A Multicenter Phase II Trial of ZD6474, a Vascular Endothelial Growth Factor Receptor-2 and Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, in Patients with Previously Treated Metastatic Breast Cancer

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Abstract

Purpose: To determine the efficacy and safety of ZD6474, an orally available inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase with additional activity against the epidermal growth factor receptor (EGFR) tyrosine kinase, in patients with previously treated metastatic breast cancer.

Patients and Methods: Eligible patients had histologically confirmed metastatic breast cancer and had received prior treatment with an anthracycline and taxane; measurable disease was required. Patients were enrolled sequentially into one of two dose cohorts, 100 or 300 mg orally once daily; 28 days defined one cycle. The primary end point was objective response rate; pharmacokinetics and serial pharmacodynamic studies were obtained.

Results: Forty-six patients were enrolled between May 2002 and April 2003, and 44 were evaluable for response. Diarrhea was the most commonly reported toxicity and seemed dose related (grade ≥2: 4.5% and 37.5% in the 100 and 300 mg cohorts, respectively). Rash was reported by 26% of patients but was never worse than grade 2. Seven patients in the 300 mg cohort had asymptomatic grade 1 prolongation of the QTc interval. Hypertension requiring treatment was not reported. There were no objective responses; one patient in the 300 mg cohort had stable disease ≥24 weeks. All patients in the 300 mg cohort and 90% of patients in the 100 mg cohort achieved steady-state concentrations exceeding the IC50 for VEGF inhibition in preclinical models.

Conclusion: ZD6474 monotherapy was generally well tolerated but had limited monotherapy activity in patients with refractory metastatic breast cancer.

Metastatic breast cancer remains a devastating disease, claiming the lives of over 40,000 women in the United States each year (1). Although improved early detection and advances in systemic therapy of early-stage breast cancer have led to a small decline in overall breast cancer mortality since 1989 (2), metastatic breast cancer remains largely incurable with a median survival of 20 to 24 months (3). Further advances require new therapeutic strategies firmly rooted in a detailed understanding of breast cancer biology.

Over the last two decades, substantial laboratory and indirect clinical evidence has accumulated to support the central role of angiogenesis in breast cancer progression (4, 5). Angiogenesis requires stimulation of vascular endothelial cells through the release of angiogenic peptides, of which vascular endothelial growth factor (VEGF) is the most potent and most frequently expressed by invasive breast cancers (6). Several studies have found an inverse correlation between VEGF expression and overall survival in both node-positive and node-negative patients (7–11).

Transforming growth factor α (TGF-α) is a critical mediator of normal mammary gland development and neoplastic transformation. TGF-α binds to the epidermal growth factor receptor (EGFR), activating EGFRs’ tyrosine kinase and stimulating growth. TGF-α expression can be detected in breast cancer cells in vivo and in vitro; overexpression of TGF-α elicits partial transformation of immortalized human and rodent mammary epithelial cells (12). EGFR or the EGFRvIII variant is overexpressed by ∼50% of breast cancers (13, 14). Expression of EGFR increases VEGF production and has been linked to resistance to tamoxifen (15, 16) and trastuzumab (17). Similarly, amplification of the EGFR receptor family member human epidermal growth factor receptor 2 (HER2) increases VEGF production in breast cancers (18); combined inhibition of HER2 and VEGF enhances response in xenograft models (19).

The biological effects of VEGF are mediated through binding to one of three endothelial surface receptors VEGFR-1 (flt-1),
VEGFR-2 (flk-1/kdr), and VEGFR-3 (flt-4); binding to the coreceptor neuropilin enhances signaling (20, 21). ZD6474 is a competitive inhibitor of the ATP-binding site of the flk-1/KDR tyrosine kinase and also inhibits the EGFR tyrosine kinase at submicromolar concentrations. ZD6474 has documented biological activity in a variety of preclinical models, including abrogation of VEGF-induced hypotension, decreased endothelial bone formation, and growth inhibition of tumor xenografts (22–24).

A phase I study of ZD6474 enrolled 49 patients with advanced solid tumors; doses ranged from 50 to 600 mg daily. Pharmacokinetics found a half-life of ~120 hours with significant interpatient variability in C_{max} and AUC. Rash and diarrhea were the most commonly reported toxicities and clearly increased with dose. Asymptomatic QTc prolongation was observed in seven patients. Although QTc prolongation was reported at a variety of dose levels, repolarization abnormalities including changes in T-wave morphology as well as QT and QTc prolongation were more frequent with doses ≥500 mg daily (25).

This phase II trial was conducted to determine the efficacy and safety of ZD6474 as monotherapy in patients with previously treated metastatic breast cancer. Pharmacokinetic analysis of the phase I study suggested that potentially therapeutic levels would be achieved with both the 100 and 300 mg doses whereas minimizing QTc prolongation. To gather further safety information, a sequential study design was chosen with patients initially entered into the 100 mg cohort; enrollment to the 300 mg cohort would proceed in the absence of grade 3/4 QTc prolongation. In addition to response and toxicity end points, detailed pharmacokinetic and pharmacodynamic studies were obtained.

Patients and Methods

Patient eligibility. Women with histologically or cytologically confirmed metastatic breast cancer were eligible if they had received prior therapy with both an anthracycline and a taxane; prior treatment with capecitabine and other cytotoxic agents was allowed but not mandated. Patients were required to have measurable disease according to the Response Evaluation Criteria for Solid Tumors, a performance status of 0, 1, or 2 on the WHO scale, as well as adequate renal, hematologic, and hepatic function. Effective contraception was required in all patients with child-bearing potential.

Patients were excluded if they had active or symptomatic central nervous system metastases, left ventricular ejection fraction of <45%, uncontrolled hypertension, clinically significant arrhythmia, chronic atrial fibrillation, or QTc ≥ 460 milliseconds. To reduce the risk of arrhythmia, patients were excluded if serum potassium was <4.0 mg/L, or calcium or magnesium was outside normal limits. Concurrent use of medications known to stimulate or inhibit CYP 3A4 was prohibited. Therapeutic anticoagulation was prohibited, but prophylactic anticoagulants to maintain a vascular access device were permitted. Patients must not have received prior cytotoxic chemotherapy or extensive radiation therapy within 4 weeks; localized palliative radiation therapy must have been completed at least 2 weeks before study entry. As the primary target of ZD6474 is the VEGF KDR receptor, prior treatment with other EGFR inhibitors was allowed.

Local institutional review boards approved the protocol and patients provided written informed consent before screening.

Treatment plan. Patients were enrolled sequentially into one of two dose cohorts, 100 or 300 mg as a single oral dose at approximately the same time each morning. Twenty-eight days defined one cycle. Patients continued therapy until disease progression or unacceptable toxicity. ZD6474 was interrupted for QT/QTc prolongation of >490 milliseconds, an increase in QTc of >60 milliseconds from baseline to >460 milliseconds, grade ≥3 diarrhea or skin rash, absolute neutrophil count of <1,000/mm^3, platelet count of <75,000/mm^3, bilirubin of >1.5 mg/dl, or aspartate aminotransferase of >7× upper limit of normal. ZD6474 treatment was resumed at a reduced dose (100 mg every other day for the 100 mg cohort, 200 mg for the 300 mg cohort) upon resolution of toxicity to grade <1.

Safety and efficacy assessments. Patients were evaluated before each treatment cycle. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. As prolonged QTc was identified in the phase I study, an electrocardiogram (12-lead) was done before treatment, 1 and 24 hours after the first dose, and thrice weekly during cycle 1. If no QTc prolongation was recorded, electrocardiograms were done weekly during cycle 2 and every 4 weeks during all subsequent cycles. Complete blood count and electrolytes (specifically potassium, calcium and magnesium) were measured weekly during cycle 1 and every 4 weeks during subsequent cycles. Weekly electrolyte monitoring continued if patients reported diarrhea or vomiting. Disease status was assessed after the first two cycles, then every other cycle until progression using the Response Evaluation Criteria for Solid Tumors.

Pharmacokinetics. The phase I study of ZD6474 found significant interpatient pharmacokinetic variability with a 3- to 8-fold difference in C_{max} and a 2- to 6-fold difference in AUC. To correlate ZD6474 exposure with toxicity and change in pharmacodynamic variables, blood samples to measure ZD6474 plasma concentrations were collected before treatment and 1, 2, 4, 6, 7, and 24 hours post-dose (before administration on day 2) on day 1 of cycle 1. Additional samples were obtained with each electrocardiogram and before and after each dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) scan. A validated high-performance liquid chromatography method with tandem mass spectrometric detection was used to determine ZD6474 plasma concentration. Population pharmacokinetic modeling was done using the population pharmacokinetic software NONMEM (26).

Pharmacodynamics. Platelet-poor plasma and urine samples were collected to measure VEGF concentration before treatment, every 2 weeks during cycle 1, and every 4 weeks in subsequent cycles. As platelets contain high levels of VEGF, platelet-poor plasma was used to limit the contribution of platelet VEGF (27–29). Two baseline samples were collected at least 24 hours apart to determine the variability of VEGF levels in the absence of treatment. VEGF was measured using a commercially available ELISA (R&D Systems, Minneapolis, MN). As urine VEGF concentration may be affected by renal function, urine VEGF levels were corrected with reference to urine creatinine.(30, 31) For all ELISAs, samples with a coefficient of variation (CV) of >15% were repeated.

Patients with tumor masses suitable for evaluation (soft tissue, liver, fixed pelvic masses, or bone) of blood flow and vascularity by DCE-MRI underwent serial imaging to assess the effect of ZD6474 on tumor vasculature. Patients were imaged within 7 days before the start of therapy, after cycles 1 and 2 and every other cycle until progression. DCE-MRI data were acquired using a fast three-dimensional spoiled gradient-echo sequence, optimized for time-resolved contrast-enhanced MR angiography and analyzed centrally (Perceptive Informatics, Boston, MA). Image data were analyzed to derive k_{trans} (transfer constant; ref. 32) and initial area under the curve (IAUC), defined over 60 seconds post-contrast arrival in the tissue (33). The reproducibility of this DCE-MRI acquisition and analysis methodology has been investigated in abdominal tumors: the 95% confidence limit was ±33% for k_{trans} and ±47% for IAUC 60 with a test-retest CV of 0.18 for IAUC 60 and 0.13 for k_{trans} (34).

Skin biopsies were obtained for assessment of EGF, phosphorylated EGFR, activation of downstream signaling pathways, and angiogenesis. Paired skin biopsies (3-4 mm of full thickness punch) were collected.
before treatment and after cycles 1 and 2. Each biopsy pair consisted of an initial and repeat biopsy harvested from the same location 7 days later. Tumor samples were collected and processed similarly in consenting patients with accessible lesions. Analysis of skin and tumor biopsies will be reported separately.

**Statistical analysis.** The primary objective was objective response rate at each of two dose levels (100 and 300 mg/d) based on the Response Evaluation Criteria for Solid Tumors. As patients were enrolled sequentially rather than randomized into the two dose cohorts, statistical comparison between the two dose levels was not planned or done. Secondary objectives included safety, disease control rate, progression-free survival, and overall survival. Disease control rate was defined as the proportion of patients with a complete response, partial response, or stable disease at 24 weeks. Progression-free survival was defined as the time from first dose until progression or death. Overall survival was defined as the time from first dose until death or last follow-up. Disease control rate was calculated from first dose to 24 weeks.

**Results**

Forty-six patients were enrolled between May 2002 and April 2003. Patient characteristics are shown in Table 1. All patients were evaluable for toxicity. Forty-four patients were evaluable for response. One patient was lost to follow-up before assessment of response. One patient, later found to have metastatic carcinoid rather than breast cancer, was excluded from efficacy analyses.

ZD6474 treatment was well tolerated with few patients experiencing grade 3 or 4 toxicities (Table 2). Diarrhea was the most commonly reported and seemed dose related. Nine (37.5%) patients in the 300 mg cohort compared with one (4.5%) patient in the 100 mg cohort developed grade ≥2 diarrhea. Rash was reported by 26% of patients overall but was never worse than grade 2. After 4 weeks of ZD6474 therapy,
mean QTc increased 5.8 ± 32.96 and 20.9 ± 18.75 milliseconds in the 100 and 300 mg cohorts, respectively (Fig. 1). Seven patients in the 300 mg cohort had asymptomatic grade 1 prolongation of the QTc interval. One patient in the 100 mg cohort died from a presumed pulmonary embolus 3 days after documented disease progression. As she had been restricted to a wheelchair for 4 weeks due to a femur fracture, the event was considered unrelated to ZD6474. Other toxicities were mild and easily managed. Seven (29.2%) patients in the 300 mg cohort and two (9.0%) in the 100 mg cohort required dose interruption or reduction but no patient discontinued treatment due to toxicity.

There were no objective responses. One patient in the 300 mg group had stable disease for ≥24 weeks. Median time to progression was similar in both groups: 45 days in the 300 mg group and 44 days in the 100 mg group (Fig. 2).

ZD6474 plasma concentrations were measured in all 46 patients. Forty patients completed sampling throughout the first 4-week treatment cycle. Four patients only underwent pharmacokinetic sampling through day 15. In two patients, both in the 300 mg dose group, samples were only provided for the first 3 and 5 days of cycle 1. The pharmacokinetics of ZD6474 was best described by a two-compartment, first-order absorption, first-order elimination with lag-time model (Table 3). Figure 3 compares the individual model predicted plasma concentrations to the observed values. The mean population absorption rate constant was 0.579 hour with a lag time of 0.851 hour, consistent with relatively slow oral absorption. Mean population total clearance was 9.39 L/h; mean volume of the central compartment was 1,630 liters and of the peripheral compartment 1,410 liters. Thus, ZD6474 is extensively distributed to the tissues and is slowly cleared from the central compartment. Based on these variables, the mean population terminal half-life is about 9 days. Consistent with the half-life, steady state was achieved in three quarters of all assessable patients by the end of cycle 1.

In the 100 mg cohort, the steady-state ZD6474 plasma concentrations for individual patients (~250-900 ng/mL) exceeded the projected IC50 for inhibition of VEGF-stimulated human umbilical vascular endothelial cell (HUVEC) proliferation (285 ng/mL; assuming ~10% free drug in human serum) in 90% of patients, half within the first 2 weeks of therapy. In contrast, only one patient ever achieved a steady-state

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**Table 3. Mean population pharmacokinetic variables**

<table>
<thead>
<tr>
<th>Mean population pharmacokinetic variable</th>
<th>Interpatient variability</th>
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<tbody>
<tr>
<td>CL/F (L/h)</td>
<td>9.39 (6.90)</td>
</tr>
<tr>
<td>V2/F (L)</td>
<td>1,830 (7.18)</td>
</tr>
<tr>
<td>Ka (L/h)</td>
<td>0.579 (17.2)</td>
</tr>
<tr>
<td>Q/F (L)</td>
<td>14.8 (26.2)</td>
</tr>
<tr>
<td>V3/F (L)</td>
<td>1,410 (16.9)</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>0.851 (4.59)</td>
</tr>
<tr>
<td>Residual error</td>
<td>Proportional %</td>
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<tr>
<td></td>
<td>CV = 11.1%</td>
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<tr>
<td></td>
<td>SD = 27.6 ng/mL</td>
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Abbreviations: CL/F, total body clearance of drug from plasma; V2/F, volume of the central compartment; Ka, absorption rate constant; Q/F, intercompartmental clearance rate after oral dose; V3/F, volume of peripheral compartment after oral dose.

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**Fig. 2.** Time to disease progression by Kaplan-Meier analysis: 100 mg group (n = 22) and 300 mg group (n = 24).

**Fig. 3.** Individual observed and predicted concentrations of ZD6474.

**Fig. 4.** Variability of VEGF concentration at baseline. Two baseline (A) plasma (n = 33) and (B) urine (n = 31) samples were obtained at least 24 hours apart before entry into the study.
concentration above the projected IC₅₀ for inhibition of EGFR-stimulated HUVEC proliferation (808 ng/mL). All patients in the 300 mg cohort reached steady-state concentrations (~550-1,750 ng/mL) greater than the projected IC₅₀ for inhibition of VEGF-stimulated HUVEC proliferation, three quarters within the first 7 days of treatment. Steady-state concentrations reached or exceeded the projected IC₅₀ for inhibition of EGFR-stimulated HUVEC proliferation in 60% of patients.

Two baseline plasma (n = 33) and urine (n = 31) samples were obtained at least 24 hours apart to determine the variability of VEGF concentrations in the absence of active treatment before entry into the study (Fig. 4). Plasma VEGF was below the limit of detection (31.5 ng/mL) in both baseline samples in 13 patients. Plasma VEGF concentration varied significantly with a mean change of –30.9% (range, 79.8% to –534%); results varied by more than ±50% in 13 (39%) patients. Urine VEGF was below the limit of detection in both baseline samples in five patients. Urine VEGF concentration was similarly variable with a mean change of 0.7% (range, 85% to –382%); results varied by more than ±50% in 16 (51.6%) patients. Given the variability of VEGF concentration and the lack of objective responses, attempts to correlate VEGF variables with efficacy were abandoned.

DCE-MRI scans were available at baseline (18 patients), 4 weeks (19 patients) and 8 weeks (five patients). Problems with image acquisition and data quality that would adversely affect estimation of IAUC 60 and Ktrans resulted in exclusion of all DCE-MRI data in eight patients; the 8-week scan only was excluded in one additional patient. DCE-MRI results for liver metastases are available at baseline and 4 weeks for six subjects at 100 mg and five subjects at 300 mg ZD6474. Plots showing individual patients’ values over time are presented for IAUC 60 and Ktrans, with data shown as absolute values over time by initial dose level (Fig. 5).
As only limited data are available at subsequent time points, further evaluation focused on change in IAUC 60 and $K_{\text{trans}}$ from baseline to week 4. At week 4 for tumor IAUC 60, the geometric mean for percentage change from baseline is 1.05 for the 100 mg group and 0.8 for the 300 mg group (i.e., an estimated percentage change of 5% and −20%, respectively). Data in the 100 mg group were more variable than the 300 mg group (CV, 63% and 23%, respectively). At week 4 for $K_{\text{trans}}$, the geometric mean for percentage change from baseline is 0.87 for the 100 mg group and 0.88 for the 300 mg group (i.e., an estimated percentage change of −13% and −12%, respectively). As with IAUC 60, $K_{\text{trans}}$ was more variable in the 100 mg group (CV, 63% versus 21%).

Individual predicted clearance (CL/F) and volume of distribution (V2/F) with the percentage change in the IAUC 60 and $K_{\text{trans}}$ from those patients that underwent a DCE-MRI scan are presented in Fig. 6.

**Discussion**

We report the first phase II study of ZD6474, an inhibitor of the VEGFR-2 and EGFR tyrosine kinases, in patients with metastatic breast cancer. All patients had received prior anthracycline and taxane therapy and most had visceral involvement. Despite the advanced and heavily treated patient population, ZD6474 treatment was generally well tolerated. ZD6474 interacts with cardiac ion channels leading to repolarization abnormalities and QTc prolongation. As expected, we observed mild, dose-dependent QTc prolongation, but no patient developed symptomatic arrhythmia. The most common toxicities seemed primarily due to EGFR inhibition but rarely limited therapy. ZD6474 monotherapy has only limited activity in patients with previously treated metastatic breast cancer. No objective responses were observed, although one patient had prolonged stable disease while receiving ZD6474 treatment.

Dose-dependent changes in the DCE-MRI variables IAUC 60 and $K_{\text{trans}}$ have been reported with other antiangiogenic drugs (35, 36) and vascular-targeting agents (33). In this study, after 4 weeks of ZD6474 treatment, two of six patients in the 100 mg cohort showed a decrease from baseline in tumor IAUC 60 values compared with four of five patients in the 300 mg cohort. However, the changes in DCE-MRI variables we detected were mainly within the range of normal variability for this technique.

We offer several possible explanations for the lack of efficacy. First, we may not have achieved adequate concentrations of ZD6474. Although the majority of patients achieved plasma concentrations above the IC50 for VEGF inhibition, toxicities commonly associated with other VEGF inhibitors (i.e., hypertension, headache, thrombosis, and bleeding; refs. 37–39) were not seen in our study. The one case of thromboembolism was not considered related to ZD6474. The lack of effects on blood pressure and coagulation combined with the lack of effect on tumor perfusion with DCE-MRI all suggest insufficient inhibition of VEGF. The lack of high-grade rash suggests suboptimal inhibition of EGFR as well. Importantly, the VEGF and EGFR IC50 values were calculated based on *in vitro* inhibition of VEGF- and EGFR-stimulated HUVEC proliferation and not *in vivo* inhibition of tumor growth. *In vitro* assays can not model the heterogeneity of tumor associated endothelial cells (40, 41) or regional differences in tumor oxygenation (42) and VEGF production (43) that might alter sensitivity to ZD6474.

Second, the weight of preclinical (44–46) and emerging clinical evidence suggest that the optimal time to intervene with an antiangiogenic agent is earlier in the course of disease. Angiogenic pathways become more numerous and redundant as breast cancer progresses (6), thereby making VEGF less critical for continued tumor growth. Thus, it is unlikely that inhibition of VEGF or any other single factor or pathway will produce an objective clinical response in patients with such extensively pretreated, refractory disease as in our study population. In comparison, addition of bevacizumab, a VEGF-targeted monoclonal antibody, to first-line chemotherapy in patients with metastatic colorectal cancer increased response rates and prolonged overall survival (47), but the addition of bevacizumab to capecitabine in patients with heavily pretreated breast cancer produced only a short-lived increase in response rates with no effect on disease-free or overall survival (48). Consequently, we suggest that future trials...
focus on patients with less advanced disease when fewer redundant angiogenic pathways exist. Third, TGF-α and EGFR are important mediators of normal mammary gland development and neoplastic transformation, but the role of EGFR inhibition in the treatment of breast cancer remains uncertain. Inhibition of EGFR as monotherapy had little effect on tumor growth in xenograft models (49). Phase II trials assessing the activity of the EGFR-directed tyrosine kinase inhibitors gefitinib (ZD1839, Iressa, AstraZeneca, Boston, MA) and erlotinib (OSI-774, Tarceva, Genentech, South San Francisco, CA) as monotherapy in advanced breast cancer yielded similar results (50–52). A gefitinib trial of 63 patients with predominantly visceral disease and extensive prior therapy reported a response rate of 1.6%, with a clinical benefit rate of 4.8% (one partial response, two stable disease; ref. 50). Of 47 patients who had had prior anthracycline, taxane, and capcitabine therapy treated with erlotinib, 2.1% of patients responded, with one partial remission lasting 23 weeks (52). The frequency and clinical relevance of the recently described EGFR-activating mutations in breast cancer remains unknown, as mutations have only been found to date in non–small cell lung carcinoma (53, 54).

Finally, this study used ZD6474 as a general therapy given on a population basis, rather than as a targeted therapy given to patients with a specific molecular phenotype. Methods to select those patients most likely to benefit from VEGF- and/or EGFR-directed therapies are clearly needed. Unfortunately, the lack of objective responses limits our ability to identify potential surrogate markers in this study. The variability of plasma and urine VEGF levels during the baseline period was somewhat unexpected and raises an important caution. Although commonly measured in early clinical trials of antiangiogenic therapies (55–57), we found no previous reports of the reproducibility of VEGF levels over short periods in the absence of treatment. Such variability could easily lead to spurious conclusions, especially in small trials. Future studies using these and other potential surrogates of biological activity should carefully define the baseline variability and magnitude of change that can be reliably detected and attributed to treatment.

References


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