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Statement of Translational Relevance

Therapies targeting molecular pathways of cell proliferation and angiogenesis represent a key advance in cancer treatment in general, and melanoma therapy in particular. This study reports the results of a phase I/II study dovitinib, a tyrosine kinase inhibitor of fibroblast growth factor (FGF) receptors (FGFRs), VEGF receptors (VEGFRs), and platelet-derived growth factor (PDGF) receptors, in patients with advanced melanoma.

The main objectives of this study were safety and efficacy, but a key additional element was the prospective exploratory evaluation of biomarkers to monitor target inhibition. During dovitinib treatment, plasma levels of FGF23, VEGF, and PDGF increased relative to baseline, and plasma levels of soluble VEGFR2 decreased. Phosphorylation of pErk and FGFR3 was reduced in tumor samples. These results indicate that dovitinib inhibited target receptors FGFR and VEGFR and downstream signaling. Monitoring of these biomarkers in future clinical studies may help identify patients who will best respond to dovitinib treatment.
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Abstract

**Purpose:** Dovitinib (TKI258) is an orally available inhibitor of fibroblast growth factor (FGF), VEGF, and platelet-derived growth factor receptors. This phase I/II dose-escalation study was conducted to evaluate the safety, pharmacodynamics, and preliminary efficacy of dovitinib in the treatment of advanced melanoma.

**Experimental Design:** Patients with advanced melanoma resistant or refractory to standard therapies or for whom no standard therapy was available were enrolled. Dovitinib was administered at doses ranging from 200 to 500 mg/day.

**Results:** Forty-seven patients were enrolled. The most frequently reported adverse events were fatigue (77%; grade ≥3, 28%), diarrhea (77%; grade ≥3, 11%), and nausea (77%; grade ≥3, 9%). Six dose-limiting toxicities were observed in the 400-mg and 500-mg dose cohorts, which consisted of grade 3 nausea, fatigue, and diarrhea and grade 4 fatigue events. The maximum tolerated dose was 400 mg/day. The best tumor response was stable disease, which was observed in 12 patients. Increases in plasma FGF23, VEGF, and placental growth factor and decreases in soluble VEGF receptor 2 were noted during the first cycle of treatment, consistent with FGF receptor (FGFR) and VEGF receptor (VEGFR) inhibition. Dynamic contrast-enhanced MRI analysis demonstrated a dose-dependent decrease in tumor blood flow and vascular permeability with dovitinib therapy. A decrease in FGFR phosphorylation was observed in paired tumor biopsy samples from a patient treated with dovitinib at a dose of 400 mg/day.

**Conclusions:** At a dose of 400 mg/day, dovitinib demonstrated an acceptable safety profile and limited clinical benefit, and inhibited FGFR and VEGFR.
Our current understanding of cancer biology has led to the discovery of specific pathophysiologic abnormalities required for the growth and proliferation of cancer cells. These abnormalities have the potential to serve as both biomarkers of disease and targets for therapeutic intervention. These include growth factors, such as VEGF, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and their receptors and downstream signaling components. In melanoma, deregulation of specific growth factors contributes to the development of progression of disease. VEGF signaling is upregulated during melanoma progression and plays a pivotal role in tumor-related angiogenesis (1). Both basic FGF (bFGF) and PDGF signaling have been associated with autocrine stimulation of melanoma growth (1, 2). bFGF and its receptors are expressed in melanoma cells but not in normal melanocytes (1, 2). Inhibition of growth factors, including bFGF, induces tumor cell apoptosis and inhibits the growth of melanoma in preclinical animal models (1). Activation of the FGF pathway also plays an important role in developing an escape mechanism to antiangiogenic drugs targeting VEGF receptors, because FGF expression increases with VEGFR-blocking treatment in vivo (3). A blockade of the FGF pathway can overcome resistance to VEGFR inhibitors (3).

Dovitinib (TKI258) is a small-molecule inhibitor of FGF receptors (FGFR)1-3, VEGFR1-3, PDGF receptor (PDGFR)β, and other class III receptor tyrosine kinases (4, 5). Inhibition of FGFR1-3 distinguishes dovitinib from other VEGFR inhibitors, such as sorafenib and sunitinib (6). Dovitinib has the potential for antitumor activity through direct inhibition of FGFR and PDGFR as well as antiangiogenic activity through inhibition of FGFR, VEGFR, and PDGFR (5). Dovitinib inhibits autophosphorylation of its target receptor tyrosine kinases in in vitro and in vivo tumor models. Tumor regression and stabilization in mouse tumor models was observed at doses of 30 to 100 mg/kg, which is consistent
with dose levels required for target modulation (5). The human absorption, distribution, metabolism, and excretion study identified the major metabolites as C-hydroxyl metabolites (in feces) and N-oxide (in plasma) and demonstrated that the majority of the total dose administered was recovered from feces, and less than 21% of the dose was recovered in urine. Dovitinib is metabolized mainly by cytochrome P450 (CYP)1A1/2 and flavin-containing monooxygenase. Flavin-containing monooxygenase is not readily induced or inhibited by any other agents. To a lesser extent, dovitinib could be metabolized by CYP3A4, CYP2C8, and CYP2D6 enzymes.

We conducted a prospective, phase I/II, dose-escalation study to evaluate the maximum tolerated dose (MTD) of dovitinib on a once-daily continuous dosing schedule. Additional endpoints included evaluation of preliminary efficacy, safety, biological activity, and potential biomarkers of angiogenesis to monitor the pharmacodynamic effect of oral dovitinib in advanced melanoma. Functional imaging with dynamic contrast-enhanced MRI (DCE-MRI) was used as a noninvasive in vivo assessment of dovitinib efficacy in patients by evaluating tumor blood flow, vascular permeability, and tumor tissue oxygenation before and after dovitinib treatment in patients with metastatic tumors (7, 8). The use of DCE-MRI was designed to provide an early pharmacodynamic assessment of the antiangiogenic effect of dovitinib.

Materials and Methods

This was an open-label, single-arm trial designed with an initial dose-escalation stage followed by a dose-expansion stage. The study was approved by the institutional review board or institutional ethics committee of each participating center and was conducted in accordance with the Declaration of Helsinki. This trial was registered at
www.clinicaltrials.gov as NCT00303251. Informed consent was obtained from each patient before participation in the study.

**Patient selection**

Patients were eligible for the study if they were $\geq 18$ years of age and had histologically or cytologically documented locally advanced or metastatic melanoma (American Joint Committee on Cancer stage IIIB, IIIC, or IV) that was refractory to or had relapsed after standard therapy or for which no curative standard therapy exists. Patients with at least one measurable or nonmeasurable lesion, as defined by Response Evaluation Criteria in Solid Tumors (RECIST), were eligible for the dose-escalation phase of the study. For the dose-expansion phase, eligible patients had at least one measurable lesion as defined by RECIST. An Eastern Cooperative Oncology Group performance status of 0 or 1 was required for the study. Additional inclusion criteria included the following: absolute neutrophil count $\geq 1,500/\text{mm}^3$, platelet count $\geq 75,000/\text{mm}^3$, hemoglobin $\geq 8.0 \text{ g/dL}$, serum creatinine $\leq 1.5 \times$ upper limit of normal (ULN), bilirubin $\leq 1.5 \times$ ULN, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 2.5 \times$ ULN, except for subjects with tumor involvement of the liver, who must have had an ALT $\leq 5 \times$ ULN. Patients with impaired cardiac function or clinically significant cardiac disease were not eligible for the study. Patients were excluded if they had received an investigational agent, chemotherapy, targeted therapy, monoclonal antibody therapy, or wide-field radiotherapy within 4 weeks prior to starting the study drug. Patients were also excluded if they had received biological therapy, received immunotherapy, received limited-field radiation for palliation, undergone major surgery, or received any hematopoietic colony-stimulating factor within 2 weeks prior to starting the study drug. Patients known to be seropositive for human immunodeficiency virus (screening was not required) or to have
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a history of another primary malignancy that was clinically significant or required active intervention were also excluded.

Study design

Prior to the start of treatment cycle 1, all patients participated in a 2-day pharmacokinetic (PK) run-in, in which they received a single dose of oral dovitinib on day 1, had blood samples drawn every 2 hours up to 10 hours post-dose, then at 24, 30, and 48 hours post-dose. Immediately following the run-in portion of the study, patients received dovitinib once daily in consecutive 28-day cycles with a starting dose of 200 mg/day. Two patients each were enrolled in the 200- and 300-mg/day cohorts, and at least four patients each were assigned to the 400- and 500-mg/day cohorts. Dose escalation was governed by a two-parameter Bayesian logistic regression model. Evaluable patients for dose-escalation decisions were required to receive a minimum of 22 doses of dovitinib during the first 30 days of the study or have been withdrawn from the study secondary to a dose-limiting toxicity (DLT) prior to receiving 22 doses of dovitinib. Patients who withdrew from the study without meeting these criteria were replaced within their cohort. At least six patients were treated at the MTD.

The primary objectives of the dose-escalation phase were to determine the MTD based on DLTs and to evaluate the PKs of oral dovitinib. The primary objective for the dose-expansion phase was to assess the preliminary efficacy based on RECIST.

Efficacy assessment

Tumor status was evaluated at baseline and every 8 weeks during treatment for all patients by computed tomography scan (conventional or spiral), MRI, or clinical examination. The primary efficacy endpoint was tumor response measured by the rates
of clinical response, stable disease, and progressive disease per RECIST. Progression-free survival and overall survival were assessed as secondary efficacy endpoints.

**Safety assessment**

All patients treated in a cohort were required to undergo safety evaluations on days 8, 15, and 28 of cycle 1 prior to the next dose cohort being enrolled. Afterward, patients underwent a safety evaluation once every cycle. Patients who experienced a DLT had their dose reduced to the next lower dose level. Up to two dose reductions were allowed. Intrapatient dose escalations were permitted only after two patients had been safely treated at the next higher doses. Adverse events (AEs) were assigned toxicity grades according to the Common Terminology Criteria for AEs version 3.0.

DLTs, defined in the protocol, included the following: grade ≥3 neutropenia for >7 consecutive days; grade 3 thrombocytopenia for >7 consecutive days; grade 4 thrombocytopenia; febrile neutropenia (absolute neutrophil count, including bands: <1.0 × 10^9/L; fever: body temperature ≥38.5°C); serum creatinine 2–3 × ULN for >7 consecutive days; grade ≥3 serum creatinine; total bilirubin 2–3 × ULN for >7 consecutive days; grade ≥3 total bilirubin; grade 3 AST or ALT for >7 consecutive days; grade 4 AST or ALT; grade 4 cardiac hypertension; grade 2 or 3 cardiac hypertension if diastolic blood pressure did not stabilize to within 20 mm Hg (or clinically acceptable range for that patient) of pretreatment measurements, despite concomitant antihypertensive treatment for ≤7 days; grade ≥3 cardiac event; grade >1 neurotoxicity event for >7 days; any grade 3 event (excluding alkaline phosphatase elevation) that caused an inability to administer dovitinib for >7 consecutive days; any grade 4 event (excluding alkaline phosphatase elevation); grade ≥3 diarrhea despite the use of optimal
antidiarrheal treatments; and any other adverse event unrelated to disease progression, intercurrent illness, or concomitant medications that caused an inability to administer more than seven doses of dovitinib within a treatment cycle.

Pharmacokinetic evaluation
The terminal half-life of dovitinib was estimated from the 2-day PK run-in. For patients who underwent intrapatient dose escalation, samples were collected on day 28 of the first cycle of the new dose, before the dose, and at 2, 4, 6, 8, 10 (optional), and 24 hours after the dose.

Plasma concentrations of dovitinib were measured by liquid chromatography–tandem mass spectrometry. The maximum concentration ($C_{\text{max}}$) and area under the curve from time zero to t ($\text{AUC}_{0-t}$) for dovitinib were estimated on full PK samples collected on days 1, 15, and 28 of cycle 1. Noncompartmental analysis (WinNonLin, V5.2) was conducted to determine key PK parameters, including $\text{AUC}_{0-4}$ and half-life.

Pharmacodynamic analysis
Plasma samples were obtained at baseline, on day 15, and on days 26–28 to assess the following plasma biomarkers: VEGF, placental growth factor (PLGF), bFGF, soluble VEGFR1 (sVEGFR1) and VEGFR2 (sVEGFR2), FGF23, and c-KIT. The Meso-Scale Discovery platform was used to analyze VEGF, PLGF, bFGF, VEGFR1, and c-KIT levels, and an ELISA (Kinos) was used to analyze FGF23 levels.

Tumor biopsy samples were collected from consenting patients at baseline and within 6 hours of the dosing on day 15 of cycle 1. Tumor cells were stained for pErk with rabbit anti-pErk monoclonal 20G11 (Cell Signaling Technologies), at a concentration of 1
μg/mL on a Ventana immunostainer, after antigen retrieval using Ventana CC1 buffer at pH 8 for 30 min at 100°C. Tumor cells were stained for pFGFR3 using custom affinity-purified rabbit anti-phospho FGFR3 (Y724-P) antibody (NVS Emeryville), at a concentration of 1 μg/mL, after antigen retrieval using Ventana CC1 at room temperature. Ventana Omnimap was used for detection.

DCE-MRI scans were performed in patients with liver metastases of ≥3 cm according to previously reported protocols (7, 9). Assessments were performed during baseline and during cycles 1, 2, and 4 for consenting patients. Tumor blood flow, vascular permeability, and tumor tissue oxygenation were quantified as a volume transfer constant ($K_{\text{trans}}$), which is proportional to the degree of tumor perfusion and vascular permeability (8). Based on previous research, a decrease in $K_{\text{trans}}$ of greater than 20% is considered to be associated with a clinically significant vascular response of the tumor (9). An objective vascular response was defined as the change in $K_{\text{trans}}$ and/or in blood-normalized AUC of greater than 2 SD. Levels of FGF23 were assessed as a pharmacodynamic marker of FGFR1 inhibition (10, 11).

**Statistical analyses**

A two-stage multinomial design (12) was used for the dose-expansion phase. The primary endpoint was tumor response. The null hypothesis of $H_0$ (response rate of ≤5% and disease progression rate at month 2 of ≥50%) was tested against the alternative hypothesis of $H_a$ (response rate of >5% and disease progression rate at month 2 of <50%). The nominal type I error rate was set at 10%. The dose-expansion phase was powered at 90% for a 15% response rate and a 25% disease progression rate. Twenty patients were needed in the first stage to provide an additional assessment of safety. If the study continued to the second stage, an additional 20 patients would be needed. The
safety analysis population comprised all patients who received any amount of study
treatment. The safety analyses were performed using descriptive statistics.

All biomarker data were log₂ transformed and subsequently baseline subtracted to
account for the skew that often occurs in these data. This has the effect of modeling the
data as the log₂ change from baseline. Because of their small sample size, the 200- and
300-mg dose cohorts were combined, while the 400- and 500-mg cohorts were
assessed separately. The changes from baseline were assessed using a two-way linear
mixed-effects model of dose group, time, and their interaction, which accounted for the
within-subject correlation using an autoregressive covariance structure and was applied
to each biomarker separately. This allowed the model-adjusted mean changes from
baseline and their statistical significance to be computed for each dose group and at the
day 15 and day 28 time points, individually. Although the original results were generated
in the log₂ space, this was easily converted to the model-adjusted average ratio from
baseline via exponentiation. A false discovery rate (13) adjustment was applied to the
P values to control for the multiple statistical tests being performed. Spearman correlations
were used to determine the degree of association between the imaging
pharmacodynamic biomarker K_{trans} and PK biomarker AUC at baseline.

Results

Between April 5, 2006, and September 28, 2008, 47 patients were treated. Of these
patients, 27 were enrolled in the dose-escalation segment and 20 were enrolled in stage
1 of the dose-expansion segment. Patient characteristics by treatment are summarized
in Table 1.
Clinical efficacy

Preliminary efficacy was a primary objective for the dose-expansion segment only and was evaluated for the full analysis set (N = 47). The best overall tumor response after 8 weeks of treatment was stable disease in 12 patients (25.5%) and progressive disease in 25 patients (53.2%); the remaining 10 patients were not assessed, or the response was unknown. Stable disease was achieved by 1 of 4 patients at the 200–300-mg dose, 10 of 36 patients at the 400-mg dose, and 1 of 7 patients at the 500-mg dose. Seven patients maintained stable disease for at least 4 months (1 of 4 at the 200–300-mg dose, 5 of 36 at the 400-mg dose, and 1 of 7 at the 500-mg dose). No patients achieved complete or partial responses. The median progression-free survival from Kaplan-Meier analysis was 2.04 months (95% CI, 1.87–3.68 months), and the median overall survival was 7.49 months (95% CI, 4.30–9.23 months). The study was completed after stage 1 of the dose-expansion phase, because the prespecified criteria for continuation were not met.

Safety and tolerability

**Drug exposure.** The median durations of treatment for the 200–300-, 400-, and 500-mg daily dosing groups were 85 (range, 42–279), 58 (range, 1–338), and 62 (range, 27–182) days, respectively. The median duration of treatment for the entire cohort was 58 (range, 1–338) days.

No dose modifications or delays occurred in the 200–300-mg dose group. Unplanned dose reductions and/or delays occurred in 13 of 36 (36.1%) patients in the 400-mg group and in 6 of 7 (85.7%) patients in the 500-mg group. The events triggering unplanned dose reductions or interruptions included the following: AEs (15 patients), laboratory abnormalities (3 patients), or medication errors (3 patients); some patients experienced
multiple events and dose changes. Dose reductions occurred in nine patients in the 400-mg group and in four patients in the 500-mg group. A total of 24 (51.1%) patients required a dose adjustment or interruption because of an adverse event of all causes; 19 (40.4%) patients required a dose adjustment or interruption because of a study drug–related adverse event.

**Dose-limiting toxicities.** During the dose-escalation phase of the study, no DLTs occurred in the 200–300-mg dose cohort ($n = 4$) or in the 400-mg cohort ($n = 6$). As a result, enrollment was opened for the 500-mg/day group. Of the seven patients treated with 500 mg/day (all evaluable), three experienced DLTs: grade 3 fatigue, grade 4 fatigue, and grade 3 diarrhea. The 500-mg/day dose was determined to exceed the MTD. Ten additional patients were then enrolled in the 400-mg/day group, of whom three experienced DLTs: grade 3 nausea (one patient) and grade 3 fatigue (two patients). Overall, of the 16 patients treated with 400 mg/day in the dose-escalation phase, DLTs were reported in 3 of 10 evaluable patients. The MTD was thus defined as 400 mg/day, and the dose-expansion phase of the study was opened to enroll an additional 20 patients at the 400-mg/day dose.

**AEs.** The AEs occurring in $\geq 10\%$ of the patients (regardless of the study drug relationship) are summarized in Table 2. The most common AEs observed were diarrhea, fatigue, and nausea (76.6% each, all grades). The most frequent grade 3 and/or grade 4 AEs were fatigue (27.7%), diarrhea (10.6%), upper abdominal pain (8.5%), dehydration (8.5%), and nausea (8.5%). Grade 3 and/or grade 4 hypertension was reported in 2 patients (4.3%). No hand-foot syndrome was observed. A total of seven patients discontinued the study because of AEs, including two patients enrolled in the dose-escalation phase, for whom the AEs were considered DLTs. Two deaths
occurred during the study; both were secondary to the progression of metastatic melanoma, and neither was considered to be related to the study drug.

The percentage of patients with newly occurring or worsening grade 3 or grade 4 abnormal results from the hematologic or clinical chemistry laboratory tests was low overall. The hematology measure associated with the highest overall percentage of patients worsening from baseline to grade 3 was the absolute lymphocyte count, which decreased in 2 of 24 (8.3%) patients. None of the measured hematologic tests worsened to grade 4. Other hematologic measures that worsened from baseline to grade 3 were platelet count and hemoglobin (both of which decreased in 3 of 44 patients, 6.5%) and the white blood cell count (which decreased in 2 of 46 patients, 4.3%). The clinical chemistry tests with the most frequent laboratory abnormalities that worsened from baseline to grade 3 were triglycerides (five patients, 11.4%) and alkaline phosphatase (six patients, 13%). Other significant laboratory abnormalities that worsened from baseline to grade 3 included AST (which was elevated in 1 of 46 patients, 2.2%) and ALT (which was elevated in 1 of 46 patients, 2.2%). No patient had a worsening of bilirubin levels to grade 2, 3, or 4. The only abnormality that worsened from baseline to grade 4 was triglycerides, which occurred in one patient receiving the 400-mg dose.

Two patients (4.3%) experienced an increase from baseline in the QT interval of >60 msec, and no patient experienced a QT prolongation >500 msec. One patient had a QT >450 msec. No patients had new QTcF increases >60 msec or an absolute QTcF >500 msec. The ejection fraction decreased from baseline by a mean of 8.3% (n = 12) based on multiple gate acquisition scans and by 1.9% (n = 16) based on echocardiograms in evaluable patients. One patient who received the 400-mg dose experienced a grade 2
first-degree atrioventricular block that was reported as a serious adverse event but was not considered to be related to the study drug according to the investigator.

**Pharmacokinetics**

Key pharmacokinetic parameters are reported in Supplementary Table 1. Large interindividual variability (CV% ~50%) was observed in the AUC$_{0-t}$ and C$_{max}$. The mean AUC$_{0-t}$ values in the 200- and 300-mg/day dose cohorts were approximately the same, possibly because of the small sample size (two patients in each dose cohort) and the large interindividual variability. Dose-dependent increases in AUC$_{0-t}$ or C$_{max}$ were observed in patients receiving 200–500 mg/day. At doses of 400 mg/day and lower, AUC$_{0-t}$ on day 15 of cycle 1 was equal to or lower than that on day 1. The half-life of dovitinib was also reduced to approximately 13 hours on day 15 (from 24 hours on day 1), possibly because of auto-induction of CYP1A1/A2 (14). At a dose of 500 mg/day, an accumulation of dovitinib following multiple doses was observed in two of five patients, suggesting that the accumulation overrode the auto-induction. The mean AUC$_{0-t}$ on day 15 was approximately 2,800 (ng/mL⋅h) in the 400-mg/day cohort. In the 500-mg/day cohort, the mean AUC$_{0-t}$ on day 15 was approximately 3,400 (ng/mL⋅h).

**Biomarker analysis**

FGFR inhibition by dovitinib was assessed by measuring levels of the ligand FGF23—a circulating factor secreted by osteocytes that plays a critical role in phosphorus homeostasis and vitamin D metabolism (15, 16). FGF23, FGFR1, and co-receptor Klotho complex formation are required for FGF23 signaling to occur (17). Elevation of plasma FGF23 has been demonstrated to be a surrogate pharmacodynamic biomarker of FGFR1 inhibition, probably from compensatory upregulation due to FGFR inhibition.
Following dovitinib treatment, plasma FGF23 levels increased significantly in all dose cohorts from baseline to day 15 and continued to rise only in the 400-mg/day cohort to day 28 (Fig. 1A).

To confirm FGFR inhibition, pairs of tumor biopsy samples obtained before and after dovitinib treatment (400 mg/day) were examined for levels of phosphorylated FGFR and pErk—a downstream component of FGFR signaling. Collection of tumor biopsies was optional in the protocol for this study, and pre- and posttreatment biopsies were ultimately obtained from only two patients. Results from the single biopsy that was of sufficient quality for analysis are presented in Fig. 2. On day 15 of cycle 1, the expression of phosphorylated FGFR3 was lower than at baseline (Fig. 2A). Additionally, FGF23 in plasma samples increased initially after dosing on days 1 to 15, then declined from days 15 to 26, but remained above baseline levels (Fig. 2B). Furthermore, in paired tumor biopsy samples, pErk levels on day 15 of cycle 1 were lower than those at baseline (400 mg/day) (Fig. 2C). These results indicate that dovitinib therapy inhibits FGFR at the levels of the tumor in patients with melanoma treated with dovitinib 400 mg/day.

Plasma levels of candidate molecules were analyzed as potential biomarkers of angiogenesis. In all dose groups, plasma levels of VEGF on days 15 and 26 in the first cycle were higher than at baseline, suggesting inhibition of VEGFR (Fig. 1D; Table 3). The 200–300- and 400-mg groups had similar fold-increases in VEGF over baseline on day 15 (1.29 and 1.23, respectively), which increased to 1.54- and 1.51-fold, respectively, on days 26 to 28. In the 500-mg dose cohort, VEGF levels increased 1.73-fold on day 15 and 3.03-fold on day 28, which was statistically significant even after correction for the false discovery rate.
In the 400- and 500-mg dose cohorts, sVEGFR2 decreased significantly from baseline on days 15 and 28 (Fig. 1C). PLGF levels increased from baseline in all dose groups on day 15 and days 26–28, with a >2-fold increase in each dose cohort on day 28, suggesting inhibition of VEGFR (Fig. 1B; Table 3).

**DCE-MRI**

A total of 15 patients with liver metastases underwent imaging using DCE-MRI. An objective vascular response (reduction in median $K_{\text{trans}}$ of ≥20%) was seen in none of three patients in the 200- and 300-mg cohorts, in three of six patients in the 400-mg cohort, and in four of six patients in the 500-mg cohort. No significant treatment effect was present in the 200- or 300-mg cohorts ($P = 0.58$). However, reductions in median $K_{\text{trans}}$ were nearly significant in both the 400-mg ($P = 0.06$) and 500-mg ($P = 0.08$) cohorts. Assessment of the median change in $K_{\text{trans}}$ on day 2 of cycle 1 in 15 patients demonstrated that the change in vascularity of the metastatic tumors with dovitinib treatment was dose-dependent. Additionally, linear regression demonstrated a correlation between a decrease in $K_{\text{trans}}$ in liver metastases at an early time point (day 2 of cycle 1) and an increase in plasma dovitinib exposure on day 1 (Fig. 3).

**Discussion**

This phase I/II study was conducted to determine the MTD, the biological activity, and the preliminary efficacy of dovitinib as a once-daily continuous oral dose in patients with advanced melanoma. Here, the MTD was defined by Bayesian methodology as 400 mg/day. During the dose-escalation phase, DLTs were observed in three patients in the 400-mg dose group (grade 3 fatigue in two patients and grade 3 nausea in one patient).
and in three patients in the 500-mg dose group (grade 3 and 4 fatigue and grade 3 diarrhea, each in one patient), consistent with other agents in this class of drugs. In a previous phase I study of dovitinib in solid tumors that assessed a once-daily continuous dosing schedule, the MTD was defined as 125 mg/day (18). DLTs in that study were as follows: grade 3 hypertension in one patient at a continuous dose of 100 mg/day, grade 3 anorexia in a second patient at 175 mg/day, and grade 3 alkaline phosphatase elevation in a third patient at 175 mg/day. In that study, definitions for DLTs were more limited compared with those in the current study. The definitions for DLTs in the current study were broad, including any grade 4 event and any grade 3 event causing an inability to administer dovitinib for 7 consecutive days. In the current study, the most frequently reported AEs were diarrhea, nausea, and fatigue, all of which occurred across dose levels and were generally mild (grade 1 or 2). These AEs are known side effects of other targeted multikinase inhibitors (19, 20). Hand-foot syndrome is a commonly observed adverse event in patients receiving small-molecule multikinase inhibitors (21, 22); notably, no incidents of hand-foot syndrome were observed in this study with dovitinib treatment.

Complete or partial responses were not observed in this study, but 25.5% and 14.9% of the patients achieved stable disease lasting at least 2 and 4 months, respectively. Similar to other antiangiogenic therapies evaluated for the treatment of melanoma (23-27), single-agent dovitinib was not sufficient to meaningfully reduce the tumor burden and provided limited clinical benefit. In a phase II study of axitinib, a VEGFR inhibitor, patients with metastatic melanoma had an objective response rate of 15.6% (28). A phase II study of E7080, another oral multikinase inhibitor with activity against FGFRs, is ongoing (ClinicalTrials.gov, identifier NCT01136967). Thus, therapies targeting VEGFR and FGFR may have clinical utility in the treatment of melanoma.
dubious that any single agent will be sufficient to treat this aggressive malignancy, and studies of dovitinib and other multikinase inhibitors as combination therapies may prove more successful.

PK results demonstrated a dose-dependent increase in AUC$_{0-t}$ and $C_{\text{max}}$ when dovitinib was administered at doses from 200 to 500 mg/day. Time-dependent PKs of dovitinib were observed, consistent with the findings of other studies (14). Following a continuous daily dose of <400 mg/day, the auto-induction of CYP1A1/A2 resulted in a lower plasma exposure of dovitinib than that on day 1 of cycle 1. However, when the daily dose was increased from 400 to 500 mg, plasma dovitinib concentrations were similar to or greater than those on day 1 of cycle 1, rather than lower, as described above for the lower dose groups. This finding suggests a more pronounced accumulation of dovitinib at higher doses. At the MTD (400 mg/day), no accumulation in exposure was observed, and steady state was achieved on day 15. An additional phase I/II study is ongoing to assess a 5-days-on/2-days-off schedule to decrease the accumulation effect. Preliminary data suggest that this dose schedule is feasible to control drug accumulation and achieve target inhibition and a biological effect (10, 29).

Previous studies have shown that the reduction in tumor vascularity upon exposure to tyrosine kinase inhibitors is significantly correlated with improved clinical outcome in patients with advanced disease (30). Levels of soluble sVEGFR2 and VEGF were inversely correlated in patients with acute lymphoblastic leukemia and a number of other conditions (31-33); similar effects have been observed with the preclinical clinical use of VEGF-targeting drugs (34-37). This observation has stimulated the investigation of these plasma proteins as possible surrogate biomarkers for targeted inhibition of angiogenesis.
Dovitinib therapy was associated with an increase in VEGF and PLGF levels and a decrease in sVEGFR2 levels; these changes are indicative of antiangiogenic action associated with inhibition of VEGFR, and VEGF, PLGF, and SVEGFR2 may be useful as potential biomarkers for dovitinib treatment. DCE-MRI analysis demonstrated a dose-dependent change in metastatic tumor vascularity with dovitinib treatment. Furthermore, a higher exposure to dovitinib was associated with a greater reduction in the vascularity of liver metastases at an early time point (day 2 of cycle 1). These data clearly demonstrated a classic vascular response comparable with that of other anti-VEGF treatments (7, 9).

Additionally, treatment with dovitinib led to an increase in plasma FGF23 levels and to an inhibition of downstream targets of FGFR activation (e.g., pErk) in tumor lesions, demonstrating dovitinib inhibition of FGFR signaling in these patients. These results indicate that the panel of circulating proteins examined in this study may have utility as pharmacodynamic biomarkers not only in dovitinib-treated patients with melanoma but also in other cancer types. Taken together, dovitinib treatment resulted in an increase from baseline in FGF23, VEGF, and PLGF and a decrease in sVEGFR2 levels, indicating that dovitinib is capable of inhibiting VEGFR in melanoma cells and suggesting the utility of VEGF, sVEGFR2, and PLGF as pharmacodynamic biomarkers of dovitinib activity.

This study demonstrated that oral dovitinib has an acceptable safety profile and provides pharmacologic inhibition of both the VEGFR and FGFR pathways at the MTD of 400 mg once daily. Future studies are ongoing with a once-daily 5-days-on/2-days-off schedule, which was predicted by modeling to eliminate the accumulation effect on exposure that was demonstrated in this study (14). Results from a phase I/II study in patients with renal
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cell carcinoma have demonstrated that this dosing schedule eliminates the accumulation
effect. Because dovitinib is an inhibitor of FGFRs and VEGFRs, in contrast with other
tyrosine kinase inhibitors (e.g., sorafenib and sunitinib), it may be beneficial in FGF-
activated cancers. These include breast cancers (due to FGFR1 amplification), bladder
cancer (due to FGFR3 mutations), multiple myeloma (due to FGFR3 overexpression),
and endometrial cancers (due to FGFR2 mutations), for which dysregulation of the
FGFR pathway is important in tumor survival (38-46). FGFR inhibition may also have
utility in renal and hepatocellular cancers, because the upregulation of FGF signaling
appears to play a role in resistance to VEGFR inhibitors (47, 48).

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References


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Figure legends

Fig. 1. Effect of dovitinib on plasma biomarkers: A, FGF23; B, PLGF; C, sVEGFR2; and D, VEGF. Longitudinal plots of the model-estimated fold change from baseline on days 15 and 26 of the first dosing cycle are shown. Thin line, 200–300 mg/day dose; bold line, 400 mg/day dose; dashed line, 500 mg/day dose; bars, SE. Mean levels at baseline were as follows: FGF23, 42.34 pg/mL; PLGF, 31.05 pg/mL; sVEGFR2, 172.99 pg/mL; and VEGF, 197.91 pg/mL.

Fig. 2. Dovitinib inhibits FGFR3 phosphorylation and pErk phosphorylation. A, a pair of tumor biopsy samples stained with anti-pFGFR3 antibody before and after treatment (day 15 of cycle 1, 400 mg/day). B, plasma FGF23 levels from the same patient receiving dovitinib (400 mg/day) on days 1, 15, and 26 of cycle 1 (C1D1, C1D15, and C1D26). C, paired tumor biopsy samples from the same patient stained with anti-pErk antibody before and after treatment (day 15 of cycle 1, 400 mg/day).

Fig. 3. Dovitinib exposure correlates with a change in $K_{trans}$ in liver metastases at an early time point (day 2 of cycle 1) measured by DCE-MRI.
Table 1. Baseline demographics and patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>200–300 mg (n = 4)</th>
<th>400 mg (n = 36)</th>
<th>500 mg (n = 7)</th>
<th>Total (N = 470)</th>
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<td>48.5</td>
<td>57.0</td>
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<td>19 (53)</td>
<td>4 (57)</td>
<td>25 (53)</td>
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<td>17 (47)</td>
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<td>2 (28.6)</td>
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<td>4 (11.1)</td>
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<td>4 (8.5)</td>
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Abbreviation: ECOG, Eastern Cooperative Oncology Group.
Table 2. Adverse events occurring in ≥10% of patients, regardless of the study drug relationship

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<th>Adverse events, n (%)</th>
<th>All grades</th>
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<th>Grade 4</th>
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<td>47 (100)</td>
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<td>6 (12.8)</td>
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<td>Diarrhea</td>
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<td>Fatigue</td>
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<td>Vomiting</td>
<td>22 (46.8)</td>
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<td>Weight decrease</td>
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<td>2 (4.3)</td>
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<td>Dysgeusia</td>
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<td>Dyspnea</td>
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<td>Dehydration</td>
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<td>Dizziness</td>
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<tr>
<td>bFGF</td>
<td>200–300 mg</td>
<td>1.23</td>
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<td></td>
<td>400 mg</td>
<td>0.66</td>
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<td>500 mg</td>
<td>1.83</td>
<td>0.30</td>
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<td>c-KIT</td>
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<td>0.23</td>
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<td>500 mg</td>
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<td>500 mg</td>
<td>1.84</td>
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<td>sVEGFR1</td>
<td>200–300 mg</td>
<td>0.93</td>
<td>0.89</td>
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<td>500 mg</td>
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<td>sVEGFR2</td>
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<td>0.76</td>
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<tr>
<td></td>
<td>500 mg</td>
<td>0.73</td>
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<td>VEGF</td>
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<td>0.59</td>
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<tr>
<td></td>
<td>500 mg</td>
<td>1.73</td>
<td>0.09</td>
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Abbreviations: bFGF, basic fibroblast growth factor; FGF fibroblast growth factor; PLGF, placental growth factor; sVEGFR, soluble VEGF receptor
Figure 1

A. Model-Estimated Fold Change From Baseline for FGF23 over time (Days)

B. Model-Estimated Fold Change From Baseline for PLGF over time (Days)

C. Model-Estimated Fold Change From Baseline for sVEGFR2 over time (Days)

D. Model-Estimated Fold Change From Baseline for VEGF over time (Days)
Figure 2

(A) Representative images of tissue sections stained for FGF23.

(B) Graph showing FGF23 levels over Treatment Cycle and Day.

(C) Additional images of tissue sections stained for FGF23.
Clinical Cancer Research

Phase I/II and Pharmacodynamic Study of Dovitinib (TKI258)—an Inhibitor of Fibroblast Growth Factor Receptors and VEGF Receptors—in Patients with Advanced Melanoma

Kevin B. Kim, Jason Chesney, Douglas Robinson, et al.

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