Targeting FGFR With Dovitinib (TKI258): Preclinical and Clinical Data in Breast Cancer

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Conflicts of interest: Stephanie Deudon, Michael Shi, Yong Zhang, Andrea Kay, Alejandro Yovine, and Diana Graus Porta are employees of Novartis Pharmaceuticals Corporation. Fabrice André, Thomas Bachelot, Nicholas Turner, and Jose Baselga have consulted with Novartis Pharmaceuticals Corporation. Mario Campone has consulted with and received research funding from Novartis Pharmaceuticals Corporation. Hope Rugo has received research funding from Novartis Pharmaceuticals Corporation. No potential conflicts of interest were disclosed by the other authors.

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ABSTRACT

Purpose: Fibroblast growth factor receptor 1 (FGFR1) and FGFR2 amplifications are observed in approximately 10% of breast cancers and are related to poor outcomes. We evaluated whether dovitinib (TKI258), an inhibitor of FGFR1, FGFR2, and FGFR3, presented antitumor activity in FGFR-amplified breast cancers.

Experimental Design: Preclinical activity of dovitinib was evaluated in breast cancer cell lines and an FGFR1-amplified xenograft model (HBCx2). Dovitinib was then evaluated in a phase II trial that included four groups of patients with human epidermal growth factor receptor 2–negative metastatic breast cancer based on FGFR1 amplification and hormone receptor (HR) status. FGFR1 amplification was assessed by silver in situ hybridization. Preplanned retrospective analyses assessed predictive value of FGFR1, FGFR2, and FGF3 amplifications by quantitative polymerase chain reaction (qPCR).

Results: Dovitinib monotherapy inhibits proliferation in FGFR1- and FGFR2-amplified but not in FGFR-normal breast cancer cell lines. Dovitinib also inhibits tumor growth in FGFR1-amplified breast cancer xenografts. Eighty-one patients were enrolled in the trial. Unconfirmed response or stable disease > 6 months was observed in five (25%) and one (3%) patients with FGFR1-amplified/HR-positive and FGFR1-nonamplified/HR-positive breast cancer. When qPCR-identified amplifications in FGFR1, FGFR2, or FGF3 were grouped to define an FGF pathway–amplified breast cancer in HR-positive patients, the mean reduction in target lesions was 21.1% compared with a 12.0% increase in patients who did not present with FGF pathway–amplified breast cancer.
Conclusion: Dovitinib demonstrated antitumor activity in FGFR-amplified breast cancer cell lines and may have activity in breast cancers with FGF pathway amplification.

**Statement of Translational Relevance:** FGFR1 amplification in breast cancer is associated with resistance to endocrine therapy and poor prognosis. Dovitinib has demonstrated antitumor activity in advanced breast cancer with FGF pathway alterations suggesting that FGFR could be a therapeutic target in these patients and warrants further investigation.
INTRODUCTION

Breast cancer is segmented into molecular subgroups defined by genomic alterations involved in tumor progression which could identify patient populations best treated with targeted agents. One such population may be patients with gene alterations in the fibroblast growth factor (FGF) pathway, which consists of 18 different FGF ligands that act on 4 transmembrane tyrosine kinase FGF receptors (FGFR 1-4) (1).

Multiple genetic alterations in FGFRs have been identified in breast cancer. For example, amplification of FGFR1 (8p11-12) is present in 8% to 15% of all breast cancer (2-4) and 16% to 27% of luminal type B breast cancer (5). These amplifications correlate with FGFR1 overexpression and are associated with resistance to endocrine therapy and poor prognosis (3, 5). Preclinical studies suggest that targeting FGFR1 could lead to antitumor effects by decreasing cell viability and restoring endocrine therapy sensitivity. Several other genomic alterations of the FGF pathway have also been observed in breast cancer (6, 7), including FGFR2 and FGF3/4/19 amplification.

Dovitinib (TKI258) is an oral tyrosine kinase inhibitor (TKI) with in vitro IC$_{50}$ values against FGFR1-3, VEGFR1-3, and platelet-derived growth factor receptor (PDGFR) of approximately 10 nM (8, 9). Phase I studies have suggested that dovitinib has a tolerable safety profile and effectively targets FGFR at therapeutic doses. In order to determine whether FGFR inhibition could lead to antitumor effects in patients with breast tumors harboring FGFR amplifications, we first evaluated the preclinical activity of dovitinib in breast cancer preclinical models, then conducted a phase II trial of dovitinib in patients with metastatic breast cancer, with and without...
**MATERIALS AND METHODS**

**Preclinical Studies**

Effects of dovitinib on cell proliferation using methylene blue staining were assessed after a 72-hour exposure in 13 breast cancer cell lines, including two with \textit{FGFR1} (MDA-MB-134) or \textit{FGFR2} (SUM52) amplification. Effects of dovitinib on FGFR signaling were assessed by detection of phosphorylated FGFR substrate 2 (pFRS2) and phosphorylated extracellular signal–regulated kinase/mitogen-activated protein kinase (pERK/MAPK) via Western blot using rabbit polyclonal anti-pFRS2 (Tyr196), anti-pMAPK (Thr202/Tyr204), or mouse monoclonal anti–β-tubulin (Cell Signaling Technology, Danvers, MA). Athymic nude mice were implanted with a human \textit{FGFR1}- or \textit{FGFR2}-amplified breast cancer xenograft, HBCx-2 or HBCx-3, respectively. Ten mice per group were treated for up to 42 days (HBCx-2) or 35 days (HBCx-3) with vehicle or dovitinib (30 or 50 mg/kg daily [HBCx-2] or 40 mg/kg daily [HBCx-3] by oral gavage) and tumors were measured twice weekly. The percent tumor volume change of the treatment over control group (T/C) was calculated by dividing the change in mean tumor volume of the drug-treated group by the change in the mean tumor volume of the control-treated group and multiplying by 100.

**Study Design and Treatment**
This nonrandomized phase II trial evaluated dovitinib in female patients with HER2-negative metastatic breast cancer. Patients were divided into 4 cohorts based on hormone receptor (HR; ER or PR) status (positive [+] and negative [-]) and FGFRI amplification status. Patients having an average of 6 or more copies of FGFRI assessed by fluorescent–, chromogenic–, or silver in situ hybridization (FISH, CISH, or SISH) were considered as FGFRI amplified (FGFRI⁺). SISH was performed centrally in formalin fixed and paraffin embedded (FFPE) tumor tissue sections for all patients using the ultraView SISH DNP Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ), either retrospectively for confirmation of local assessments, if available, or prospectively for those centers where local assessment was not performed. The study results are presented using central SISH. The study followed a Simon 2-stage design (10) for each cohort to test the null hypothesis that the response rate (confirmed complete response + partial response) was ≤5% versus the alternative hypothesis that the response rate was >5% using a 1-sided test with 10% level of significance and 78% power at the alternative response rate of 15%. Twenty patients were planned to be enrolled in each cohort for each stage. All patients provided informed consent. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, with the protocol and all amendments reviewed by the independent ethics committee or institutional review board for each center. The trial was registered on www.clinicaltrials.gov (NCT00958971).

Dovitinib was administered orally 500 mg/day (5 days on/2 days off) in 28-day cycles (11). The primary objective was to determine the overall response rate in patients with measurable disease at baseline according to Response Evaluation Criteria In Solid Tumors (RECIST) v1.0 (12). Complete response and partial response (PR) had to be confirmed in a second assessment after 4-6 weeks.
Similarly, stable disease (SD) had to last at least 6 weeks. Secondary objectives and exploratory analyses included progression-free survival (PFS), safety, and analysis of predictive value for FGFR1, FGF3, and FGFR2 as determined by quantitative polymerase chain reaction (qPCR). Tumor measurability at baseline, responses, and PFS were assessed locally and centrally. A second independent central review (adjudication) was performed for discordant cases between local and first central review. The adjudicating radiologist was blinded to treatment arm and prior assessment. Adjudicated results are reported here.

Eligibility Criteria

Women ≥18 years old with histologically confirmed HER2-negative metastatic breast cancer were eligible for the trial. The primary tumor, metastatic axillary lymph nodes, or biopsy of metastatic tumor must have been tested by FISH, CISH, or SISH for FGFR1 amplification (defined as ≥6 copies) by a designated local investigator or a reference laboratory before study entry with archival tumor tissue available for central confirmation. Patients with HR+ disease had to have received at least 1 line of endocrine therapy, and less than 3 lines of chemotherapy in the metastatic setting, whereas patients with HR− disease had to have received between 1 and 3 lines of chemotherapy in the metastatic setting. Additional inclusion criteria included measurable disease, World Health Organization (WHO) performance status of 0 or 1, left ventricular ejection fraction (LVEF) ≥45%, and adequate bone marrow, hepatic, and renal function. Patients with liver metastases were eligible if they had ≤ grade 2 alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Patients on chronic treatment with corticosteroids or other immunosuppressive agent or those
with brain metastases or significantly decreased cardiac function were excluded from study participation.

**Biomarker Analyses**

Blood samples were collected predose on days 1, 5, and 26 of cycle 1, day 26 of cycle 2, and day 1 of odd numbered cycles beginning at cycle 5. A 6-hour postdose sample was also collected on cycle 1, day 5. Plasma FGF23 was measured as a surrogate marker of FGFR1 inhibition using a commercial enzyme linked immunosorbent assay (Kinos Laboratories, Inc., Tokyo, Japan). Tumor copy number variations of FGFR1, FGFR2, and FGF3 were quantified by qPCR to test the predefined hypothesis that FGFR1, FGFR2, or FGF3 amplifications would identify patients who are highly sensitive to dovitinib. Briefly, DNA was extracted from FFPE sections using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). TaqMan® Copy Number Assays (Life Technologies Corporation, Grand Island, NY) for FGFR1, FGFR2, and FGF3 were normalized with the copy number obtained from a simultaneous analysis using the TaqMan® Copy Number Reference Assay (ribonuclease P [RNAase P]) in a duplex real-time PCR. The cutoff for FGFR1 and FGF3 gene amplification was ≥6 copies, consistent with the predefined FGFR1 gene amplification threshold (by hybridization) in the trial protocol. A threshold of ≥4 FGFR2 copies by qPCR was predefined based on the previous report of Turner, et al (7).

**RESULTS**

*Dovitinib Had Antitumor Activity in FGFR-Amplified Breast Cancer Models*
Dovitinib decreased the concentrations of pFRS2 and pERK/MAPK in a dose-dependent manner in MDA-MB-134 (FGFR1 amplified) and SUM52 (FGFR2 amplified) cell lines (Fig 1A). Because these data indicated that dovitinib effectively inhibited FGFR signaling in vitro, we further explored whether dovitinib preferentially inhibited proliferation of FGFR1- and FGFR2-amplified breast cancer cell lines. The IC50 for cell growth inhibition was 190 nM and 180 nM in MDA-MB-134 and SUM52, respectively (Fig 1B; Supplementary Table S1). Conversely, IC50 values were more than 2,000 nM in the 11 breast cancer cell lines that had neither FGFR1 nor FGFR2 amplification. We then tested the antitumor activity of dovitinib in a FGFR1-amplified in vivo model (HBCx-2 breast cancer primary xenograft, with eight FGFR1 gene copies (13). As shown in Fig 1C, dovitinib prevented tumor growth at the 30 mg/kg dose and caused tumor regression at the 50 mg/kg dose. On day 28, T/C calculations showed that the mean tumor volume was 24.6% and 7.2% of the vehicle control for the 30- and 50-mg/kg groups, respectively (P < .001 by Mann-Whitney nonparametric test). We also evaluated dovitinib in a FGFR2-amplified in vivo model (HBCx-3; ten FGFR2 copies). Similar to the results above, dovitinib caused tumor regression in HBCx-3 xenografts when administered at 40 mg/kg daily until day 35 (Fig 1D). On day 28, T/C calculations showed a mean tumor volume for the dovitinib-treated group was 23.4% of the vehicle control group (P < .001). Overall, these data suggested that dovitinib had antitumor activity in FGFR-amplified breast cancer models.

*Patient Characteristics*
Based on the data observed in cell lines, together with consistent results from other groups (5, 14), we conducted a phase II trial to test the hypothesis that dovitinib has antitumor activity in patients with FGFR1-amplified breast cancer. In addition to enrolling patients with FGFR1-amplified tumors, patients with nonamplified tumors were enrolled to determine if dovitinib’s ability to inhibit other targets (i.e., VEGFR and PDGFR) would mediate FGFR-independent antitumor activity. A retrospective analysis was also preplanned to evaluate the activity of dovitinib in patients with FGFR2- and FGF3-amplified breast cancers. Of the 243 HER2– patients who underwent molecular prescreening (i.e., tumor sample analyzed for FGFR1 assessment), 116 did not continue to the protocol specific screening phase due to nonassessable FGFR1 status (insufficient tumor or insufficient SISH signal, n = 24) or the assigned treatment arm being closed to additional enrollment (n = 92) (Figure 2). For example, enrollment to the FGFR1– arms was completed early and closed to accrual, with screening continued in order to complete accrual of the FGFR1+ arms. Of the 127 patients who entered the protocol specific screening phase, 46 were excluded from study entry due to not meeting inclusion criteria, most commonly due to hematology and biochemistry laboratory values outside the requested range. The remaining 81 patients were enrolled and received treatment, with a median time of 18.3 months from first treatment to analysis cutoff. These 81 patients were divided into 4 cohorts based on FGFR1 amplification and HR status: FGFR1+/HR+ (n = 23), FGFR1+/HR− (n = 2), FGFR1−/HR+ (n = 34), and FGFR1−/HR− (n = 22) (Table 1). Of note, there were very few cases of FGFR1-amplified HR− disease (n = 2), confirming there is a very low incidence of FGFR1 amplification in patients with triple-negative breast cancer, and this arm was stopped prior to completion of enrollment.
Patient characteristics for the fully enrolled cohorts (\textit{FGFR1}+/HR+, \textit{FGFR1}−/HR+, and \textit{FGFR1}−/HR−) are reported in Table 1. Most patients had late-stage breast cancer and were heavily pretreated. For example, 70% and 78% of the \textit{FGFR1}+/HR+ patients presented with ≥3 organs involved and liver metastases, respectively. Additionally, 95% of all patients were previously treated with chemotherapy in the metastatic setting and all but 10 HR+ patients previously received therapeutic endocrine therapy. Two patients who did not receive any endocrine therapy (adjuvant, neoadjuvant, or therapeutic) were already refractory to prior chemotherapy at study entry and were not considered candidates for endocrine therapy as per the investigator’s judgment.

\textit{Dovitinib Effectively Increases FGF23 Plasma Levels, Indicative of FGFR1 Inhibition}

FGF23 has been identified as a target gene of FGF signaling \textit{in vitro} (15). Furthermore, increases in FGF23 have previously been reported as a surrogate for FGFR1 inhibition in clinical trials of dovitinib in renal cell carcinoma and melanoma (11, 16, 17). In these trials, 50% to 100% increases of plasma FGF23 from baseline were detected at cycle 1, day 15 and were correlated with reduced pERK (17). In the data presented here, plasma FGF23 levels peaked ≈ 100% above baseline at cycle 1, day 8 and were sustained over time, suggesting that dovitinib effectively inhibited FGFR1 signaling (Supplementary Figure S1). The level of increase was similar among the 3 groups of patients (\textit{FGFR1}+/HR+, \textit{FGFR1}−/HR+, and \textit{FGFR1}−/HR−), suggesting that there were no major differences in pharmacodynamic activity between the 3 groups.

\textit{Dovitinib Exhibits Greater Antitumor Effects in FGFR1-Amplified as Compared to Nonamplified Breast Cancers}
No complete responses or confirmed PRs were observed (overall response rate 0%) as per the adjudicated central review (Table 2). Three patients with FGFR1-amplified /HR⁺ breast cancer achieved an initial objective response, although these responses were not confirmed at a subsequent assessment. One patient demonstrated a complete disappearance of liver lesions with persistence of nontarget bone and lymph node lesions in the first postbaseline evaluation. This would have qualified as a PR, but was not confirmed in the second evaluation (day 109) because the investigator assessed disease progression in the bone and discontinued therapy, while the liver disease remained in complete response. The second patient showed a reduction of 30.8% in target lesions in the liver, retroperitoneum, and ovaries in the second postbaseline evaluation, but was not confirmed in the 3 subsequent evaluations which showed 25.8%, 27.1%, and 28.5% reductions from baseline. The patient experienced disease progression (observed on day 274; new peritoneal lesion). The third patient had a reduction of 40.3% in target lesions. However, this was not confirmed due to clinical disease progression at day 87 (increased markers cancer antigen 15.3 and carcinoembryonic antigen, and decline in performance status). The patient refused further assessment.

Two additional FGFR1⁺/HR⁺ evaluable patients had SD for ≥24 weeks. Therefore, 5 of 20 FGFR1⁺/HR⁺ patients (25%) achieved either an unconfirmed PR or SD ≥24 weeks. On the contrary, only 1 patient (3%) in the FGFR1⁻/HR⁺ cohort and 2 patients (13%) in the FGFR1⁻/HR⁻ cohort achieved long term (≥24 weeks) SD and none had tumor shrinkage >30%. None of the study arms proceeded to the second stage since the predefined criteria (at least 2 confirmed objective responses) was not met.
We further explored whether higher levels of FGFR1 amplification detected by qPCR are predictive for dovitinib sensitivity in patients with HR⁺ disease. Tissue for qPCR assessment was available for 42 of 51 HR⁺ patients with measurable disease. Thirty-eight of these patients had assessable disease, and in 35 of them the reported change in tumor size was in accordance with the overall tumor response; these are included in this analysis. Thirteen and 22 of these patients were FGFR1 amplified and nonamplified by SISH, respectively. Using qPCR, with a cutoff of 6 copies for FGFR1, a total of 7 patients were identified with FGFR1 amplification. Interestingly, qPCR data suggested that six cases presented FGFR1-amplification by SISH but not by qPCR. There was full concordance for the remaining 22 patients with no amplification of FGFR1 by SISH (all of them nonamplified by qPCR as well). In the 7 patients amplified by qPCR (and SISH), a 20.2% reduction in the mean tumor size was observed (range, 100% reduction to 28.4% increase, with 6 of the 7 patients showing tumor shrinkage and only 1 showing tumor increase) compared with a 14.2% increase in mean tumor size for the 6 patients with FGFR1 amplification detected by SISH but not qPCR (range, 11.6% reduction to 54.0% increase). As shown in Figure 3a, dovitinib was more effective in FGFR1-highly amplified (≥6 gene copies) breast cancer as compared with tumors with lower levels of FGFR1-amplification in this population of HR⁺ patients. In the FGFR1-amplified breast cancer group (n = 7), dovitinib induced a mean 20.2% reduction in tumor size (range, 28.4% increase to 100% reduction). Conversely, tumors with <6 FGFR1 copies (n = 28) had a mean 8.3% increase (range, 54.2% increase to 28.2% reduction). Overall, these data suggested that dovitinib demonstrates more potent antitumor activity in patients with high levels of FGFR1 amplification compared to those tumors without FGFR1 amplification by qPCR.
**FGF3 and FGFR2 Amplification to Complement FGFR1 Amplification in Selecting Individuals Most Likely to Respond to Dovitinib**

Since **FGF3** and **FGFR2** amplifications have also been reported in breast cancers (6, 18, 19), we further evaluated by qPCR whether such amplifications could define additional subsets of sensitive patients. Four patients had **FGF3** amplification as measured by qPCR, with tumor reductions of 100%, 30.8%, 23.0%, and 7.5%, respectively. Interestingly, 3 of these 4 patients also had a high level of **FGFR1** amplification (≥6 copies by qPCR) and the fourth presented with **FGFR1**-gene gain (3.4 copies of **FGFR1** by qPCR and SISH negative).

**FGFR2** amplification was also assessed by qPCR and detected (≥4 copies) in 2 of the HR+ patients (both **FGFR1**-nonamplified by SISH and qPCR). These 2 patients with **FGFR2+/FGFR1−/HR+** breast cancer had tumor reductions of 28.2% and 18.5%. Two additional patients with HR− disease had **FGFR2**+ amplification by qPCR but discontinued due to adverse events (AEs) prior to a postbaseline assessment.

We further analyzed the impact of amplification in **FGFR1** (≥6 copies), **FGFR2** (≥4 copies), or **FGF3** (≥6 copies) by qPCR in the above described group of patients with assessable and HR+ disease (n = 38). Ten of these 38 HR+ patients were defined as having FGF-pathway–amplified breast cancer (**FGFR1** and/or **FGFR2** and/or **FGF3** amplification). Interestingly, the mean reduction in target lesions was 21.1% from baseline (range, 28.4% increase to 100% reduction) in these 10 patients. Conversely, target lesions in the remaining 28 patients who did not present with FGF-pathway–amplified breast cancer had a mean 12.0% increase from baseline (range, 54.2% increase to 15.4% reduction). Best tumor response according to the presence of gene amplification on FGF-pathway is reported in Figure 3b. Interestingly, this
analysis showed that FGF pathway amplification–negative patients were unlikely to respond to dovitinib, as 16 out of 28 patients (57.1%) without FGF-pathway amplification presented either a new lesion or tumor size increase as best response, compared with only 1 out of 10 (10.0%) FGF-pathway–amplified patients. Similar results were obtained when amplification of \( FGFR1 \) or \( FGFR2 \) was used to define FGF-pathway amplification since only 1 patient presented with \( FGF3 \) amplification but not \( FGFR1 \) or \( FGFR2 \) amplification.

**Safety**

All patients eventually discontinued the study, with disease progression as the most common reason reported for discontinuation (\( n = 47, \) 58.0%). AEs regardless of relationship to dovitinib were reported as the primary reason for discontinuation in 22 patients (27.2%). AEs leading to discontinuation were most commonly grade 3, the most common being asthenia/fatigue (\( n = 7 \)), gastrointestinal disorders (\( n = 5 \)), and investigations of laboratory abnormalities (\( n = 6 \), mainly liver function test abnormalities).

All patients (100.0%) experienced at least 1 AE regardless of relationship to study drug, most commonly vomiting (77.8%), diarrhea (76.5%), asthenia (67.9%), and nausea (67.9%) (Table 3). Sixty-three patients (77.8%) experienced a grade 3/4 AE. The most common grade 3/4 AEs regardless of study drug relationship were asthenia (23.5%), ALT increase (11.1%), diarrhea (8.6%), and vomiting, fatigue, and AST increase (7.4% each). No notable differences across the groups were observed (data not shown).
Patients were also monitored for clinical laboratory abnormalities. Overall, new or worsened grade 3 and 4 alkaline phosphatase was observed in 21.1% and 1.3% of patients, respectively, and was more common in the FGFR1+/HR+ group (43.5%). New or worsened grade 3 or 4 AST increase was observed in 16.7% of the patients (all grade 3), and new or worsened grade 3 and 4 ALT increase was also observed in 15.4% and 1.3% of patients, respectively. New or worsening grade 3 and 4 total bilirubin was observed in only 2.6% and 1.3% of patients, respectively.

The most frequently observed grade 3 or 4 hematological clinical laboratory abnormality was lymphopenia (16.9%, all grade 3). The majority of new or worsened abnormalities in absolute neutrophils, hemoglobin, white blood cell, and platelet counts were grade 1 and 2, with few patients exhibiting a shift to grade 4. No major difference in hematological clinical laboratory abnormalities was observed between the treatment groups.

Eight patients died on the study or during the 28-day follow-up period. In 3 cases, the fatal event was assessed by the investigator as being related to the study drug, and in the remaining 5 cases, the death was deemed as secondary to progression of underlying disease. A brief description of the 3 deaths not related to progressive disease follows. Patient #1 was a 62-year-old female with antecedent of hypertension, diabetes mellitus, and heavily pretreated (anastrozole, tamoxifen, fulvestrant, paclitaxel, bevacizumab, and capecitabine) metastatic breast cancer (widespread metastatic liver and bone disease). After 4 weeks of therapy, the patient experienced grade 3 AST increase and grade 4 ALT, grade 4 alkaline phosphatase increase, and grade 4 total bilirubin increase. Dovitinib treatment was discontinued due to these events, and the patient died 25 days after the last dose of therapy due
to acute liver toxicity (cholestatic liver damage). Patient #2, a 44-year-old female who was heavily pretreated (doxorubicin, cyclophosphamide, docetaxel, cisplatin, vinorelbine, tamoxifen, and anastrozole) had breast cancer with multiple mediastinal, pulmonary, and bone metastases at baseline. She received 8 weeks of therapy until discontinuation due to disease progression (new pleural mass and subpleural nodes). The patient started paclitaxel 12 days after study discontinuation and died of pleural hemorrhage and acute respiratory failure 15 days later (27 days after the last dose of dovitinib). Patient #3, a 62-year-old female with metastatic breast cancer (single measurable liver lesion at baseline) had previously been treated with doxorubicin, cyclophosphamide, docetaxel, bevacizumab, and capecitabine. The patient received 25 doses of dovitinib and died at day 51, four days after the last dose. The death was secondary to listeria infection and sepsis following treatment emergent grade 3 neutropenia.

DISCUSSION

We examined the activity of dovitinib in preclinical models and patients with breast cancer. Our findings suggest that dovitinib could have modest antitumor activity in FGF-pathway amplified tumors, but not in FGF-pathway non-amplified tumors. These data suggest that future trials testing FGFR inhibitors should focus on FGF-pathway-amplified breast cancer.

This study was conducted in a population who was unlikely to respond. These patients had advanced disease (70% had ≥3 organs involved and 78% had liver metastases) and were heavily pretreated, with most having received more than 1 line
of chemotherapy in the metastatic setting in addition to endocrine therapy. In addition, the expected response rates in patients with breast cancer treated with targeted agents alone is generally low. For example, single-agent treatment of advanced breast cancer patients with lapatinib was associated with a low response rate but was improved when used in combination with trastuzumab (20). Therefore, the observed results with dovitinib in patients with FGFR1-amplified breast cancer may be consistent with results obtained in the same setting with successful targeted therapies in breast cancers. Additional data obtained with qPCR suggested that within FGFR1-amplified cancers detected by SISH, those who presented higher levels of FGFR1-amplification (qPCR, number copies ≥6) could be even more sensitive to dovitinib. In these latter 7 patients, a 20.2% reduction in the mean tumor size was observed, as opposed to 14.2% increase in the mean tumor size for the 6 patients with FGFR1-amplification detected by SISH, but not qPCR. Finally, FGFR1 was not the only genomic alteration identified within the FGF pathway. In our study, FGFR2 amplifications were also associated with a higher number of responders. Indeed, tumor reductions of 28.2% and 18.5% were observed in 2 evaluable patients with FGFR2 amplification, suggesting that dovitinib could have antitumor activity in this small subset of patients. FGF3 amplification was also associated with tumor shrinkage, with 2 of the 4 FGF3-amplified patients achieving an unconfirmed partial response. Whether FGF3 is a biomarker by itself or a surrogate of high-level FGFR1 amplification is unclear since amplification of the 11q12 amplicon is more likely to occur when FGFR1 is amplified (6). In the present study, 3 of 4 patients with FGF3 amplification also had a high level of FGFR1 amplification and the fourth patient had a FGFR1-gene gain by qPCR (3.4 copies). Also, the precise role of FGF3 in the amplicon and oncogenesis is unclear. Indeed, several other candidate oncogenic
drivers are present on the amplicon including CCND1 and PAK1. Overall, the present study could not address whether FGF3 amplification by itself contributed to the definition of dovitinib-sensitive patients. Another limitation was that the study was not sufficiently powered for statistical analyses on FGF-pathway-amplified vs non-amplified patients.

Further studies of dovitinib will be in combination with endocrine therapy, as targeted therapies present optimal efficacy when combined with other agents. In a similar fashion as the synergy that was observed with everolimus and aromatase inhibitors (21, 22), FGFR1 inhibition has been shown to reverse endocrine resistance in preclinical models (5). Since the present study did not address the role of sensitization to endocrine therapy, future combination trials should include both FGF-pathway amplified and non-amplified patients. Finally, additional molecular analyses will determine whether FGFR-amplifications could be associated with additional mutations on oncogenes. This biomarker work could provide some rationale for combining dovitinib with other targeted agents with the aim of delaying resistance.

The safety profile of dovitinib includes gastrointestinal toxicity (nausea, vomiting), liver toxicity, and asthenia. This profile is comparable with TKIs (e.g., pazopanib, sunitinib, and lapatinib) but higher than monoclonal antibodies (23-25). Increasing awareness of these effects amongst physicians should accelerate detection and reduce severity of these events.

Overall, the present study provides the first detailed report examining FGFR-pathway status and response in a phase II trial testing an FGFR-inhibitor. The results suggest that targeting FGFR could lead to modest antitumor activity in patients with FGF-pathway–deregulated breast cancer. Based on these results, together with
biomarker exploration on FGFR2, FGF3, and preclinical data, dovitinib is being studied in combination with fulvestrant in a phase II randomized trial in patients with breast cancer who have FGF-pathway amplifications (FGFR1, FGFR2, or FGF3) as determined by qPCR (www.clinicaltrials.gov registry number NCT01528345). Further studies will show whether the FGF pathway should be targeted in other cancers with a deregulated pathway. For example, a phase II trial of dovitinib in metastatic endometrial cancer (www.clinicaltrials.gov registry number NCT01379534) is screening and grouping patients based on FGFR2-mutation status (26). These data also open new avenues in the field of FGFR targeting in other tumor types including lung, and gastric cancers.

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REFERENCES


Table 1: Patient Characteristics

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<td>WHO performance status, n (%)</td>
<td>13 (56.5)</td>
<td>20 (58.8)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>0</td>
<td>13 (56.5)</td>
<td>20 (58.8)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>1</td>
<td>10 (43.5)</td>
<td>14 (41.2)</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>≥3 organs involved, n (%)</td>
<td>16 (69.6)</td>
<td>15 (44.1)</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>Histology/cytology, n (%)</td>
<td>19 (82.6)</td>
<td>25 (73.5)</td>
<td>18 (81.8)</td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>19 (82.6)</td>
<td>25 (73.5)</td>
<td>18 (81.8)</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>1 (4.3)</td>
<td>3 (8.8)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (13.0)</td>
<td>6 (17.6)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Poorly differentiated/undifferentiated histology, n (%)</td>
<td>10 (43.5)</td>
<td>16 (47.1)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>Hormone receptor status, n (%)</td>
<td>–</td>
<td>2 (5.9%)</td>
<td>–</td>
</tr>
<tr>
<td>Progesterone receptor positive only</td>
<td>–</td>
<td>2 (5.9%)</td>
<td>–</td>
</tr>
<tr>
<td>Estrogen receptor positive only</td>
<td>7 (30.4)</td>
<td>15 (44.1)</td>
<td>–</td>
</tr>
<tr>
<td>Both progesterone and estrogen receptor positive</td>
<td>16 (69.6)</td>
<td>17 (50.0)</td>
<td>–</td>
</tr>
<tr>
<td>Metastatic sites, n (%)</td>
<td>Bone 19 (82.6)</td>
<td>25 (73.5)</td>
<td>7 (31.8)</td>
</tr>
<tr>
<td>Liver 18 (78.3)</td>
<td>24 (70.6)</td>
<td>4 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Lung 8 (34.8)</td>
<td>9 (26.5)</td>
<td>8 (36.4)</td>
<td></td>
</tr>
<tr>
<td>Median time from initial diagnosis to first relapse, months (range)*</td>
<td>33.8 (14.1-178.1)</td>
<td>45.1 (3.6-177.3)</td>
<td>19.0 (4.3-216.6)</td>
</tr>
<tr>
<td>Patients with stage IV at diagnosis, n (%)</td>
<td>7 (30.4%)</td>
<td>6 (17.6%)</td>
<td>1 (4.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure to prior chemotherapy, n (%)</th>
<th>FGFR1+/HR+</th>
<th>FGFR1+/HR+</th>
<th>FGFR1−/HR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant/neoadjuvant</td>
<td>15 (65.2)</td>
<td>21 (61.8)</td>
<td>17 (77.3)</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>22 (95.7)</td>
<td>32 (94.1)</td>
<td>21 (95.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prior therapeutic chemotherapy regimens</th>
<th>Median</th>
<th>FGFR1+/HR+</th>
<th>FGFR1+/HR+</th>
<th>FGFR1−/HR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2, n (%)</td>
<td>17 (73.9)</td>
<td>23 (67.6)</td>
<td>15 (68.2)</td>
<td></td>
</tr>
<tr>
<td>≥3, n (%)</td>
<td>5 (21.7)</td>
<td>9 (26.5)</td>
<td>6 (27.3)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure to prior hormone therapy, n (%)</th>
<th>FGFR1+/HR+</th>
<th>FGFR1+/HR+</th>
<th>FGFR1−/HR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant/neoadjuvant</td>
<td>13 (56.5)</td>
<td>20 (58.8)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Therapeutic</td>
<td>19 (82.6)</td>
<td>28 (82.4)</td>
<td>2 (9.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prior therapeutic hormone therapy regimens</th>
<th>Median</th>
<th>FGFR1+/HR+</th>
<th>FGFR1+/HR+</th>
<th>FGFR1−/HR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2, n (%)</td>
<td>12 (52.2)</td>
<td>20 (58.8)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>≥3, n (%)</td>
<td>7 (30.4)</td>
<td>8 (23.5)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
</tbody>
</table>

WHO, World Health Organization.

* Excludes patients with stage IV disease at diagnosis.
Table 2: Best Overall Response Summary Based on Adjudicated Data According to RECIST Criteria

<table>
<thead>
<tr>
<th></th>
<th>FGFR1⁺/HR⁺ n = 20</th>
<th>FGFR1⁻/HR⁺ n = 31</th>
<th>FGFR1⁻/HR⁻ n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best overall RECIST response, n (%)</td>
<td>PRnc</td>
<td>SD</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>3 (15)</td>
<td>9 (45)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>PD per clinical evaluation but not RECIST</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Not assessable</td>
<td>1</td>
<td>6*</td>
<td>2</td>
</tr>
<tr>
<td>Clinical benefit (CR/PR/SD ≥24 weeks)†</td>
<td>3 (15)</td>
<td>1 (3)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>PRnc or SD ≥24 weeks†</td>
<td>5 (25)</td>
<td>1 (3)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>PFS median by Kaplan-Meier estimates, months [range]</td>
<td>3.6 [0–9.0]</td>
<td>3.5 [0–5.5]</td>
<td>2.1 [0–9.2]</td>
</tr>
</tbody>
</table>

CR, complete response; PD, disease progression; PFS, progression-free survival; PRnc, partial response not confirmed after 4 weeks; RECIST, Response Evaluation Criteria In Solid Tumors; SD, stable disease.

* Three FGFR1⁻/HR⁺ patients did not have any postbaseline assessments (2 patients withdrew consent after 1 and 3 doses, respectively, and another patient discontinued due to liver function test abnormalities at day 15 of cycle 1), and 3 patients had SD that was assessed <6 weeks from start of treatment but then discontinued due to AE or PD not confirmed by central adjudicator.

† A 2-week window was applied to SD ≥24-week calculations.
Table 3: Adverse Events (≥15% Any Grade) Regardless of Study Drug Relationship
(N = 81)

<table>
<thead>
<tr>
<th>Event</th>
<th>All Grades n (%)</th>
<th>Grade 3 n (%)</th>
<th>Grade 4 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>63 (77.8)</td>
<td>6 (7.4)</td>
<td>–</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>62 (76.5)</td>
<td>7 (8.6)</td>
<td>–</td>
</tr>
<tr>
<td>Asthenia</td>
<td>55 (67.9)</td>
<td>19 (23.5)</td>
<td>–</td>
</tr>
<tr>
<td>Nausea</td>
<td>55 (67.9)</td>
<td>4 (4.9)</td>
<td>–</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>31 (38.3)</td>
<td>3 (3.7)</td>
<td>–</td>
</tr>
<tr>
<td>Headache</td>
<td>27 (33.3)</td>
<td>1 (1.2)</td>
<td>–</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>23 (28.4)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fatigue</td>
<td>21 (25.9)</td>
<td>6 (7.4)</td>
<td>–</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>19 (23.5)</td>
<td>5 (6.2)</td>
<td>–</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>17 (21.0)</td>
<td>3 (3.7)</td>
<td>–</td>
</tr>
<tr>
<td>Abdominal pain upper</td>
<td>16 (19.8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ALT increased</td>
<td>15 (18.5)</td>
<td>8 (9.9)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Constipation</td>
<td>15 (18.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>15 (18.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rash</td>
<td>15 (18.5)</td>
<td>3 (3.7)</td>
<td>–</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>14 (17.3)</td>
<td>1 (1.2)</td>
<td>–</td>
</tr>
<tr>
<td>AST increased</td>
<td>13 (16.0)</td>
<td>6 (7.4)</td>
<td>–</td>
</tr>
<tr>
<td>Dry skin</td>
<td>13 (16.0)</td>
<td>1 (1.2)</td>
<td>–</td>
</tr>
<tr>
<td>Weight decreased</td>
<td>13 (16.0)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
FIGURE LEGENDS

Figure 1. Dovitinib inhibits FGFR downstream signaling and cell proliferation in FGFR1 or FGFR2 amplified breast cancer cell lines and growth of FGFR1-amplified HBCx-2 xenografts. A) Effects of dovitinib on FGFR signaling were assessed by Western blot detection of pFRS2 and pERK/MAPK in FGFR1-amplified MDA-MB-124 and FGFR2-amplified SUM52 cells. B) Dovitinib inhibited cell proliferation in FGFR-amplified but not FGFR-nonamplified breast cancer cell lines. C, D) Dovitinib demonstrated tumor growth inhibition in C) FGFR1-amplified HBCx-2 and D) FGFR2-amplified HBCx-3 mouse xenograft models. Error bars indicate standard error of the mean. The T/C values and P values for the Mann-Whitney nonparametric tests vs vehicle control are shown below each graph.

Figure 2. Study design. Patients were prescreened to determine FGFR1 status, then stratified into 4 cohorts based on HR status and FGFR1 amplification. The study was designed as a Simon 2-stage with 20 patients planned for enrollment in each cohort for each stage. No arm proceeded to stage 2.

Figure 3. Dovitinib induces target lesion reductions in FGF-pathway–amplified tumors. A) Box plot showing the best tumor change of the target lesions based on FGFR1 copy number as assessed by qPCR in HR+ patients with measureable disease at baseline and evaluable for this plot (n = 35). The line and + symbol within each box represents the median and mean value for that group. Whiskers above and below each box indicate the maximum and minimum value in that group, respectively. B) Waterfall plot of HR+ FGF-pathway–amplified (black bars; FGFR1, FGFR2, or FGF3 amplification by qPCR) or FGF-pathway–nonamplified (grey bars) patients evaluable for this plot (n = 38). The asterisk (*) denotes patients whose
overall lesion response is progressive disease, but the target lesion response is PR or SD. The dagger (†) denotes the $FGFR1^+/FGF3^+$ patient with 3.4 copies of $FGFR1$ by qPCR, the double dagger (‡) denotes the $FGFR1^+/FGFR2^+$ patients, and the section mark (§) denotes the $FGFR1^+/FGFR3^+$ patients.
Figure 1

A

Dovitinib - 50 nM 250 nM

pFRS2

pErk/MAPK

β-Tubulin

MDA-MB-134

SUM52

B

Breast Cancer Cell Line

IC₅₀ dovitinib (nM)

No FGFR amplification
FGFR1 amplification
FGFR2 amplification

C

Vehicle qdx42

Dovitinib 30 mg/kg qdx42

Dovitinib 50 mg/kg qdx42

Tumor Volume (mm³)

Time (Days)

D

Control

Dovitinib 40 mg/kg qdx35

Tumor Volume (mm³)

Time (Days)
*FGFR1* arms were closed to accrual due to completed enrollment to stage 1, with prescreening continued to complete accrual of *FGFR1*+ arms.
Figure 3

A

![Box plot showing Best % Change From Baseline for different FGF pathway copy numbers.

- B

![Graph showing Best % Change From Baseline for FGF pathway amplified and nonamplified.

Legend:
- Black: FGF pathway amplified
- Gray: FGF pathway nonamplified

Data points:
- < 4 copies (n = 24)
- 4 to < 6 copies (n = 4)
- ≥ 6 copies (n = 7)
Targeting FGFR With Dovitinib (TKI258): Preclinical and Clinical Data in Breast Cancer

Fabrice Andre, Thomas Bachelot, Mario Campone, et al.

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