Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

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

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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 both breast cancer cell lines and an *FGFR1*-amplified xenograft model (HBCx2). Dovitinib was then evaluated in a phase II trial that included 4 groups of patients with human EGF receptor 2–negative metastatic breast cancer on the basis of *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 PCR (qPCR).

Results: Dovitinib monotherapy inhibits proliferation in *FGFR1*- and *FGFR2*-amplified, but not *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 for more than 6 months was observed in 5 (25%) and 1 (3%) patient(s) 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 showed antitumor activity in *FGFR*-amplified breast cancer cell lines and may have activity in breast cancers with FGF pathway amplification. *Clin Cancer Res; 19(13); 3693–702. ©2013 AACR.*

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; ref. 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 nmol/L (8, 9). Phase I studies have suggested that dovitinib has a tolerable safety profile and

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Translational Relevance

FGFR1 amplification in breast cancer is associated with resistance to endocrine therapy and poor prognosis. Dovitinib has shown 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.

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, and then conducted a phase II trial of dovitinib in patients with metastatic breast cancer, with and without *FGFR1* amplification. Importantly, this is the first report of a study selecting patients on the basis of *FGFR1*-amplification status.

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 2 with *FGFR1* (MDA-MB-134) or *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). Athymic nude mice were implanted with a human *FGFR1*- or *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 percentage 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 on the basis of hormone receptor (HR; ER or PR) status (positive [$^+$] and negative [$^-$]) and *FGFR1* amplification status. Patients having an average of 6 or more copies of *FGFR1* assessed by fluorescent–, chromogenic–, or silver *in situ* hybridization (FISH, CISH, or SISH) were considered as *FGFR1* amplified (*FGFR1*$^+$). SISH was carried out 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.), either retrospectively for confirmation of local assessments, if available, or prospectively for those centers where local assessment was not done. 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% or less versus the alternative hypothesis that the response rate was greater than 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 at 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 to 6 weeks. Similarly, stable disease (SD) had to last for 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 PCR (qPCR). Tumor measurability at baseline, responses, and PFS were assessed locally and centrally. A second independent central review (adjudication) was conducted for discordant cases between the local and first central reviews. The adjudicating radiologist was blinded to treatment arm and previous assessment. Adjudicated results are reported here.

Eligibility criteria

Women 18 years or older 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 $\geq$6 copies) by a designated local investigator or a reference laboratory before study entry with archival tumor tissue available for central confirmation. Patients with HR$^+$ 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% or higher, and adequate bone marrow, hepatic, and renal function. Patients with liver metastases were eligible if they had grade 2 or lower 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 ELISA (Kinos Laboratories, Inc.). 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). TaqMan Copy Number Assays (Life Technologies Corporation) 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 or more, consistent with the predefined *FGFR1* gene amplification threshold (by hybridization) in the trial protocol. A threshold of 4 *FGFR2* copies or more by qPCR was predefined on the basis of a previous report of Turner and colleagues (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 IC_{50} for cell growth inhibition was 190 and 180 nmol/L in MDA-MB-134 and SUM52, respectively (Fig. 1B and Supplementary Table S1). Conversely, IC_{50} values were more than 2,000 nmol/L 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 8 *FGFR1* gene copies; ref. 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 ($P < 0.001$ by Mann–Whitney nonparametric test). In addition, we evaluated dovitinib in an *FGFR2*-amplified *in vivo* model (HBCx-3; 10 *FGFR2* copies). Similar to the results described earlier, dovitinib caused tumor regression in HBCx-3 xenografts when administered at a dose of 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 that was 23.4% of the vehicle control group ($P < 0.001$). Overall, these data suggested that dovitinib had antitumor activity in *FGFR*-amplified breast cancer models.

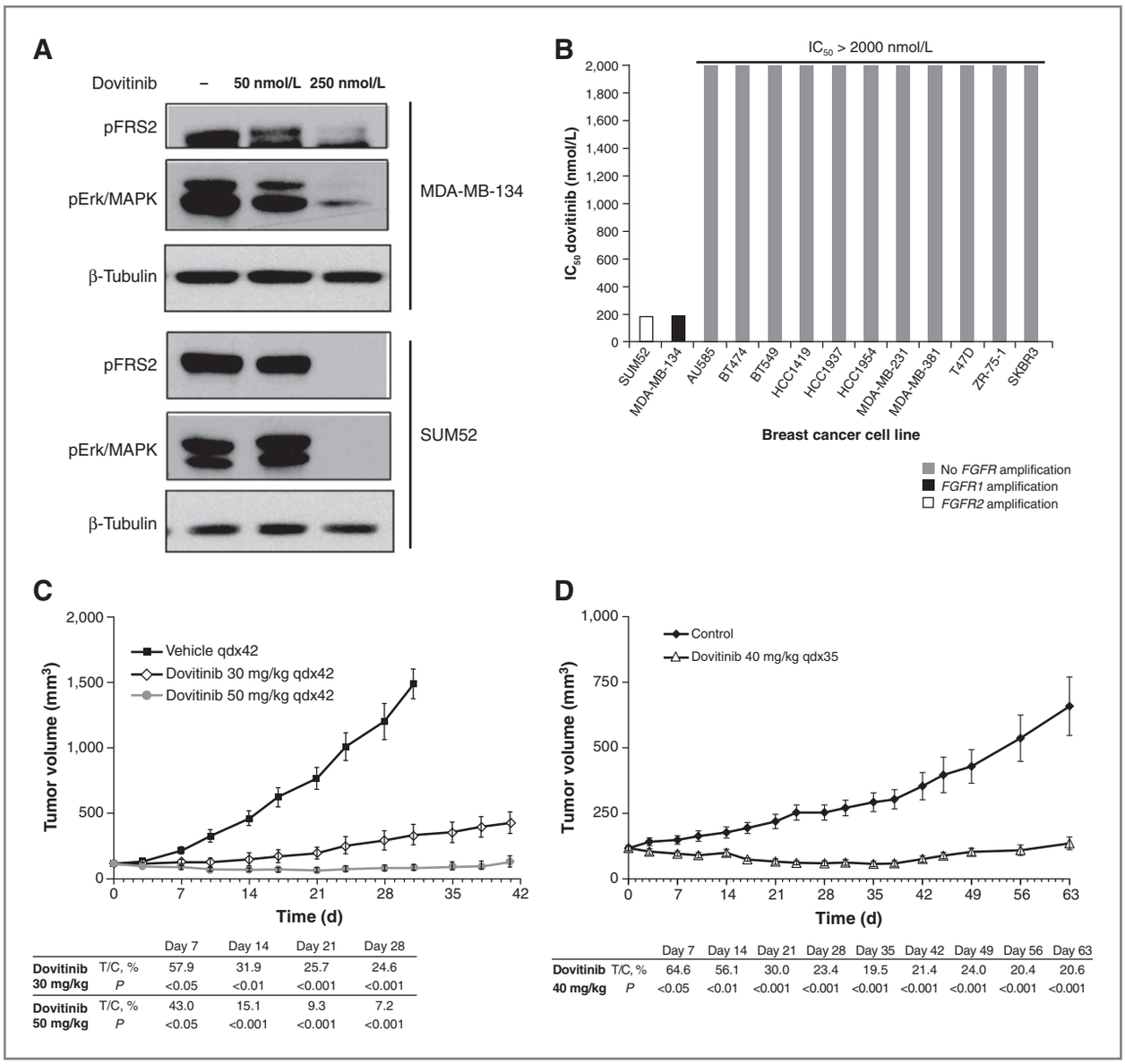
Patient characteristics

On the basis of 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 ($n = 92$; Fig. 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 hematologic and biochemical 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 on the basis of *FGFR1* amplification and HR status: $FGFR1^+/HR^+$ ($n = 23$), $FGFR1^+/HR^-$ ($n = 2$), $FGFR1^-/HR^+$ ($n = 34$), and $FGFR1^-/HR^-$ ($n = 22$; Table 1). Notably, 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 before completion of enrollment.

Patient characteristics for the fully enrolled cohorts ($FGFR1^+/HR^+$, $FGFR1^-/HR^+$, and $FGFR1^-/HR^-$) are reported in Table 1. Most patients had late-stage breast cancer and were heavily pretreated. For example, 70% and 78% of the $FGFR1^+/HR^+$ patients presented with 3 organs or more involved and liver metastases, respectively. In addition, 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 previous chemotherapy at study entry and were not considered as candidates for endocrine therapy as per the investigator's judgment.

Dovitinib effectively increases FGF23 plasma levels, indicative of FGFR1 inhibition

FGF23 has been identified as a target gene of FGF signaling *in vitro* (15). Furthermore, increases in FGF23 have previously been reported as a surrogate for FGFR1



		Day 7	Day 14	Day 21	Day 28
Dovitinib 30 mg/kg	T/C, %	57.9	31.9	25.7	24.6
	P	<0.05	<0.01	<0.001	<0.001
Dovitinib 50 mg/kg	T/C, %	43.0	15.1	9.3	7.2
	P	<0.05	<0.001	<0.001	<0.001

		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63
Dovitinib 40 mg/kg	T/C, %	64.6	56.1	30.0	23.4	19.5	21.4	24.0	20.4	20.6
	P	<0.05	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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 and D, dovitinib showed 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 compared with vehicle control are shown below each graph.

inhibition in clinical trials of dovitinib in renal cell carcinoma and melanoma (11, 16, 17). In these trials, 50% to 100% increases in 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 Fig. S1). The level of increase was similar among the 3 groups of patients (*FGFR1*$^+$/HR$^+$, *FGFR1*$^-$/HR$^+$, and *FGFR1*$^-$/HR$^-$), suggesting that there were no major differences in pharmacodynamic activity between the 3 groups.

Dovitinib exhibits greater antitumor effects in *FGFR1*-amplified as compared with 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*$^+$/HR$^+$ breast cancer achieved an initial objective response, although

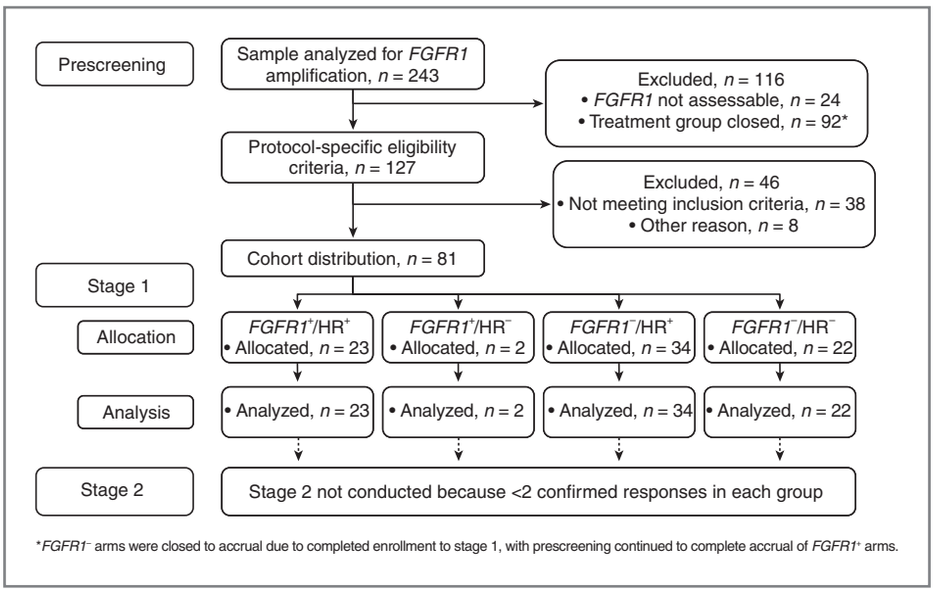


Figure 2. Study design. Patients were prescreened to determine *FGFR1* status, then stratified into 4 cohorts on the basis of HR status and *FGFR1* amplification. The study was designed as a Simon 2-stage with 20 patients planned for enrollment in each cohort for each stage. No arm proceeded to stage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

these responses were not confirmed at a subsequent assessment. One patient showed 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 that 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 or longer. Therefore, 5 of 20 $FGFR1^+/HR^+$ patients (25%) achieved either an unconfirmed PR or SD 24 weeks or longer. On the contrary, only 1 patient (3%) in the $FGFR1^-/HR^+$ cohort and 2 patients (13%) in the $FGFR1^-/HR^-$ cohort achieved long-term ($\geq$24 weeks) SD and none had tumor shrinkage greater than 30%. None of the study arms proceeded to the second stage because the predefined criteria (at least 2 confirmed objective responses) were 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 6 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 Fig. 3A, dovitinib was more effective in *FGFR1*-highly amplified ($\geq$6 gene copies) breast cancer as compared with tumors with lower levels of *FGFR1* amplification in this population of HR^+ patients. In the *FGFR1*-amplified breast cancer group ($n = 7$), dovitinib induced a mean 20.2% reduction in tumor size (range, 28.4% increase to 100% reduction). Conversely, tumors with less than 6 *FGFR1* copies ($n = 28$) had a mean 8.3% increase (range, 54.2% increase to 28.2% reduction). Overall, these data suggested that dovitinib shows more potent antitumor activity in patients with high levels of *FGFR1* amplification compared with those tumors without *FGFR1* amplification by qPCR.

FGF3 and *FGFR2* amplification to complement *FGFR1* amplification in selecting individuals most likely to respond to dovitinib

Because *FGF3* and *FGFR2* amplifications have also been reported in breast cancers (6, 18, 19), we further evaluated by qPCR whether such amplifications could define

Table 1. Patient characteristics

	FGFR1$^+$/HR$^+$ $n = 23$	FGFR1$^-$/HR$^+$ $n = 34$	FGFR1$^-$/HR$^-$ $n = 22$
Median age, years (range)	56.0 (25–72)	55.0 (32–78)	59.5 (37–78)
Age ≥65 years, *n* (%)	2 (8.7)	10 (29.4)	5 (22.7)
WHO performance status, *n* (%)			
0	13 (56.5)	20 (58.8)	15 (68.2)
1	10 (43.5)	14 (41.2)	6 (27.3)
2	–	–	1 (4.5)
≥3 organs involved, *n* (%)	16 (69.6)	15 (44.1)	6 (27.3)
Histology/cytology, *n* (%)			
Invasive ductal carcinoma	19 (82.6)	25 (73.5)	18 (81.8)
Invasive lobular carcinoma	1 (4.3)	3 (8.8)	1 (4.5)
Other	3 (13.0)	6 (17.6)	3 (13.6)
Poorly differentiated/undifferentiated histology, *n* (%)	10 (43.5)	16 (47.1)	15 (68.2)
Hormone receptor status, *n* (%)			
Progesterone receptor positive only	–	2 (5.9%)	–
Estrogen receptor positive only	7 (30.4)	15 (44.1)	–
Both progesterone and estrogen receptor positive	16 (69.6)	17 (50.0)	–
Metastatic sites, *n* (%)			
Bone	19 (82.6)	25 (73.5)	7 (31.8)
Liver	18 (78.3)	24 (70.6)	4 (18.2)
Lung	8 (34.8)	9 (26.5)	8 (36.4)
Median time from initial diagnosis to first relapse, months (range)[a]	33.8 (14.1–178.1)	45.1 (3.6–177.3)	19.0 (4.3–216.6)
Patients with stage IV at diagnosis, *n* (%)	7 (30.4%)	6 (17.6%)	1 (4.5%)
Exposure to previous chemotherapy, *n* (%)			
Adjuvant/neoadjuvant	15 (65.2)	21 (61.8)	17 (77.3)
Therapeutic	22 (95.7)	32 (94.1)	21 (95.5)
Previous therapeutic chemotherapy regimens			
Median	2	2	2
1–2, *n* (%)	17 (73.9)	23 (67.6)	15 (68.2)
≥3, *n* (%)	5 (21.7)	9 (26.5)	6 (27.3)
Exposure to previous hormone therapy, *n* (%)			
Adjuvant/neoadjuvant	13 (56.5)	20 (58.8)	3 (13.6)
Therapeutic	19 (82.6)	28 (82.4)	2 (9.1)
Previous therapeutic hormone therapy regimens			
Median	2	2	0
1–2, *n* (%)	12 (52.2)	20 (58.8)	1 (4.5)
≥3, *n* (%)	7 (30.4)	8 (23.5)	1 (4.5)

Abbreviation: WHO, World Health Organization.
[a]Excludes patients with stage IV disease at diagnosis.

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) before 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

Table 2. Best overall response summary on the basis of adjudicated data according to RECIST criteria

	FGFR1$^+$/HR$^+$ $n = 20$	FGFR1$^-$/HR$^+$ $n = 31$	FGFR1$^-$/HR$^-$ $n = 16$
Best overall RECIST response, *n* (%)			
PRnc	3 (15)	–	–
SD	9 (45)	15 (48)	4 (25)
PD	5 (25)	9 (29)	5 (31)
Unknown	3 (15)	7 (23)	7 (44)
PD per clinical evaluation but not RECIST	2	1	5
Not assessable	1	6[a]	2
Clinical benefit (CR/PR/SD ≥24 weeks)[b]	3 (15)	1 (3)	2 (13)
PRnc or SD ≥24 weeks[b]	5 (25)	1 (3)	2 (13)
PFS median by Kaplan–Meier estimates, months (range)	3.6 (0–9.0)	3.5 (0–5.5)	2.1 (0–9.2)

Abbreviations: 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.

[a]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.

[b]A 2-week window was applied to SD ≥24-week calculations.

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 Fig. 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 as 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 hematologic 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 grades 1 and 2, with few patients exhibiting a shift to grade 4. No major difference in hematologic 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 history 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 and grade 4 ALT increases, 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).

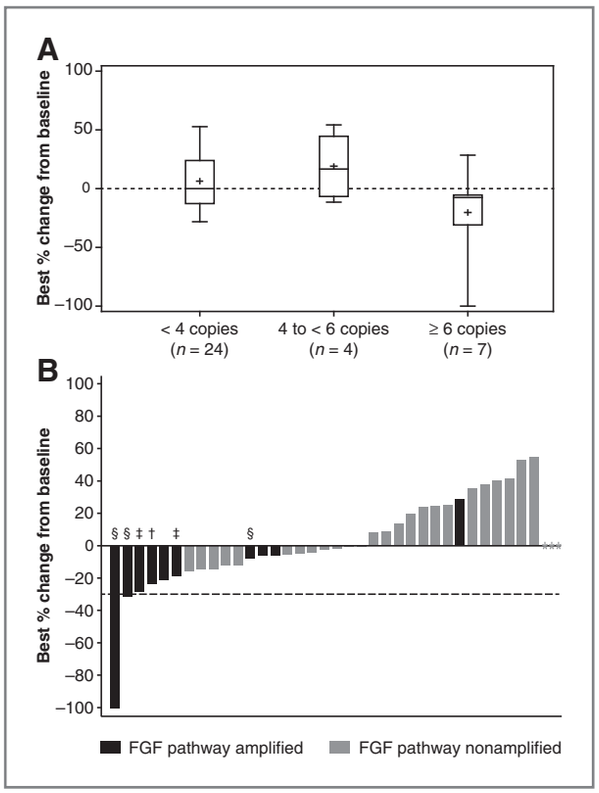


Figure 3. Dovitinib induces target lesion reductions in FGF-pathway–amplified tumors. A, box plot showing the best tumor change of the target lesions on the basis of *FGFR1* copy number as assessed by qPCR in HR^+ patients with measureable disease at baseline and evaluable for this plot ($n = 35$). The 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 values 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 (gray 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.

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, 4 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–nonamplified 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, ≥6 copies) could be even more sensitive to dovitinib. In these latter 7 patients, a 20.2% reduction in the mean tumor size was observed, as

Table 3. Adverse events (≥15% any grade) regardless of study drug relationship ($N = 81$)

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

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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 because 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 an *FGFR1*-gene gain by qPCR (3.4 copies). In addition, 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 versus nonamplified 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). As the present study did not address the role of sensitization to endocrine therapy, future combination trials should include both FGF-pathway–amplified and –nonamplified 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 among 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. On the basis of 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 on the basis of *FGFR2*-mutation status (26). In addition, these data open new avenues in the field of FGFR targeting in other tumor types including lung and gastric cancers.

Disclosure of Potential Conflicts of Interest

S. Deudon, M. Shi, Y. Zhang, A. Kay, A. Yovine, and D. Graus Porta are employees, and F. André, T. Bachelot, N. Turner, J. Baselga, and M. Campone are consultants of Novartis Pharmaceuticals Corporation. M. Campone and H. Rugo have received commercial research grants from Novartis Pharmaceuticals Corporation. No potential conflicts of interest were disclosed by the other authors.

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