumor viability and blood vessel area were assessed by a Student's *t* test. In all analyses, $P < 0.05$ was considered statistically significant. All data are expressed as mean ± SE.

## Results

*Selection of human breast cancer cell line models.* Based on gene expression profiling studies similar to the classification system used for breast cancer subtypes, human breast cancer cell lines are categorized as luminal or nonluminal (5, 25). The effects of motesanib treatment on tumor growth *in vitro* and *in vivo* were studied using three different human breast cancer cell line models. We tested the estrogen receptor–positive cell line MCF-7, a prototypical luminal model (25), and the estrogen receptor–negative, vimentin-positive cell line MDA-MB-231 and the Cal-51 cell line, both of which represent the nonluminal subtype. More specifically, MDA-MB-231 has been classified as mesenchymal or post–epithelial-mesenchymal transition (25), whereas Cal-51 has been included in the experiments as a representative of a "mixed/progenitor" phenotype. Previously, the Cal-51 cell line has been characterized as post–epithelial-mesenchymal transition in microarray analyses of bulk mRNA expression (26). However, we have found that these cells do not express high levels of vimentin, and immunofluorescent staining using various differentiation markers has provided evidence that the Cal-51 cell line is multipotent, giving rise to multiple cell lineages, including the highly aggressive basal cell type. These findings will be reported in a separate publication.

*Effect of motesanib treatment on proliferation of breast cancer cells.* The cell lines characterized above were used to investigate whether motesanib had any direct inhibitory activity on breast cancer cell growth *in vitro*. In all three cell lines, we did not detect quantifiable levels of VEGFR2 or Kit mRNA; platelet-derived growth factor receptor β mRNA was only detected in the MDA-MB-231 cells and at quantities that were within the lowest 25% of mRNA expression levels of all genes examined (data not shown; ref. 25). Treatment with motesanib at concentrations up to 3 μmol/L for 3 days did not significantly affect the proliferation of MCF-7, MDA-MB-231, or Cal-51 cells (Table 1). In contrast, treatment with the chemotherapy agent docetaxel significantly inhibited the proliferation of cells from all three breast cancer cell lines ($IC_{50}$ = 0.5-0.6 nmol/L). These results are consistent with the

lack of a direct antitumor effect of motesanib on the tumor cells themselves.

*Effect of motesanib on growth of human breast cancer xenografts.* To determine whether motesanib has antitumor activity *in vivo*, the growth of MCF-7, MDA-MB-231, and Cal-51 tumor xenografts in response to increasing doses of the drug was measured. In all three xenograft models, treatment with motesanib resulted in a dose-dependent inhibition of tumor growth. In mice implanted with MCF-7 tumor pieces, mean tumor volume in vehicle-treated animals increased ∼3-fold from day 20 (first day of treatment) after xenograft implantation to day 37. In motesanib-treated mice receiving 7.5 mg/kg twice daily, the mean tumor volume on day 37 was not significantly different from the control (16% reduction compared with vehicle; $P = 0.37$). However, higher doses of motesanib resulted in significant inhibition of tumor growth. In animals receiving 25 or 75 mg/kg twice daily, the mean tumor volume on day 37 was reduced by 44% ($P = 0.0004$) and 65% ($P < 0.0001$), respectively, compared with vehicle-treated controls. The antitumor activity observed with motesanib treatment was not associated with any overt toxicity as judged by the lack of significant changes in body weight (Fig. 1A).

In mice implanted with MDA-MB-231 tumor pieces, mean tumor volume in vehicle-treated animals rapidly increased ∼7-fold from day 15 (first day of treatment) after xenograft implantation to day 31. In this model, only the highest dose of motesanib (75 mg/kg twice daily) resulted in significant inhibition of tumor growth: mean tumor volume on day 31 was reduced by 64% compared with vehicle-treated controls ($P < 0.0001$). In animals receiving lower doses of motesanib, mean tumor volume on day 31 was not significantly different from the control [reductions of 28% ($P = 0.21$) and 24% ($P = 0.33$) compared with vehicle in the 7.5 and 25 mg/kg twice daily groups, respectively]. As observed in the previous experimental group, no notable effect on body weight occurred during treatment with motesanib (Fig. 1B).

In animals bearing Cal-51 tumor xenografts, mean tumor volume in the control group increased ∼4-fold from day 22 (first day of treatment) after xenograft implantation to day 41. In this model, treatment with motesanib significantly inhibited tumor growth in a dose-dependent manner. In the 7.5 mg/kg twice daily dose group, mean tumor volume on day 41 was reduced by 38% ($P < 0.0314$) compared with vehicle-treated animals and by 74% ($P < 0.0001$) and 81% ($P = 0.0001$) in the 25 and 75 mg/kg twice daily groups, respectively. Compared with the control animals, no effect of motesanib treatment on body weight was observed at any dose level (Fig. 1C). For each of the three breast cancer models tested in this study, similar tumor growth inhibition was observed when motesanib (75 mg/kg twice daily) was administered.

*Effect of motesanib in combination with cytotoxic chemotherapy agents on the growth of MDA-MB-231 and Cal-51 human breast cancer xenografts.* The antitumor activity of motesanib was further evaluated in combination with docetaxel and doxorubicin, cytotoxic chemotherapy agents commonly used in breast cancer therapy. Motesanib plus docetaxel significantly reduced tumor volume compared with either agent alone in mice bearing MDA-MB-231 tumor xenografts. Treatment with motesanib (75 mg/kg twice daily) began on day 20 and treatment with docetaxel (20 mg/kg) on day 21 after xenograft implantation. Mean tumor volume on day 48 was significantly

**Table 1.** Effect of motesanib treatment on breast cancer cell proliferation *in vitro*

| IC$_{50}$ (nmol/L) | Breast cancer cell line | | |
|---|---|---|---|
| | MCF-7 (luminal) | MDA-MB-231 (mesenchymal) | Cal-51 (mixed/progenitor) |
| Motesanib | >3,000 | >3,000 | >3,000 |
| Docetaxel | 0.5 | 0.5 | 0.6 |

less in mice receiving the combination than in animals treated with either agent alone: 41% reduction compared with motesanib alone (*P* = 0.028) and 59% reduction compared with docetaxel alone (*P* = 0.0002; Fig. 2A). A small decrease in body weight was observed in animals receiving motesanib plus docetaxel (92% of the body weight maintained on day 41 compared with day 19; *P* ≤ 0.0004 versus motesanib alone). In contrast, no reduction in body weight was seen in animals treated with either motesanib (106% on day 41) or docetaxel (104% on day 41) alone. It is unlikely that the effect of motesanib in combination with docetaxel was due to a modulation of plasma exposure of either agent as we have not observed any consistent or significant effects on the pharmacokinetics of either agent in xenograft models of other tumor types (data not shown).

In animals bearing Cal-51 tumor xenografts, treatment with motesanib (7.5 mg/kg twice daily; the lowest concentration at which a significant decrease in tumor volume was previously observed) in combination with doxorubicin (2.5 mg/kg once weekly) did not result in a significant reduction in tumor volume compared with animals treated with either agent alone (Fig. 2B). Of note, the Cal-51 model was relatively resistant to a variety of chemotherapeutic agents, including docetaxel, cyclophosphamide, and doxorubicin (data not shown). Treatment of Cal-51 xenografts with higher doses of doxoru-

bicin (up to 7.5 mg/kg) did not result in greater single-agent efficacy compared with the 2.5 mg/kg dose (data not shown). Combination experiments using higher doses of motesanib in Cal-51 xenografts were not done.

*Effect of motesanib in combination with tamoxifen on the growth of MCF-7 human breast cancer xenografts.* We also assessed the antitumor effect of motesanib in combination with the selective estrogen receptor modulator tamoxifen in mice bearing estrogen-sensitive MCF-7 tumors. Treatment with motesanib plus tamoxifen significantly reduced tumor volume compared with either agent alone. Animals received motesanib beginning on day 23 following tumor implantation. Compared with vehicle-treated controls, tumor volume on day 40 was reduced by 55% in mice administered motesanib (25 mg/kg twice daily) and by 52% in animals administered tamoxifen (30 mg/kg per mouse five times per week i.p.). In mice receiving both motesanib and tamoxifen, tumor volume on day 40 was reduced by 72% compared with vehicle-treated controls and was significantly less than tumor volumes in animals administered either motesanib (*P* = 0.005) or tamoxifen (*P* = 0.023) alone. Treatment with motesanib and tamoxifen, either alone or in combination, had no effect on the body weight of the animals (Fig. 3). The data shown represent one of three experiments done. In the remaining experiments, treatment with combination therapy resulted in
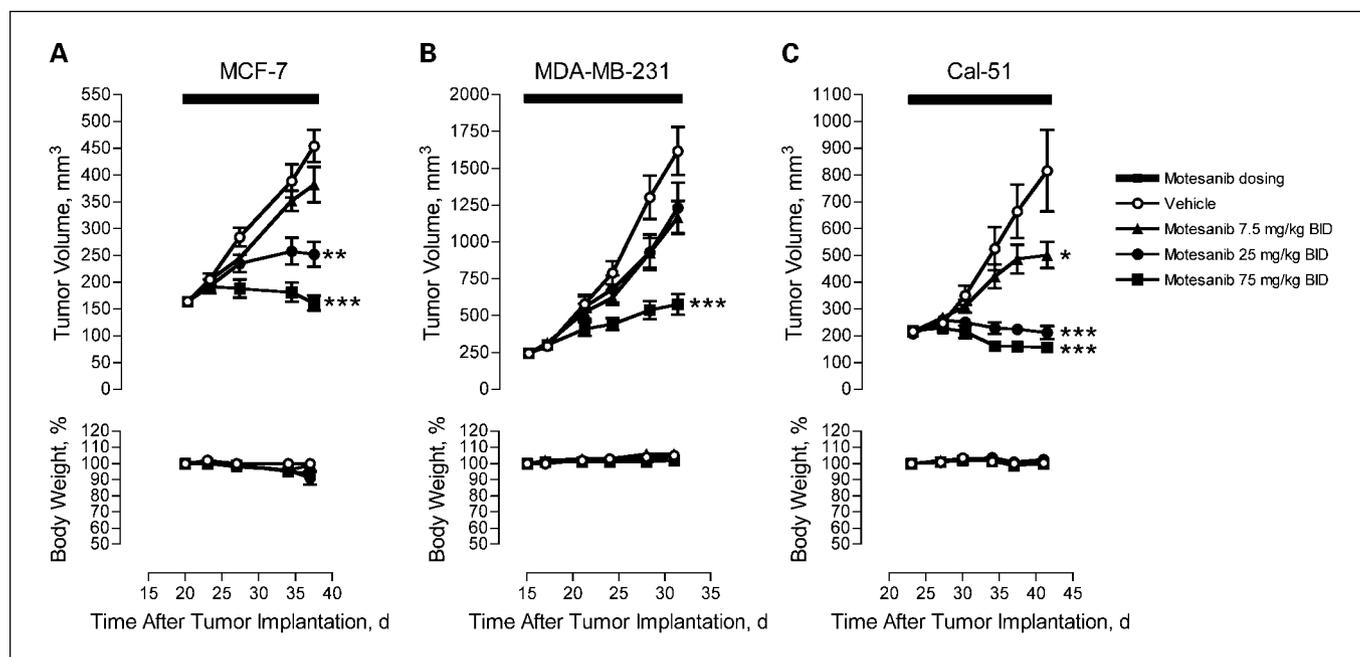


**Fig. 1.** Effect of motesanib on tumor volume and body weight in MCF-7 tumor xenografts (*A*), MDA-MB-231 tumor xenografts (*B*), and Cal-51 tumor xenografts (*C*). One Cal-51 tumor xenograft–bearing animal in the 25 mg/kg twice daily group died before the day 34 assessment. *, *P* < 0.05 versus vehicle; **, *P* < 0.01 versus vehicle; ***, *P* < 0.0001 versus vehicle. Points, mean; bars, SE.
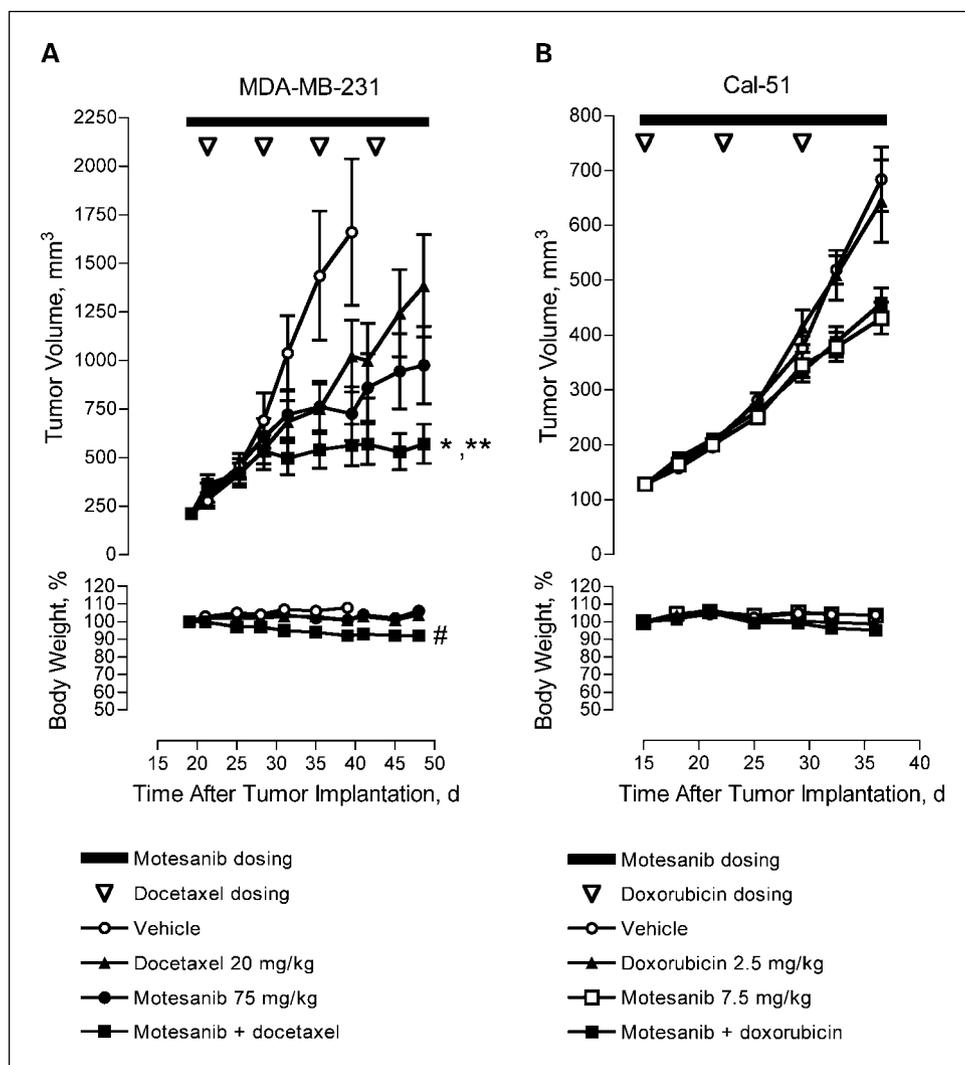
**Fig. 2.** Effect of motesanib, docetaxel, or doxorubicin alone, or motesanib in combination with either docetaxel or doxorubicin on tumor volume and body weight in MDA-MB-231 and Cal-51 tumor xenografts. *A,* effect of motesanib (75 mg/kg twice daily) either alone or in combination with docetaxel (20 mg/kg once weekly) on tumor volume and body weight in mice bearing MDA-MB-231 tumor xenografts. *B,* effect of motesanib (7.5 mg/kg twice daily) either alone or in combination with doxorubicin (2.5 mg/kg once weekly) on tumor volume and body weight in mice bearing Cal-51 tumor xenografts. *, $P < 0.05$ versus motesanib alone; **, $P < 0.01$ versus docetaxel alone; #, $P < 0.01$ versus motesanib. Results from all treatment groups were significant compared with vehicle ($P < 0.05$). Points, mean; bars, SE.

enhanced antitumor activity but the results did not reach statistical significance compared with either agent alone.

***Effect of motesanib on viable tumor fraction and blood vessel density in human breast cancer xenografts.*** To explore the mechanism of antitumor activity of motesanib on breast cancer tumors, the histology of xenografts from the control and treatment groups was assessed. Microscopically, all three untreated xenografts (MDA-MB-231, MCF-7, and Cal-51) consisted of focal, ovoid, well-demarcated masses that caused compression of the adjacent connective tissue. The masses were often surrounded by a thin, discontinuous band of mononuclear cells and scattered neutrophils. Additionally, the xenografts contained a central, irregular area of necrosis characterized by neoplastic cells with nuclear pyknosis, densely eosinophilic cytoplasm, and ghost cells. Following treatment with motesanib, there was robust expansion of the central necrotic area that extended circumferentially at the expense of viable neoplastic cells, frequently reaching the adjacent subcutis. Remaining viable neoplastic cells were located along the periphery of the mass in thin, discontinuous bands directly opposed to the subcutis (Fig. 4A).

Compared with vehicle-treated animals, administration of motesanib (75 mg/kg twice daily) significantly reduced the viable tumor fraction in all three tumor models. In the MCF-7 xenograft group, viable tumor fraction on day 37 after implantation was 21% in motesanib-treated animals and 59% in vehicle-treated controls ($P < 0.0001$); in the MDA-MB-231 group, it was 25% and 67%, respectively, on day 31 ($P < 0.0001$); and in the Cal-51 group, viable tumor fraction was 6% and 28%, respectively, on day 41 ($P < 0.0001$; Fig. 4B). We also examined the blood vessel area in each tumor by measuring the CD31 immunostaining-positive area and expressing it as a percentage of the viable tumor area. Compared with vehicle-treated controls, blood vessel area of tumor xenografts in animals receiving motesanib (75 mg/kg twice daily) was significantly reduced across experimental groups: 4.8% (control) versus 0.8% (motesanib) in the MDA-MB-231 xenograft group ($P < 0.0001$), and 1.2% (control) versus 0.5% (motesanib) in the Cal-51 xenograft group ($P = 0.0013$). Although size-matched tumors were not compared, our own historical data have shown that once these tumors have established, the blood vessel area relative to the area of viable tumor in each xenograft model remains relatively constant throughout the range of tumor volumes observed in the experiments. Quantitative vessel analysis could not be done in MCF-7 tumor xenografts because of widespread severe

necrosis. However, as shown in the representative sections, there was a visible reduction in blood vessel density in these tumor xenografts (Fig. 5). We were unable to characterize differences in the vascularity of the tumors in the combination treatment groups due to the lack of viable tumor remaining at the end of each experiment.

## Discussion

In this study, treatment with motesanib of both luminal and nonluminal breast tumor xenografts resulted in significant, dose-dependent decreases in tumor volume and decreased viable tumor fraction. The concomitant reductions in blood vessel density suggest that the observed antitumor effect was mediated in part by inhibition of angiogenesis. Consistent with an antiangiogenic mechanism, motesanib did not inhibit the growth of these three breast cancer cell lines *in vitro*, suggesting that it does not exert a direct effect on either the proliferation or the survival of the cells. In contrast, the commonly prescribed cytotoxic chemotherapy agent docetaxel markedly inhibited breast cancer cell growth *in vitro*.

Most of the proangiogenic effects of VEGF seem to be mediated by activation of VEGFR2 (14), and VEGFR2



**Fig. 3.** Effect of motesanib alone, tamoxifen alone, or motesanib (25 mg/kg twice daily) in combination with tamoxifen (30 mg/kg five times per week) on tumor volume and body weight in MCF-7 tumor xenografts. *, $P < 0.05$ versus tamoxifen alone; **, $P < 0.01$ versus motesanib alone. Results from all treatment groups were significant compared with vehicle ($P < 0.05$). Points, mean; bars, SE.

phosphorylation (i.e., activation) has been shown in some breast tumor xenografts (27). Motesanib selectively inhibits the activity of VEGFR1, VEGFR2, and VEGFR3 ($IC_{50} = 2, 3$, and 6 nmol/L, respectively; ref. 23), with its antiangiogenic action likely mediated by inhibition of VEGFR2. The data presented here show significant reductions in tumor volume, viable tumor fraction, and microvessel density with motesanib treatment. Consistent with our findings, several other studies have reported that inhibition of VEGF receptor activity attenuates tumor xenograft growth in preclinical models of breast cancer (27–31). It is tempting to speculate that breast tumor xenografts that express VEGFR2 might respond even more favorably to treatment with motesanib due to the effect both directly on the tumor cells themselves and on its associated vasculature.

Although breast cancer can be described as several distinct malignancies (4, 5), previous studies investigating the effect of targeted therapies on tumor xenografts (27–31) have not included multiple models of individual tumor subtypes. In this study, we showed that motesanib inhibited the growth of xenografts derived from three different breast cancer cell lines, including estrogen-dependent MCF-7 tumor cells and estrogen-independent tumor cells (32). This finding is important as it has been shown that the tumor subtype has a significant effect on disease outcomes. For example, patients with basal-like tumors, compared with luminal subtype tumors, develop distant metastases earlier and have reduced overall survival (5), whereas mesenchymal breast cancer cells may represent the final stages of basal tumor differentiation (33). Furthermore, the type of malignancy can have a significant effect on the response to therapy (2, 34). The ability of motesanib to inhibit growth of xenografts derived from diverse breast cancer cell lines suggests that, in contrast to other targeted therapies such as Herceptin or tamoxifen, motesanib may have broad applicability in the treatment of diverse breast cancer subtypes. In particular, the ability of motesanib to inhibit the growth of Cal-51 tumor xenografts suggests that it may be useful in the treatment of patients with basal-like tumors, which, as mentioned above, have a high relapse rate and are associated with poor outcomes (5). We have tested a variety of chemotherapeutic agents in the Cal-51 xenograft model; all showed minimal activity (data not shown). The lack of activity of many of these agents, including doxorubicin, is consistent with the highly aggressive nature of this type of breast cancer. However, the underlying mechanisms leading to the observed resistance are not known. We tested the combination of motesanib and doxorubicin to determine whether inhibition of tumor growth by motesanib-induced inhibition of angiogenesis could sensitize the tumor cells to doxorubicin. Clearly, this was not observed, suggesting that further studies are needed to better understand the nature of these tumors on a molecular level, which may allow for the identification of better treatment strategies.

Combination treatments of targeted therapies plus conventional chemotherapy agents are currently being investigated and have proven clinically successful in several cancers, including lung cancer and breast cancer (35, 36). By targeting both cell proliferation and tumor vascularization, synergistic antitumor activity may be achieved while minimizing the potential for acquired drug resistance. Our data show that an increased reduction in tumor volume was achieved when
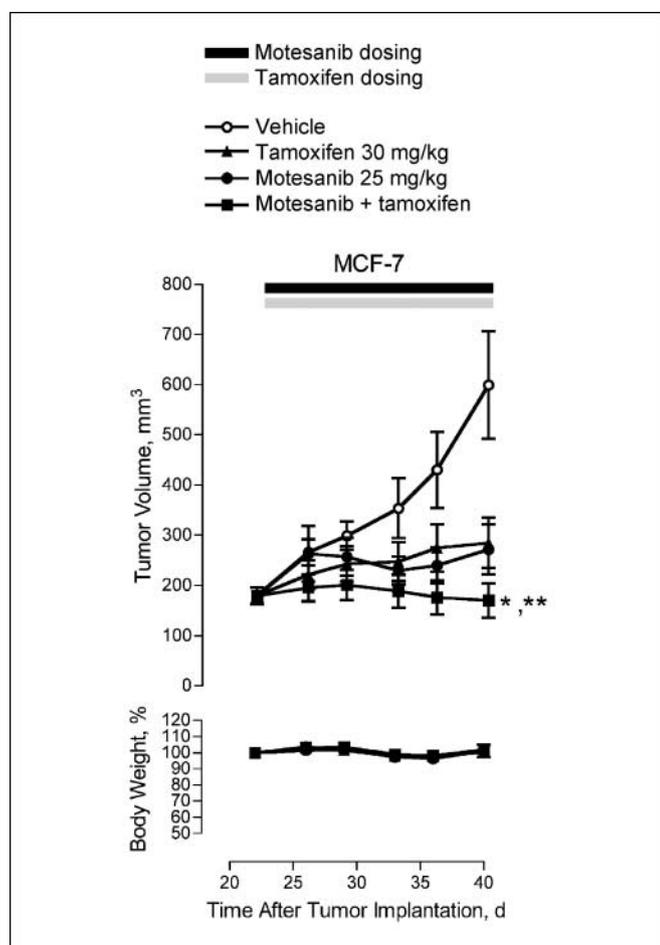
motesanib was combined with the cytotoxic agent docetaxel and with the estrogen receptor modulator tamoxifen compared with either treatment alone. Docetaxel has been previously shown to have antiangiogenic properties that can be enhanced by inhibition of VEGFR2 signaling (37). The additive effect on reduction in tumor volume observed with the combination of motesanib plus docetaxel may be due, at least in part, to inhibition of VEGF signaling, which may increase the anti-tumor activity of docetaxel. The potential use of motesanib in combination with taxanes is currently being explored further in xenograft models of various tumor types. It should be noted that our combination studies used submaximal doses of both motesanib and the tested chemotherapy agents to investigate additive activity. It is possible that even greater antitumor activity could have been achieved if maximal doses were used. However, a more thorough understanding of the observed additive antitumor effect of motesanib plus chemotherapy in mouse models of breast cancer might require survival comparison studies, which were beyond the scope of the current experiments.

Overall, these data are consistent with initial clinical studies that have investigated the safety and efficacy of motesanib in combination with chemotherapy agents in patients with solid tumors, showing no significant drug interaction or toxicity (38–40). Specifically, combination treatment of locally recurrent or metastatic breast cancer with motesanib plus docetaxel is tolerable, with no marked effect on the pharmacokinetic profile of either agent (41). In the study reported here, we noted small reductions in body weight during coadministration of motesanib with docetaxel. Because the pharmacokinetic properties of either agent in mice are different from that in humans, it cannot be concluded that the observed change in the body weight of the animals was a result of alterations in docetaxel exposure (23, 24, 42).
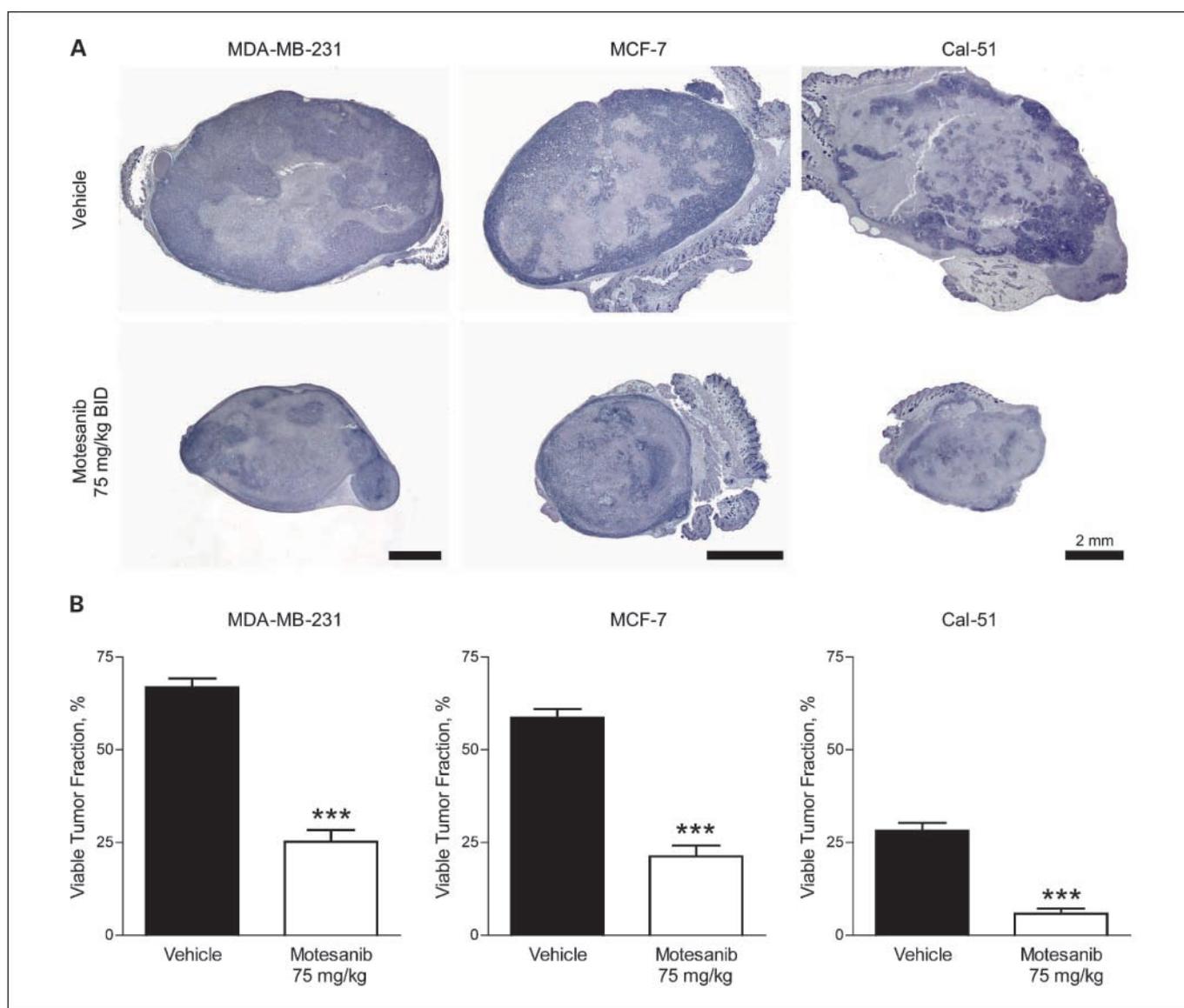


**Fig. 4.** Effect of motesanib treatment (75 mg/kg twice daily) on viable tumor fraction in MDA-MB-231, MCF-7, and Cal-51 human breast cancer xenografts. *A,* representative hematoxylin-stained sections from each treatment group. *B,* quantitation of viable tumor volume by RGB thresholding. ***, $P < 0.0001$. Columns, mean; bars, SE.

**Fig. 5.** Effect of motesanib treatment (75 mg/kg twice daily) on blood vessel area in MDA-MB-231, MCF-7, and Cal-51 human breast cancer xenografts. *A*, representative sections from each treatment group showing CD31 visualized immunohistochemically using 3,3′-diaminobenzidine as the chromogen. *B*, quantitation of tumor blood vessel density. **, $P < 0.01$; ***, $P < 0.0001$. Columns, mean; bars, SE.
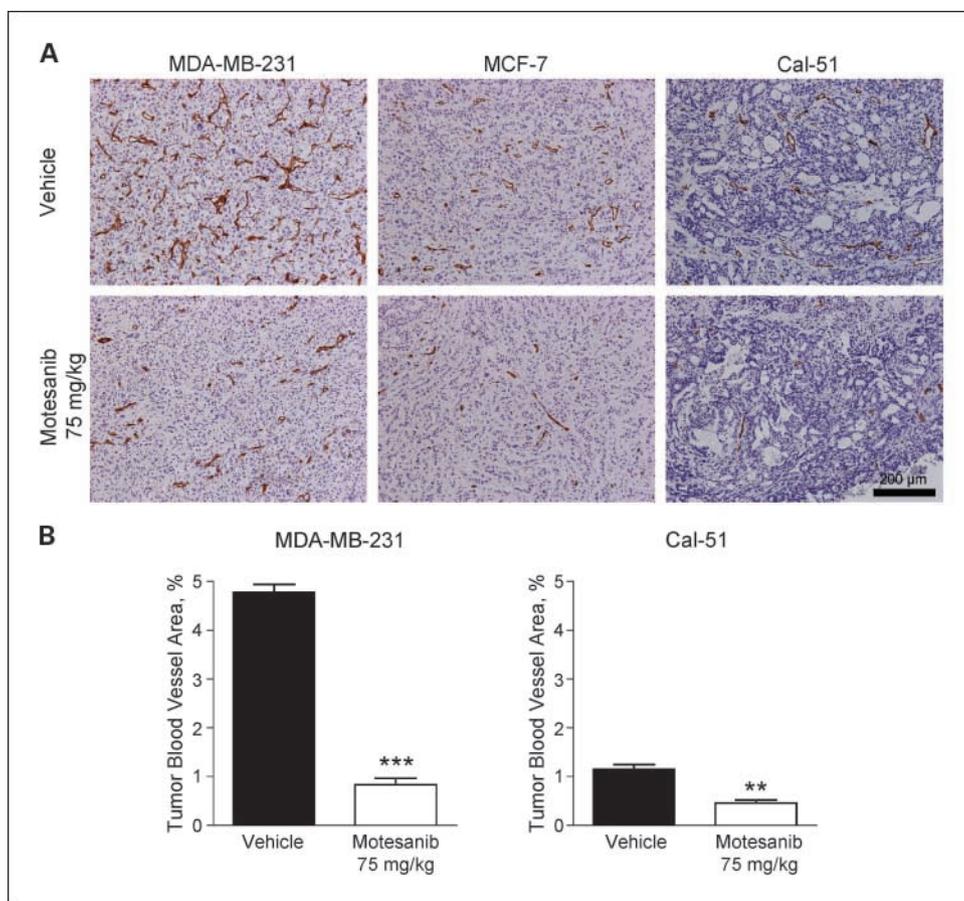
In summary, the data presented here show that treatment with motesanib resulted in a significant, dose-dependent inhibition of tumor growth in xenografts derived from human luminal (MCF-7), mesenchymal (MDA-MB-231), and mixed/progenitor (Cal-51) breast cancer cell lines. Furthermore, treatment with motesanib in combination with the cytotoxic chemotherapy agent docetaxel or with the estrogen receptor modulator tamoxifen resulted in a significantly greater reduction in tumor volume than treatment with either agent alone. The data suggest broad applicability of motesanib treatment alone or in combination with chemotherapy in the management of different breast cancer subtypes and support further development of these treatment regimens in clinical studies.

## Disclosure of Potential Conflicts of Interest

## Acknowledgments

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# Broad Antitumor Activity in Breast Cancer Xenografts by Motesanib, a Highly Selective, Oral Inhibitor of Vascular Endothelial Growth Factor, Platelet-Derived Growth Factor, and Kit Receptors

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