Cancer Therapy: Preclinical

Biological Evidence for Dual Antiangiogenic-Antiaromatase Activity of the VEGFR Inhibitor PTK787/ZK222584 In vivo

Susana Banerjee1, Roger A’Hern2, Simone Detre2, Amanda J. Littlewood-Evans3, Dean B. Evans3, Mitchell Dowsett1,2, and Lesley-Ann Martin1

Abstract

Purpose: Targeting vascular endothelial growth factor (VEGF) and estrogen receptor signaling pathways concomitantly may enhance benefit in estrogen receptor–positive breast cancer. We had shown previously that the VEGF receptor tyrosine kinase inhibitor PTK787/ZK222584 (PTK/ZK) is a competitive aromatase inhibitor in vitro. Here we investigated (a) whether PTK/ZK shows both antiangiogenic and aromatase inhibitory properties in vivo, and (b) whether the combination of PTK/ZK and letrozole is superior to letrozole alone.

Experimental Design: Estrogen-dependent human breast cancer cells engineered to express aromatase (MCF7 AROM 1 and BT474 AROM) were used. Mice were treated with vehicle, PTK/ZK (25, 50, or 100 mg/kg), letrozole, or PTK/ZK in combination with letrozole.

Results: In MCF7 AROM 1 tumors, all treatments induced growth suppression and were associated with a reduction in cell turnover index, a composite measurement of both proliferation and apoptosis. PTK/ZK significantly reduced vessel density. Whereas letrozole caused tumor regression, PTK/ZK stabilized tumor volumes. The growth suppressive and antiangiogenic effects of PTK/ZK were confirmed in BT474 AROM xenografts. The addition of PTK/ZK did not enhance the growth-suppressive effects of letrozole. However, PTK/ZK decreased progesterone receptor (PgR) and TFF1 expression and uterine weight, indicating that PTK/ZK decreases 17β-estradiol (E2) signaling in vivo.

Conclusion: The VEGF receptor inhibitor PTK/ZK showed effects on E2-dependent gene expression consistent with aromatase inhibition as well as antiangiogenesis in xenograft models of breast cancer. The combination with letrozole was not superior to letrozole alone. Overall, these results provide further support for a potential therapeutic approach of dual inhibition of VEGF and E2 signaling using a single agent. Clin Cancer Res; 16(16); 4178–87. ©2010 AACR.

Aromatase inhibitors have improved the treatment of estrogen receptor (ER)-positive breast cancer; aromatase inhibitors show superior efficacy to tamoxifen in both early and advanced breast cancer (1–4). Despite these advances, the efficacy of aromatase inhibitors, at least in the metastatic setting, is limited by inevitable disease progression or relapse. The combination of aromatase inhibitors with agents targeting other key biochemical pathways required for tumor viability has been proposed as a potentially successful strategy to enhance tumor shrinkage and delay endocrine resistance (5). The majority of preclinical models and clinical trials have investigated the combination of aromatase inhibitors with epidermal growth factor receptor/human epidermal growth factor receptor 2 (HER2) inhibitors (6–8). There is extensive evidence supporting the role of angiogenesis in breast cancer, and vascular endothelial growth factor (VEGF) is a critical regulator of this process (9). VEGF and VEGF receptors (VEGFR) have been implicated in resistance to hormonal therapies (10). This has led to the hypothesis that concomitantly targeting the VEGF and ER signaling pathways may provide enhanced therapeutic benefit.

PTK787/ZK222584 (PTK/ZK) was developed as a potent and selective tyrosine kinase inhibitor of VEGFR1-3 (11) and has undergone clinical evaluation for the treatment of breast and other cancers. Although PTK/ZK has been studied in other in vivo models of cancer including melanoma, renal, prostate, and hepatocellular carcinoma (11–13), it had not been previously investigated in models of ER-positive breast cancer. Furthermore, the in vivo effects of the combination of VEGF inhibitors and aromatase inhibitors had not previously been reported. Our primary

Authors’ Affiliations: 1Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, and 2The Royal Marsden Hospital, London, United Kingdom; and 3Novartis Institutes for BioMedical Research, Basel, Switzerland

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org).

Corresponding Author: Lesley-Ann Martin, Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, SW3 6JB, UK. Phone: 44-(0)-207-153-5239; Fax: 44-(0)-207-153-5340; E-mail: Lesley-Ann.Martin@icr.ac.uk.

doi: 10.1158/1078-0432.CCR-10-0456
©2010 American Association for Cancer Research.
Translational Relevance

Although effective treatments are available for estrogen receptor–positive breast cancers, drug resistance and relapse are common. Targeting tumor vasculature by blockade of vascular endothelial growth factor (VEGF) is being explored as a therapeutic strategy for a number of cancers. Concomitant targeting of the VEGF and estrogen signaling pathways may provide enhanced therapeutic benefit in estrogen receptor–positive breast cancer, and this approach is under evaluation in clinical trials. Here we explore this approach in vivo breast cancer models. We show that PTK787/ZK222584 (PTK/ZK), a dual VEGF receptor tyrosine kinase inhibitor and aromatase inhibitor, has dual antiangiogenic and antian- drogenic activity in vivo and stabilizes tumor growth. As PTK/ZK has entered clinical trials for a variety of tumors, these findings may have important clinical implications. Furthermore, these observations may provide the impetus to the development of related compounds that have increased potency and specificity for both targets allowing the exploitation of novel therapeutic approaches.

Human tumor xenografts

All experiments were carried out in accordance with Home Office guidelines and the Institute of Cancer Research Ethics Committee. Female, ovariectomized, athymic (CtTac:NCr-Fox1[Nu]) mice of 6 to 8 weeks of age were kept under sterile conditions with free access to food and water. MCF7 AROM 1 and BT474 AROM xenografts were initiated by inoculation of 100 μl cell suspension containing 10^7 cells in matrigel into the right flank. Subcutaneous xenografts were maintained by daily injection of androstenolone (100 μg/day) or slow-release estradiol pellets (0.75 mg 60-day release pellets, Innovative Research) implanted 14 days prior to cell inoculation. Once tumors reached a diameter of ~7 mm, the mice were randomized to receive daily concentrations of vehicle [10% N-methyl-pyrollidone (NMP)/90% polyethylene glycol (PEG300, Fluka)], PTK/ZK (25, 50, or 100 mg/kg), letrozole (1 mg/kg), or PTK/ZK (50 mg/kg) in combination with letrozole (1 mg/kg). The dose ranges used had previously been described (11, 16).

All drugs were administered by oral gavage and were given daily for 28 days. Tumor growth was assessed twice weekly by caliper measurements of the two largest diameters. Volumes were calculated according to the formula: \( V = \frac{a \times b^2 \times \pi}{6} \), where \( a \) and \( b \) are orthogonal tumor diameters (17). Tumor volumes were expressed as fold change compared with the volume at the start of treatment. Mice from each group were sacrificed before treatment (baseline) and at weekly intervals (n = 5). Tumors were removed and halved, and one half was placed in liquid nitrogen and the other half in formalin-buffered saline. In an additional experiment, mice harboring BT474 AROM tumors were treated with vehicle or PTK/ZK 50 mg/kg twice daily. The uteri from mice in this experiment were harvested and weights were determined.

Immunohistochemistry for Ki67, apoptosis, ER, and PgR

For Ki67, ER, and progesterone receptor (PgR), sections were dewaxed in xylene, gradually rehydrated to water, and endogenous peroxidase activity was blocked with hydrogen peroxidase. Antigen retrieval was achieved by microwaving at 750 watts for 10 minutes in citrate buffer (pH 6.0). Sections were pretreated with avidin using the avidin/biotin blocking kit (Vector Laboratories). For ER, the clone 6F11 (Novostra) primary antibody was used at a dilution of 1:40 for 20 minutes. The PgR 312 (Novostra) antibody was used at a dilution of 1:100 for 20 minutes. For Ki67, the primary antibody was MIB-1 (Dako) at a dilution of 1:50 for 20 minutes. Detection was achieved with the Dako REAL detection system (Dako). The terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay was used to identify apoptotic cells as previously described (18). All sections were examined by light microscopy. ER and PgR immunostaining was quantified by quickscore (range, 0-18; ref. 19). For Ki67, at least 1,000 tumor cells were counted in randomly selected fields throughout the section. Ki67-positive nuclear staining cells
were recorded as percentages. The apoptotic index was calculated from the number of brown staining cells displaying apoptotic bodies of 3,000 cells per section counted under high power and expressed as a percentage. Necrotic areas were excluded.

**CD31 and lectin**

Cryosections (7 μmol/L) were stained for CD31. Briefly, sections were fixed in 4% paraformaldehyde, blocked with normal horse serum in PBS, incubated overnight at 4°C with rat anti-mouse CD31 (BD Pharmingen) followed by an immunoglobulin G conjugated to Alexa Fluor 488 (Molecular Probes), and counterstained with TO-PRO-3. To assess vessel perfusion, mice from each group received FITC-lectin [120 μg; 1 μg/μL; Lucopencin Esculentum (Tomato) Lectin, Vector Labs] via tail vein injection. After 5 minutes mice were sacrificed, and tumors were resected immediately and stored in liquid nitrogen. Lectin staining was visualized by direct immunofluorescence. Images were collected sequentially in three channels on a Leica TCS SP2 confocal microscope. Lectin staining was indicated in green (FITC 488), CD31 and lectin in red (FITC 555), and TO-PRO-3 (633) in blue. The number of CD31-positive vessels in 6 to 8 fields of each section at ×200 magnification were recorded. All discrete staining structures were considered as individual vessels for this purpose. Sections were analyzed by an operator blinded to treatment assignment.

**Quantification of TFF1 expression**

RNA was extracted from xenografts and TFF1 mRNA expression was determined. Real-time quantitative reverse transcriptase-PCR was done on the 7900HT Fast Real-Time PCR System (Applied Biosystems). TFF1 was expressed relative to endogenous controls, KIAA000674 and TBP.

**Statistical analyses**

Growth for individual tumors was assumed to be exponential. The growth rate was estimated by using general estimating equations with log of the volume at a time point (day x) divided by the baseline volume as the dependent variable. Day was a covariate and treatment was included as a factor variable. The treatment by day interaction was computed to estimate the volume changes (relative to the vehicle group) in the different treatment groups. The growth rates were compared among the six groups using the Kruskal-Wallis ANOVA and between-group comparisons were then undertaken using the Mann-Whitney U test. For the analysis of Ki67, apoptosis, cell turnover index (CTI), ER, PgR, and CD31, the Kruskal-Wallis test was applied to check if there was evidence of a difference between groups. This was then explored using a Mann-Whitney test to compare groups. Unpaired one-tailed t-tests done using log transformed values were used for TFF1 expression and uterine weight analyses. Scores were expressed for each group and time point as the arithmetic mean ± SE. Stata 10.1 for Window and GraphPad Prism were used for analysis.

**Results**

**PTK/ZK reduces tumor growth of MCF7 and BT474-derived human breast cancer xenografts**

The MCF7 aromatase-transfected cell line (MCF7 AROM 1) modeling endocrine-sensitive breast cancer was used for in vivo studies (15). These cells, when injected s.c. into immunocompromised mice, form tumors under androstenedione support (20). MCF7 AROM 1 cells convert androstenedione to estradiol providing an intratumoral source of estrogen representative of postmenopausal, hormone-dependent breast cancer. With the use of this model, the effects of increasing doses of PTK/ZK, letrozole, and PTK/ZK in combination with letrozole compared with control on MCF7 AROM 1 tumor growth were studied.

The mean fold change in tumor volume for each treatment is shown in Fig. 1A and B. Tumor volumes in the vehicle-treated mice increased over the study period (mean 2.1 ± 0.16-fold on day 27). The daily growth rate, expressed as daily volume change relative to the vehicle group over the study period, was significantly reduced in all treatment groups compared with vehicle (P < 0.05). However, a clear PTK/ZK dose-dependent effect was not evident (Fig. 1A). Assessment of PTK/ZK and letrozole as monotherapies versus the combination revealed fold changes in tumor volume of 0.98 ± 0.1 for PTK/ZK 50 mg/kg, 0.71 ± 0.1 for letrozole, and 0.60 ± 0.09 for the combination at the end of the study (Fig. 1B). The growth rate over the study period was not significantly different between the PTK/ZK 50 mg/kg and the letrozole group (P = 0.14; Fig. 1B). Growth inhibition was greater in mice treated with the combination of PTK/ZK and letrozole compared with mice treated with PTK/ZK alone (P = 0.03; Fig. 1B). However, there seemed to be no statistical difference between letrozole and the combination of PTK/ZK 50 mg/kg and letrozole. This suggests that the addition of PTK/ZK to letrozole does not enhance tumor growth suppression, at least at this dose.

The effects of PTK/ZK and letrozole were also investigated in a second xenograft model of ER-positive breast cancer using BT474 AROM [ER+, HER2+] cells. This model was chosen because HER2-positive breast cancer has been associated with elevated levels of VEGF (21) and reduced sensitivity to endocrine therapy (22, 23). In the BT474 AROM xenograft, the mean tumor volume fold change for the vehicle group was 2.43 ± 0.27 at day 28 (Fig. 1C). In keeping with the findings of the MCF7 AROM 1 xenograft model, a dose-dependent effect of PTK/ZK was not seen (Fig. 1C). Similar to the MCF7 AROM 1 model, letrozole significantly inhibited growth for the duration of the experiment. In contrast to the previous model, however, letrozole treatment resulted in only disease stabilization (P < 0.001; Fig. 1D), which may be attributed to the increased HER2 expression. In the 50 mg/kg PTK/ZK- and the combination-treated groups, growth inhibition was also achieved (P = 0.002
and \( P = 0.015 \), respectively) resulting in disease stabilization (Fig. 1D). Furthermore, there were no significant differences in tumor volume among mice treated with 50 mg/kg PTK/ZK, letrozole alone, or combination (Fig. 1D). There were no significant alterations in body weight between the vehicle and any of the treatment arms (Supplementary Fig. S1).

**PTK/ZK induces vascular regression in breast cancer xenografts**

To determine the effects of PTK/ZK on the vasculature of breast cancer xenografts, MCF7 AROM 1 tumors were stained for CD31 (Fig. 2A). The number of CD31 staining vessels per high power field (hpf) in the vehicle-treated group remained relatively constant throughout the duration of the study (\( P = 0.14 \); Fig. 2B). The effect of the treatments on the number of vessels was analyzed at individual time points. The number of CD31 staining vessels per hpf was significantly reduced (>50%) in the PTK/ZK (50 mg/kg and 100 mg/kg) and combination groups compared with the vehicle group from as early as day 7, and the effect was maintained throughout the study (\( P < 0.05 \)). A decrease in the number of CD31 staining vessels per hpf was also seen in the 25 mg/kg PTK/ZK group, but this effect occurred at later time points (days 21 and 28). Letrozole had no significant effect on the vessel number at day 7 to 21. At day 28, however, a 42% reduction in vessel number was seen in the letrozole group compared with the vehicle group (\( P < 0.05 \)). Overall, similar effects of PTK/ZK on CD31 staining vessels were seen in the BT474 AROM model. Letrozole, however, had no effect on vessel number in BT474 AROM tumors throughout the study (Fig. 2C). These data from two independent breast cancer cell lines indicate that PTK/ZK can cause significant vascular regression in breast tumors.
PTK/ZK decreases the cell turnover index in vivo

Ki67 staining scores following 7 and 21 days of treatment of MCF7 AROM 1 tumors were assessed to determine whether PTK/ZK affected tumor cell proliferation. Representative images are shown in Fig. 3A. The Ki67 scores pretreatment, at day 7, and at day 21 did not differ significantly in the vehicle-treated tumors (P = 0.10). As expected, following 1 week of treatment with letrozole...
there was a significant reduction in mean Ki67 score to 27 ± 2.6% compared with the vehicle group (Fig. 3A). In contrast, Ki67 did not change significantly in any of the PTK/ZK-treated groups following both 7 and 21 days of treatment (Fig. 3A). In the combination group, there was a significant reduction in Ki67 scores at both time points ($P = 0.016$; Fig. 3A). However, there was no significant difference between the Ki67 values in the letrozole and the combination groups ($P = 0.19$), suggesting that PTK/ZK at 50 mg/kg had no additional effect on cell proliferation compared with letrozole alone.

The mean percentage of apoptotic cells detected by TUNEL pretreatment (day 0) was low (1.6% ± 0.29%). Following therapy for only 1 week, the apoptosis index increased significantly in the 50 mg/kg ($P = 0.016$) and 100 mg/kg ($P = 0.016$) PTK/ZK, letrozole ($P = 0.029$), and combination groups ($P = 0.016$) compared with the vehicle-treated mice (Fig. 3B). After 3 weeks of treatment (day 21), the apoptosis index in the 25 mg/kg PTK/ZK group was significantly increased compared with the vehicle group ($P = 0.016$). However, the apoptosis index in the 50 mg/kg, 100 mg/kg PTK/ZK, and combination groups was reduced back to baseline levels and was no longer significantly different from the vehicle-treated group at day 21. On the other hand, the apoptosis index remained significantly higher than the vehicle group in the letrozole-treated mice ($P = 0.008$).

To take into account the combined effect of proliferation and apoptosis, the cell turnover index (CTI) was calculated by dividing the Ki67 index by the apoptosis index (Fig. 3C). However, there was no significant difference between the CTI at day 7 and day 21 in the vehicle-treated

---

**Fig. 3.** Changes in cell proliferation, apoptosis, and cell turnover index following PTK/ZK, letrozole, or the combination. A, cell proliferation (% of cells positive for Ki67). Representative images of Ki67 staining are shown. B, apoptosis index (% of cells positive for TUNEL). C, CTI. CTI was calculated as % Ki67 positive cells/apoptosis index. Scores pretreatment, and following 7 and 21 days of the indicated treatments in the MCF7 AROM 1 xenograft model are shown. Mean values ± SE are shown.
There was a significant decrease in CTI (>50%) following 1 week of treatment with all treatments (PTK/ZK 50 mg/kg, \(P = 0.016\); PTK/ZK 100 mg/kg, \(P = 0.016\); letrozole, \(P = 0.029\); combination, \(P = 0.016\)), except for 25 mg/kg PTK/ZK, compared with the vehicle group (\(P = 0.41\)). The decrease was greatest with letrozole treatment (vehicle mean CTI, 39; letrozole, 5.6). However, following 21 days’ treatment, the decrease in CTI with 25 mg/kg PTK/ZK became significant (\(P = 0.016\)). The effects of PTK/ZK on CTI did not seem to be dose dependent. The CTI was decreased in the combination group compared with 50 mg/kg PTK/ZK alone (\(P = 0.06\) at day 7; \(P = 0.03\) at day 21). However, the addition of PTK/ZK did not lower the CTI further compared with letrozole alone. These data showed that PTK/ZK, like letrozole, decreased the CTI whereas the combination treatment was not superior to letrozole alone, and are consistent with the effects seen on androstenedione-induced tumor growth (Fig. 1A).

**PTK/ZK suppresses estrogen-dependent events in vivo**

Having established that PTK/ZK inhibits angiogenesis in an ER-positive breast cancer model, we next addressed whether the novel *in vitro* aromatase activity we had identified (14) was sufficient to suppress E2-dependent effects *in vivo*. The plasma levels of E2 were too low to be reliably detected in this model and therefore the level of PgR was measured as a marker of estrogenic stimulation (24). By immunohistochemistry, maximal PgR expression was seen in the vehicle-treated tumors. Following 1 week of therapy, the addition of PTK/ZK at all doses did not significantly affect PgR expression compared with the vehicle group (7.5 ± 0.96; Fig. 4A). In contrast, the mean quick scores for PgR were reduced significantly by almost 50% in the letrozole- (mean score 4.0 ± 0.0; \(P = 0.029\)) and combination-treated arms (letrozole and PTK/ZK 50 mg/kg; mean score, 4.4 ± 0.4; \(P = 0.032\)) as early as day 7, and this effect was maintained following 21 days of treatment.

**Fig. 4.** PTK/ZK displays aromatase inhibitory properties *in vivo*. A, PTK/ZK suppresses PgR expression. MCF7 AROM1 tumors under androstenedione support were treated daily with the indicated treatments. Columns, mean quickscores; bars, ± SE (n = 5). Nonparametric statistical comparisons were made by the Mann-Whitney U test. Representative images of PgR staining are shown. PTK/ZK reduces TFF1 expression (B) and uterine weight (C). BT474 AROM xenografts were established and maintained androstenedione support. Mice were treated with vehicle (n = 3) or 50 mg/kg PTK/ZK (twice daily; n = 3) by oral gavage. Following 3 days of treatment, 6 hours after the last dose, the tumors and uteri were harvested. RNA was extracted from frozen tumors, and quantitative reverse transcriptase-PCR was done. Expression of TFF1 is shown as fold change compared with the vehicle-treated mice. Mean values ± SE are shown. An unpaired one-tailed \(t\)-test was done using log transformed values. *, \(P < 0.05\) compared with the vehicle-treated mice.
of treatment (26). The mean uterine weight in the PTK/ZK-treated mice was significantly reduced compared with the vehicle-treated group (25). The major initial reduction in vascular density following PTK/ZK treatment did not seem to be increased over the time studied. This suggests that the effects of PTK/ZK on the vasculature occur early on and are maintained rather than progressive. This is in contrast to the effects of AZD2171 in the lung tumor model in which vascular regression increased over time (27). A possible explanation for the increased vascular regression over time seen with AZD2171 but not PTK/ZK is that AZD2171 also targets the fibroblast growth factor receptor, which has been implicated in resistance to antiangiogenic therapy (9). However, in a study of AZD2171 in breast cancer xenografts following 4 weeks of therapy, the microvessel density in MCF7VEGF tumor xenografts was not different from that of the vehicle-treated tumors (28) and suggests that the effects of antiangiogenic agents is likely to be dependent on multiple factors including tumor type.

There is preclinical evidence that VEGF may be regulated by estrogen at a transcriptional level, and possible mechanisms include an imperfect estrogen response element (ERE) on the VEGF promoter (29). In addition, estrogen has been shown to increase the extracellular levels of VEGF in both in vitro and in vivo breast cancer models (30). Therefore, by reducing estradiol levels, letrozole treatment may theoretically suppress VEGF production and lead to regression of the vasculature. In the MCF7 AROM xenografts, there was no difference in the number of vessels in the luteozone-vehicle-treated tumors at days 7, 14, and 21. After chronic treatment, however, at day 28, the vascular density was significantly reduced compared with the vehicle-treated group (P < 0.05). This effect was not seen in the BT474 AROM xenograft model and may reflect the fact that MCF7 cells are more sensitive to estrogen deprivation than are BT474 cells as a result of increased HER2 expression. Furthermore, in addition to estrogen, there is evidence supporting VEGF regulation by HER2 (31), and this mechanism may predominate in the BT474 model.

PTK/ZK inhibited tumor growth in the MCF7 AROM and BT474 AROM xenografts. Interestingly, a dose-dependent effect was not seen, and the optimal dose that resulted in disease stabilization in the breast cancer models seems to be 50 mg/kg, which is an effective dose range seen in in vivo models of other tumor types (11, 12). It is noteworthy that, although letrozole has been reported to cause tumor regression (32), xenograft studies of the aromatase inhibitor anastrozole only achieved disease stabilization (33). Therefore, our observation that PTK/ZK failed to cause tumor regression does not exclude the possibility of estrogen-independent mechanisms.

PTK/ZK significantly reduced the vascular density in two models of ER-positive breast cancer: MCF7 AROM 1 and BT474 AROM xenografts. To investigate the temporal effects, the vessels were assessed at several time points up to 28 days of treatment. Significant effects were seen at the early time point, after 7 days of treatment. The rapid vascular regression induced by PTK/ZK is likely to be due to VEGFR inhibition and subsequent VEGF signaling disruption in the endothelial cells affecting endothelial cell proliferation and survival. The early effect on vascular density seen with PTK/ZK is consistent with the results seen with a different VEGFR inhibitor, AZD2171, in a Calu-6 lung tumor model (27) and a xenograft study of MCF7 cells that were transfected with a vector expressing VEGF (MCF7VEGF, ref. 28). The major initial reduction in vascular density following PTK/ZK treatment did not seem to be increased over the time studied. This suggests that the effects of PTK/ZK on the vasculature occur early on and are maintained rather than progressive. This is in contrast to the effects of AZD2171 in the lung tumor model in which vascular regression increased over time (27). A possible explanation for the increased vascular regression over time seen with AZD2171 but not PTK/ZK is that AZD2171 also targets the fibroblast growth factor receptor, which has been implicated in resistance to antiangiogenic therapy (9). However, in a study of AZD2171 in breast cancer xenografts following 4 weeks of therapy, the microvessel density in MCF7VEGF tumor xenografts was not different from that of the vehicle-treated tumors (28) and suggests that the effects of antiangiogenic agents is likely to be dependent on multiple factors including tumor type.

There is preclinical evidence that VEGF may be regulated by estrogen at a transcriptional level, and possible mechanisms include an imperfect estrogen response element (ERE) on the VEGF promoter (29). In addition, estrogen has been shown to increase the extracellular levels of VEGF in both in vitro and in vivo breast cancer models (30). Therefore, by reducing estradiol levels, letrozole treatment may theoretically suppress VEGF production and lead to regression of the vasculature. In the MCF7 AROM xenografts, there was no difference in the number of vessels in the luteozone-vehicle-treated tumors at days 7, 14, and 21. After chronic treatment, however, at day 28, the vascular density was significantly reduced compared with the vehicle-treated group (P < 0.05). This effect was not seen in the BT474 AROM xenograft model and may reflect the fact that MCF7 cells are more sensitive to estrogen deprivation than are BT474 cells as a result of increased HER2 expression. Furthermore, in addition to estrogen, there is evidence supporting VEGF regulation by HER2 (31), and this mechanism may predominate in the BT474 model.

PTK/ZK inhibited tumor growth in the MCF7 AROM and BT474 AROM xenografts. Interestingly, a dose-dependent effect was not seen, and the optimal dose that resulted in disease stabilization in the breast cancer models seems to be 50 mg/kg, which is an effective dose range seen in in vivo models of other tumor types (11, 12). It is noteworthy that, although letrozole has been reported to cause tumor regression (32), xenograft studies of the aromatase inhibitor anastrozole only achieved disease stabilization (33). Therefore, our observation that PTK/ZK failed to cause tumor regression does not exclude the in vivo aromatase inhibition
capability of this drug in contributing to part of its overall in vivo profile.

The combination of PTK/ZK and letrozole, at the doses indicated, was not superior in terms of reduction of tumor volume compared with letrozole alone. This is despite the fact that the vasculature seemed more suppressed with the combination treatment compared with letrozole. Although PTK/ZK had little or no antiproliferative effect, it was proapoptotic and the CTI was significantly reduced as early as day 7. In spite of the fact that tumor growth inhibition was maintained with PTK/ZK following 3 weeks of treatment, the increase in apoptosis was lost by day 21 in the PTK/ZK-treated mice but remained in the letrozole- and the combination-treated groups. These seemingly contradictory results may reflect the early onset of resistance, which with time could be manifested as increased tumor growth. These findings are important for the design of clinical trials, because they do not provide support for combination treatment of a VEGFR inhibitor and an aromatase inhibitor. However, traditional measures of tumor volume change are unlikely to be the best predictor of therapeutic efficacy for antiangiogenic agents because these agents may provide benefit by slowing or halting the expansion of tumors rather than causing large changes in tumor volume. In the clinical setting, progression-free survival may be a better indicator of efficacy. The study duration limit of the xenograft model does not allow for the investigation of such long-term growth effects.

Finally, the xenograft models provided opportunities to investigate whether there was evidence for the novel antiaromatase activity in vitro holding true in vivo (14). The level of circulating E2 in the ovariectomized, athymic mice was too low to be reliably detected. Therefore, surrogate markers of estrogen-dependent effects, TFF1 mRNA expression, PgR protein expression, and uterine weight were chosen to investigate whether PTK/ZK exhibited its antiaromatase activity in vivo. The decreases in PgR expression, TFF1 expression, and uterine weight following PTK/ZK (50 mg/kg twice daily) support a significant decrease in estrogen signaling consistent with antiaromatase activity being achieved in vivo.

To date, there are no previous reports of the effects of VEGF pathway inhibition and E2 deprivation in in vivo models. However, dual inhibition of VEGF and ER signaling is an attractive strategy, and phase II clinical trials are ongoing (sunitinib with exemestane; vandetanib with aromatase). Coxon and colleagues reported on the activity of motesanib, a VEGFR 1-3, platelet-derived growth factor receptor, and c-Kit inhibitor, in combination with tamoxifen in MCF7-derived tumors (34). Treatment with motesanib plus tamoxifen significantly reduced tumor volume compared with each drug alone at day 17 after commencing treatment. In that study, however, analysis of the vasculature was not done. Although a statistically significant difference was only seen at one individual time point, this result is provocative and further studies of motesanib and other VEGFR inhibitors, in combination with endocrine therapy, are warranted to clarify whether this approach is potentially successful.

In conclusion, we have shown that PTK/ZK inhibits breast tumor growth and reduces vascularization in ER-positive breast cancer models in vivo. At the dose used, the combination of PTK/ZK with an aromatase inhibitor is not superior to an aromatase inhibitor alone. To our knowledge, this is the first report of the combination of VEGFR inhibitors and an aromatase inhibitor in an in vivo model. Finally, our data support PTK/ZK having aromatase inhibitory activity in vivo.

Disclosure of Potential Conflicts of Interest

M. Dowsett: commercial research grant, Novartis; consultant/advisory board, Novartis, AstraZeneca, Roche.

Acknowledgments

We thank the Mary-Jean Mitchell Green Foundation, Breakthrough Breast Cancer, and Novartis Pharma AG for funding. S. Banerjee is a recipient of the Avon Breast Cancer Crusade Fellowship. We also acknowledge National Health Service funding to the National Institute of Health Research Biomedical Research Centre.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 02/22/2010; revised 05/03/2010; accepted 05/27/2010; published OnlineFirst 08/03/2010.

References


Clinical Cancer Research

Biological Evidence for Dual Antiangiogenic-Antiaromatase Activity of the VEGFR Inhibitor PTK787/ZK222584 In vivo

Susana Banerjee, Roger A’Hern, Simone Detre, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-0456

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2010/08/17/1078-0432.CCR-10-0456.DC1

Cited articles
This article cites 34 articles, 24 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/16/16/4178.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/16/16/4178.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.