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Cancer Therapy: Clinical

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A Phase I Study of Foretinib, a Multi-Targeted Inhibitor of c-Met and Vascular Endothelial Growth Factor Receptor 2

Joseph Paul Eder[1], Geoffrey I. Shapiro[1], Leonard J. Appleman[3], Andrew X. Zhu[2], Dale Miles[4], Harold Keer[4], Belinda Cancilla[4], Felix Chu[4], Suzanne Hitchcock-Bryan[1], Laurie Sherman[5], Stewart McCallum[5], Elisabeth I. Heath[6], Scott A. Boerner[6], and Patricia M. LoRusso[6]

Abstract

Purpose: Foretinib is an oral multikinase inhibitor targeting Met, RON, Axl, and vascular endothelial growth factor receptor. We conducted a phase I, first-time-in-human, clinical trial using escalating doses of oral foretinib. The primary objectives are to identify a maximum tolerated dose and determine the safety profile of foretinib. Secondary objectives included evaluation of plasma pharmacokinetics, long-term safety after repeated administration, preliminary antitumor activity, and pharmacodynamic activity.

Experimental Design: Patients had histologically confirmed metastatic or unresectable solid tumors for which no standard measures exist. All patients received foretinib orally for 5 consecutive days every 14 days. Dose escalation followed a conventional "3+3" design.

Results: Forty patients were treated in eight dose cohorts. The maximum tolerated dose was defined as 3.6 mg/kg, with a maximum administered dose of 4.5 mg/kg. Dose-limiting toxicities included grade 3 elevations in aspartate aminotransferase and lipase. Additional non–dose-limiting adverse events included hypertension, fatigue, diarrhea, vomiting, proteinuria, and hematuria. Responses were observed in two patients with papillary renal cell cancer and one patient with medullary thyroid cancer. Stable disease was identified in 22 patients. Foretinib pharmacokinetics increased linearly with dose. Pharmacodynamic evaluation indicated inhibition of *MET* phosphorylation and decreased proliferation in select tumor biopsies at submaximal doses.

Conclusions: The recommended dose of foretinib was determined to be 240 mg, given on the first 5 days of a 14-day cycle. This dose and schedule were identified as having acceptable safety and pharmacokinetics, and will be the dose used in subsequent phase II trials. *Clin Cancer Res; 16(13); 3507–16.* ©*2010 AACR.*

Hepatocyte growth factor (HGF) and its receptor tyrosine kinase signaling axis, Met, have been associated with several types of cancer (1, 2). Evidence supports roles for HGF-Met signaling in the regulation of three conspicuous properties of tumor cells: cell proliferation, tissue invasion, and metastasis (2–4). HGF-Met signaling synergistically mediates angiogenesis through downregulation of thrombospondin-1, a major endogenous angiogenesis inhibitor, and by upregulation of the highly angiogenic vascular endothelial growth factor (VEGF; ref. 5). Overexpression of HGF and Met is indicative of increased aggressiveness of tumors and poor prognosis in cancer patients (5–11). Recent evidence implicates upregulation of *HGF* and *MET* after VEGF-inhibitory therapy as a mechanism of resistance to angiogenesis inhibitors (12). Foretinib (formerly XL880) was developed as a small-molecule receptor tyrosine kinase inhibitor with a dual purpose: to target abnormal signaling of HGF through Met and simultaneously target several receptor tyrosine kinases involved in tumor angiogenesis. Foretinib has nanomolar *in vitro* and *in vivo* inhibitory activity for Met and VEGF receptor-2 (VEGFR2). It also has high *in vitro* affinity for platelet-derived growth factor receptor-β (PDGFRβ), Tie-2, RON, Kit, and FLT3 and kinases. Foretinib binds avidly and deeply in the adenosine triphosphate pocket of both *MET* and VEGFR2, inducing a conformational change with a mean cellular target residence time of >24 hours (13, 14). Foretinib caused tumor hemorrhage and necrosis in human xenografts within 2 to 4 hours, and maximal tumor necrosis was observed at 96 hours (after five daily doses),

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Translational Relevance

Foretinib was developed to test the hypothesis that antitumor activity could be combined in a single agent through inhibition of both tumor proliferation (Met) and angiogenesis (vascular endothelial growth factor receptor 2, Tie-2, and platelet-derived growth factor). This first-time-in-human, phase I, dose-escalation trial of oral foretinib established that a dose up to 3.6 mg/kg for 5 consecutive days of a 14-day cycle was acceptable for phase II trials and showed significant evidence of biological activity and clinical efficacy. Reversible elevations in serum aspartate aminotransferase and lipase were the dose-limiting toxicities. The clinical activity in this phase I trial supports plans to investigate alternative schedules and to explore the mechanism by which Met or angiogenesis drives tumors. This activity further supports additional investigation into agents with extended target spectrums of activity, selected for potentially significant interdependency in the neoplastic progression found in refractory solid tumor malignancies.

resulting in complete regression in tumors ranging in size from 100 to 1,000 mm^3. Peak plasma levels required for optimal efficacy were 1 to 3 μmol/L, but associated trough levels were 0.02 to 0.1 μmol/L, consistent with prolonged biological effects (13). Because preclinical toxicities associated with foretinib were generally reversible with no evidence of tumor regrowth during off-drug periods, 5 days of treatment followed by 9 days of observation was chosen as the schedule for this first phase I clinical trial.

Patients and Methods

Patient selection

Eligible subjects were 18 years or older, with an Eastern Cooperative Oncology Group performance status of 0 to 2 and had histologically confirmed metastatic or unresectable solid tumors for which no standard curative or palliative measures exist. Patients were excluded if they had received chemotherapy, immunotherapy, radiotherapy, or an investigational agent within 4 weeks; had received radiation to ≥25% of bone marrow; were pregnant or lactating; had known brain metastases; or had uncontrolled intercurrent illness. The medical ethical committees of all participating institutions approved the study, and all patients gave written informed consent according to institutional regulations before participation.

Study design

Foretinib was administered for 5 consecutive days every 14 days in a phase I, nonrandomized, dose-finding study (MET111647, NCT00742131). The primary objectives were to determine the maximum tolerated dose and to assess safety and tolerability. Additional objectives included evaluation of plasma pharmacokinetics, long-term safety after repeated administration, preliminary antitumor activity, and pharmacodynamic activity.

Dose escalation followed a conventional "3+3" design using an escalation scheme with dose doubling until the first foretinib-related grade 2 toxicity was observed. Then, doses were increased by 50% until the first instance of grade 3 toxicity was observed, and doses were subsequently increased by 25%. The decision to proceed to the next dose cohort was based on events in the first cycle. All patients received an oral dose of study medication on day 1 followed by a 72-hour observation period. If there were no dose-limiting toxicities, patients received five consecutive daily doses of foretinib on days 4 to 8. The dosage of foretinib in cohort 1 was 0.1 mg/kg (15). Twenty-one days after the initial dose, patients were eligible to participate in the treatment extension period to continue foretinib on the 5-days-on, 9-days-off schedule without planned interruption. Dose-limiting toxicities were based on clinical observations during the entire treatment period and formed the basis for defining the maximum tolerated dose. Once the maximum tolerated dose was determined, additional patients were treated at that dose to confirm safety.

Study medication

Foretinib was provided as a powder-in-bottle formulation and as 20, 100, and 200 mg capsules. The capsule strengths and dose amounts used in this study are expressed as the bisphosphate salt formulation of foretinib (molecular weight 828.64; free-base molecular weight 632.66 Da).

Safety assessments

Safety was assessed through standard clinical and laboratory tests and collection of clinical adverse events and serious adverse events. The National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 was used for grading (16). Dose-limiting toxicity was defined as any clinically significant treatment-related adverse event that met any of the following criteria: (*a*) grade ≥3 if nonhematologic (including grade 3 nausea and/or vomiting and diarrhea despite treatment and grade 3 hypertension despite optimal antihypertensive therapy) or grade ≥4 if hematologic, or (*b*) an indication that subsequent patients were at enhanced risk of harm or the need for increased medical treatment. The maximum tolerated dose was the dose level below the maximum administered dose at which no more than one dose-limiting toxicity was seen among six treated patients. Subjects were considered evaluable for safety analyses if they received at least one dose of study drug.

Pharmacokinetic sampling and analysis

Whole blood samples were collected, using K^+-EDTA as an anticoagulant, at predose and at specified time points

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up to ~72 hours after the first dose (day 1) and predose and up to ~96 hours after dose administration on day 8 (fifth consecutive daily dose). Samples were centrifuged within 30 minutes of collection, and plasma was stored at −20°C or below until assayed. On day 1, urine was collected over 24 hours after the initial dose to measure renal excretion of drug.

Foretinib concentrations in plasma were determined by a validated high-performance liquid chromatography method with tandem mass spectrometric detection. Foretinib and the internal standard, gefitinib, were extracted from plasma by solid phase extraction. The standard curve range for foretinib was 0.50 to 500 ng/mL, using a plasma sample volume of 100 μL.

The validation protocol established the inter- and intra-assay precision (less than ±15% relative SD) and accuracy (less than or equal to ±15% deviation of mean from theoretical), selectivity, carryover, linearity, sensitivity, freeze/thaw stability, absolute recovery, room temperature stability, and long-term stability. The lower limit of quantitation for foretinib is 0.50 ng/mL, with linearity shown to the upper limit of quantitation of 500 ng/mL.

Pharmacokinetic parameters in plasma were determined by noncompartmental analysis and included maximum plasma concentrations (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24}$), and terminal half-life ($t_{1/2}$). Multiple-dose accumulation of foretinib was evaluated through the ratio of exposure parameters (i.e., C_{max} and $AUC_{0\text{-}24}$) on day 8 to corresponding values on day 1. Urinary recovery of foretinib was determined from pooled samples collected over 24 hours after the first dose.

Pharmacodynamics

Three patients were biopsied for evidence of biological efficacy of foretinib activity. All three patients had superficial tumor amenable to noninvasive serial biopsies. Serial biopsies of tumor and skin samples were analyzed for the target receptors of foretinib (Met, phospho-Met, RON, and phospho-RON), downstream signaling molecules (phospho-ERK$^{Thr202/Tyr204}$, phospho-Akt^{ser473}), and cell proliferation (Ki67) by immunohistochemistry, whereas apoptosis was analyzed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining. Immunohistochemistry results were scored, and percent changes were calculated. Fresh biopsies of tumor and skin were collected and frozen in OCT-embedding compound, and serial 10-μm cryosections were prepared. Sections were fixed with acetone and stained by immmunofluorescence for *MET* (Zymed), phospho-*MET*$^{Y1230/4/5}$ (Biosource), RON (Santa Cruz), phospho-RON$^{Y1238/9}$ (Exelixis), phospho-ERK$^{T202/Y204}$ (Cell Signaling), phospho-AKT^{S473} (Cell Signaling), Ki67 (Lab Vision), and TUNEL (Roche), with 4′,6-diamidino-2-phenylindole (DAPI; Molecular Probes) as a nuclear counterstain. Eight to 10 nonoverlapping fields were imaged per sample per readout (covering the majority of the section) and analyzed using MetaMorph. Levels of immunofluorescence staining were determined in the epithelium of skin biopsies (dermis and keratinized layer were excluded from analysis) and tumor cells of tumor biopsies (nontumor cells were excluded from analysis). Positive staining for each readout was calculated as area of red pixels (readout)/area blue pixels (DAPI) (%). For Ki67 and TUNEL, positive staining was calculated as number of red nuclei/number of blue nuclei (%). Changes were expressed relative to baseline samples.

Tumor response after repeat dosing of foretinib

For subjects with measurable lesions, tumor assessments were done within 30 days of first dose and approximately every 8 weeks thereafter using the Response Evaluation Criteria in Solid Tumors (17).

Statistical methods

Descriptive statistics were used for baseline characteristics, safety assessments, pharmacokinetic variables (C_{max}, T_{max}, $AUC_{0\text{-}24}$, and $t_{1/2}$), and exploratory assessments (time to progressive disease, clinical symptoms, tumor markers, and tumor response).

Table 1. Patient characteristics at baseline

Characteristic	Results (*N* = 40)
Mean age, y (range)	54.8 (30–79)
Sex (male/female), *n* (%)	18/22 (45.0/55.0)
Race, *n* (%)	
Asian	1 (2.5)
Black or African American	4 (10.0)
White	35 (87.5)
Mean years since diagnosis (SD)	3.3 (4.62)
Mean years since metastasis (SD)	2.6 (4.57)
Prior antitumor therapy, *n* (%)	
Prior chemotherapy or radiotherapy	36 (90.0)
Prior chemotherapy and radiotherapy	12 (30.0)
Median prior chemotherapy regimens, *n* (range)	3.0 (0–7)
Tumor type, *n* (%)	
Colorectal cancer	14 (35.0)
Papillary renal carcinoma	4 (10.0)
Ovarian adenocarcinoma	5 (12.5)
Renal cell carcinoma	3 (7.5)
Melanoma	3 (7.5)
Thyroid cancer	3 (7.5)
Sarcoma	2 (5.0)
Neuroendocrine metastatic	
Pancreatic	1 (2.5)
Pulmonary carcinoid (atypical)	1 (2.5)
Adenocarcinoma, clear cell (urethral)	1 (2.5)
Breast carcinoma (invasive ductal)	1 (2.5)
Squamous cell carcinoma (anorectal)	1 (2.5)
Hepatocellular	1 (2.5)

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Table 2. Treatment-related adverse events occurring in ≥20% of treated patients

	No. of events (no. of patients)								
	Cohort 1, 0.1 mg/kg (*n* = 3)	Cohort 2, 0.2 mg/kg (*n* = 3)	Cohort 3, 0.4 mg/kg (*n* = 7)	Cohort 4, 0.8 mg/kg (*n* = 3)	Cohort 5, 1.6 mg/kg (*n* = 3)	Cohort 6, 2.4 mg/kg (*n* = 3)	Cohort 7, 3.6 mg/kg (*n* = 14)	Cohort 8, 4.5 mg/kg (*n* = 4)	All cohorts (*N* = 40)
Hypertension,* any grade	0	0 (0)	0	3 (2)	2 (2)	1 (1)	8 (6)	2 (1)	16 (12)
Grades 1–2	0	0	0	3 (2)	1 (1)	1 (1)	6 (5)	2 (1)	13 (10)
Grades 3–4	0	0	0	0	1 (1)	0	2 (2)	0	3 (3)
Elevated LDH, any grade	0	0	1 (1)	1 (1)	0	4 (1)†	9 (6)	16 (3)	31 (12)
Grades 1–2	0	0	1 (1)	0	0	1 (1)	9 (6)	14 (3)	25 (11)
Grades 3–4	0	0	0	1 (1)	0	3 (1)	0	2 (2)	6 (4)
Proteinuria,‡ any grade	0	0	0	0	3 (1)	2 (1)	8 (4)	8 (3)	21 (9)
Grades 1–2	0	0	0	0	3 (1)	2 (1)	8 (4)	8 (3)	21 (9)
Grades 3–4	0	0	0	0	0	0	0	0	0
Elevated AST, any grade	0	0	0	0	0	0	6 (6)	5 (4)	11 (10)
Grades 1–2	0	0	0	0	0	0	6 (6)	4 (4)	10 (10)
Grades 3–4	0	0	0	0	0	0	0	1 (1)	1 (1)
Fatigue, any grade	2 (1)	0	0	0	2 (1)	0	8 (6)	2 (1)	13 (9)
Grades 1–2	2 (1)	0	0	0	2 (1)	0	8 (6)	2 (1)	13 (9)
Grades 3–4	0	0	0	0	0	0	0	0	0
Diarrhea, any grade	0	0	0	0	1 (1)	1 (1)	25 (6)	0	27 (8)
Grades 1–2	0	0	0	0	1 (1)	1 (1)	25 (6)	0	27 (8)
Grades 3–4	0	0	0	0	0	0	0	0	0
Vomiting, any grade	0	0	0	0	1 (1)	1 (1)	10 (5)	2 (1)§	14 (8)
Grades 1–2	0	0	0	0	1 (1)	1 (1)	10 (5)	1 (1)	13 (8)
Grades 3–4	0	0	0	0	0	0	0	1 (1)	1 (1)

Abbreviation: LDH, lactate dehydrogenase.

*Hypertension includes the preferred terms "hypertension" and "blood pressure increase."

†A single patient experienced all lactate dehydrogenase elevations of any grade reported for cohort 6.

‡Proteinuria includes proteinuria and protein urine.

§A single patient experienced all vomiting episodes of any grade reported for cohort 8.

Results

Patient characteristics and maximum tolerated dose determination

Forty patients were treated in eight cohorts between April 2005 and May 2008. Demographic characteristics, prior treatment history, and cancer types for the study participants are provided in Table 1. Cohorts of patients were enrolled and dosed at the following levels: 0.1, 0.2, 0.4, 0.8, 1.6, 2.4, 3.6, and 4.5 mg/kg. The 0.1 and 0.2 mg/kg cohorts received foretinib as a liquid formulation from the powder-in-bottle formulation. Two cohorts were dosed at 0.4 mg/kg, one receiving foretinib as a powder-in-bottle formulation, the other as capsules. The maximum administered dose was 4.5 mg/kg. Two patients developed symptomatic brain metastases and discontinued study drug as a result; therefore, they did not receive all scheduled doses in cycle 1 but were evaluable for toxicity.

Dose-limiting toxicities were seen in two of four patients receiving the maximum administered dose (4.5 mg/kg): grade 3 elevated levels of aspartate aminotransferase (AST) and lipase. Additional patients were then enrolled at the preceding dose level (3.6 mg/kg), expanding the cohort to 14 patients. Toxicity of foretinib at 3.6 mg/kg (median 240 mg) was tolerable, with protocol-defined dose-limiting toxicities developing in only one patient (central nervous system hemorrhage into previously unsuspected brain metastasis); 3.6 mg/kg was designated as the maximum tolerated dose.

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Thirty-three patients continued into the treatment extension period, receiving treatment for an additional 1 to >97 cycles (14 days to >48 months with a median of 66.5 days on therapy). Seven patients did not receive additional treatment with foretinib after cycle 1 due to either progressive disease (n = 6) or adverse event (n = 1).

Safety

All 40 patients were included in the safety evaluation. Table 2 lists treatment-related adverse events by cohort and severity. Thirty-nine (98%) patients reported at least one adverse event. Hypertension was an expected and frequently reported treatment-related adverse event (16 events in 12 patients; ref. 18). Hypertension was most often grade 1 or 2, with one grade 3 event at 1.6 mg/kg and two at 3.6 mg/kg. Elevations in AST occurred in 10 patients (25%), and all but one event were less than grade 3. Proteinuria was common, all grade 1 or 2, and resolved spontaneously in all but three of nine patients.

Events in cycle 1 did not reflect all the toxicities encountered. With prolonged exposure in patients demonstrating clinical benefit, delayed therapy-related events were noted. Fatigue was frequent, leading to discontinuation in one patient due to grade 2 toxicity. Two patients reported reversible confusion. One patient had recently completed a trial of cedaranib for medullary thyroid cancer, and the patient's spouse reported an episode of expressive aphasia and apraxia in repetitive executive functions (shaving, dressing) that subsided within 8 to 12 hours on the second course, on the 3rd day. The second patient had completed therapy for colon cancer with bevacizumab and FOLFOX 6 weeks before receiving foretinib. He became disoriented to time and place in his first and third cycles, initially during a dose increase in major opiates that resolved without need for foretinib dose modification and, again, at a time when he was developing worsening hepatic dysfunction. However, he improved briefly after stopping foretinib before the onset of progressive portosystemic encephalopathy. Additional events, namely pedal edema, fluid retention, nausea, and diarrhea, were bothersome, if not limiting, and resolved with reduction in the dose of foretinib.

Treatment-related adverse events leading to study drug discontinuation occurred in three patients. These were elevated lipase (grade 3), tumor hemorrhage (grade 3), and hemorrhage into central nervous system metastasis (grade 4); these patients were receiving 4.5, 3.6, and 0.4 mg/kg foretinib, respectively. Three deaths occurred within 30 days of the last dose of foretinib. These were assessed as unrelated to foretinib and attributed to progression of metastatic cancer, as documented clinically and by imaging studies.

Pharmacokinetics

All 40 patients were included in the pharmacokinetic analysis. Concentration-time plots of plasma foretinib by cohort after a single dose (day 1) or after five daily doses (day 8) are shown in Fig. 1, and summary pharmacokinetic parameters for the maximum tolerated dose cohort are provided in Table 3. Observed T_{max} concentrations occurred at ~4 hours on both days 1 and 8, with no differences between dose levels. Foretinib concentrations

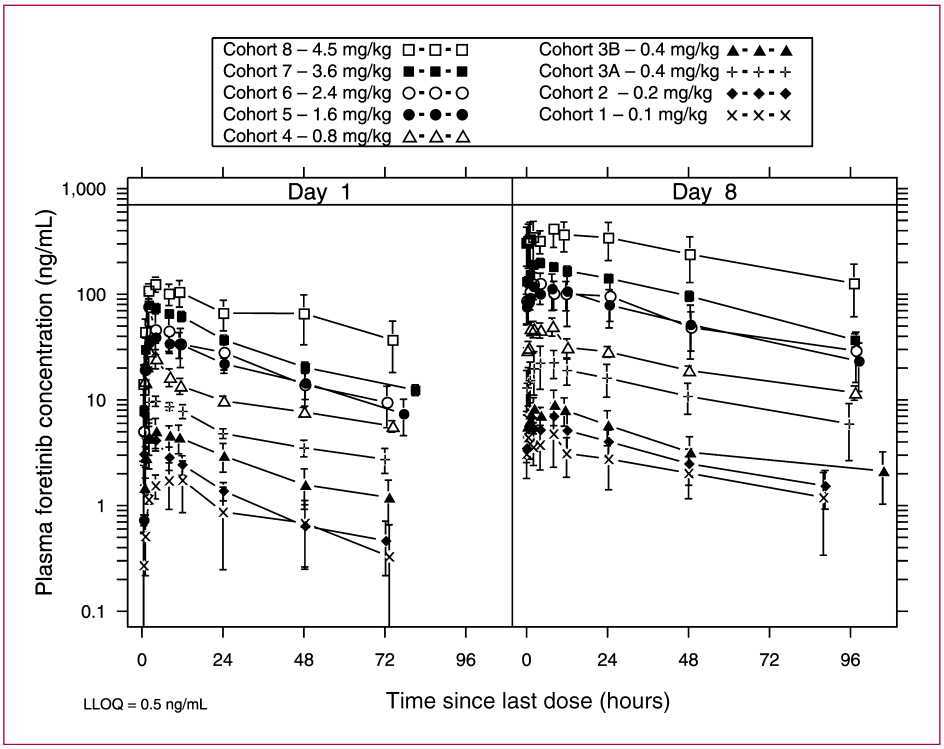

Fig. 1. Foretinib pharmacokinetics. Mean (±SE) foretinib plasma concentration-time profiles on day 1, after the first dose in each cohort, and on day 8, after the fifth consecutive daily dose in each cohort. Samples were taken for ≈72 hours after the day 1 dose and for ≈96 hours after the day 8 dose. Cohorts 1 to 3A received powder-in-bottle formulations; cohorts 3B to 8 received capsules. LLOQ, lower limit of quantitation.

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Table 3. Plasma pharmacokinetic parameters following one or five daily doses of foretinib at the maximum tolerated dose of 3.6 mg/kg (median 240 mg)

Plasma pharmacokinetic parameter	Mean (CV%)
T_{max}, h	
Day 1	5.01 (64.0)
Day 8	3.76 (82.5)
C_{max}, ng/mL	
Day 1	90.5 (40.1)
Day 8	218 (28.9)
Day 8/day 1	2.71 (41.7)
AUC_{0-24}, ng·h/mL	
Day 1	1,300 (32.5)
Day 8	4,050 (30.8)
Day 8/day 1	3.32 (35.2)
$t_{1/2}$, h	
Day 1	36.2 (29.3)
Day 8	40.5 (29.4)

NOTE: Day 1 results (n = 14) follow first dose of foretinib; day 8 results (n = 13) follow five daily doses of foretinib.
Abbreviations: CV%, percent coefficient of variation; T_{max}, observed time to maximum plasma concentration; C_{max}, maximum plasma drug concentration; AUC_{0-24}, area under the plasma drug concentration-time curve from time 0 to 24 hours; $t_{1/2}$, terminal-phase half-life.

declined in approximate monoexponential fashion after the attainment of C_{max}. Mean cohort C_{max} and AUC_{0-24} values on day 8 exceeded those on day 1 by 1.36 to 3.02 and 1.94 to 3.50, respectively, with a trend for higher ratios at higher dose levels. For subjects receiving capsules, exposure increased approximately in proportion to total dose on days 1 and 8 (based on AUC_{0-24}). At the maximum tolerated dose (3.6 mg/kg; median 240 mg), the mean (SD) C_{max} and AUC_{0-24} values were estimated to be 90.5 ng/mL (0.14 μmol/L) and 1,300 ng·hours/mL (2.05 μmol/L·hours) on day 1. On day 8, after the administration of five consecutive daily doses, mean C_{max} and AUC_{0-24} were increased to 218 ng/mL (0.34 μmol/L) and 4,050 ng·hours/mL (6.40 μmol/L·hours). The median $t_{1/2}$ across all cohorts was ~40 hours and was similar on days 1 and 8. There were no apparent differences in foretinib pharmacokinetics based on age, sex, or body weight. Urinary excretion of intact foretinib was <1% of the administered dose over 24 hours after the initial dose.

Pharmacodynamics

Serial tumor biopsies and control skin samples were collected from three patients with three different tumor types, all at or below the maximum tolerated dose. These patients were selected for biopsies because they agreed to the procedures, which in all cases were of superficial skin lesions, and the procedure was judged by the investigators to be of minimal risk and inconvenience to the patients. They were not selected by tumor type or dose, but merely by accessibility. One patient with melanoma was treated at 0.8 mg/kg, and one patient with medullary thyroid cancer and one with triple negative breast cancer were treated at 3.6 mg/kg. Immunohistochemistry staining of the tumor biopsies after foretinib treatment revealed minimal changes in total Met and total RON; however, kinase activity as measured by phosphorylation status, as well as the downstream signaling molecules pAkt and pERK, was reduced in the tumors of all three patients [Figs. 2 and 3 (represents data for a melanoma patient); Table 4]. In addition, marked decreases in proliferation and increases in apoptosis in the tumor biopsies were observed post-treatment. Although all of these effects were seen during cycle 1, effects on signaling, proliferation, and apoptosis increased markedly over time in one patient biopsied twice (Fig. 2A). No changes were seen in skin samples from these three patients (Fig. 2B). All three patients had minor responses (stable disease) to foretinib.

Tumor response

The 40 treated patients were included in assessment of tumor response. Three confirmed partial responses (7.5%) were observed, including two partial responses in patients with papillary renal carcinoma at 0.2 and 3.6 mg/kg dose levels and one in a patient with medullary thyroid carcinoma at a 3.6 mg/kg dose level. Both patients with papillary renal carcinoma, who had confirmed partial responses of >48 and 12 months duration, presented with symptomatic metastatic disease and received no prior therapy except surgery. The patient with medullary thyroid carcinoma, who had previously received treatment with cediranib and doxorubicin, had a 10-month response. Twenty-two additional patients had stable disease (55%), with a duration range of 1 to 10 months (mean 4 months).

Discussion

Foretinib was developed to test the hypothesis that antitumor activity could be combined in a single agent through inhibition of both tumor proliferation (Met) and angiogenesis (VEGFR2, Tie-2, and PDGF). This first-time-in-human, phase I, dose-escalation trial of oral foretinib established that a dose up to 3.6 mg/kg for 5 consecutive days of a 14-day cycle was acceptable for phase II trials and showed significant evidence of biological activity and clinical efficacy. Reversible elevations in serum AST and lipase were the dose-limiting toxicities.

The most common adverse events observed were hypertension, fatigue, diarrhea, and vomiting. Laboratory abnormalities included proteinuria, hematuria, and elevations in AST and lactate dehydrogenase. These changes were generally of grade 1 or 2, and resolved with scheduled dose delay or dose reduction. Two commonly observed events, hypertension and proteinuria, are linked to inhibition of VEGF-mediated angiogenesis (18).

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Patient-specific management of hypertension proved to be effective without disrupting drug administration. A unique toxicity shown in two patients was a transient (<24 hours) confusional state, with an expressive language disorder and apraxia in one. These occurred in the setting of grade 2 hypertension and proteinuria and were considered related to the inhibition of the VEGF axis (18, 19). No specific dose-limiting toxicity could be ascribed solely to inhibition of the HGF-Met axis, although fatigue, nausea, diarrhea, and elevations of hepatic transaminases are frequently seen in patients treated with several tyrosine kinase inhibitors of differing target specificity (20).

Pharmacokinetic data showed that foretinib has a long half-life in humans, approximating 40 hours. There was dose-proportional accumulation in plasma over the days of consecutive dosing; $AUC_{0\text{-}24}$ values across dose cohorts were increased by ~2- to 3.5-fold on day 8 versus day 1. The high plasma levels after five consecutive daily doses, along with the relatively long elimination half-life, result in continued plasma exposure even into the nondosing period. Plasma concentrations after a 9-days-off period were very low and essentially the same as the start of a 14-day cycle, suggesting that investigation of a lower dose administered more frequently throughout a treatment

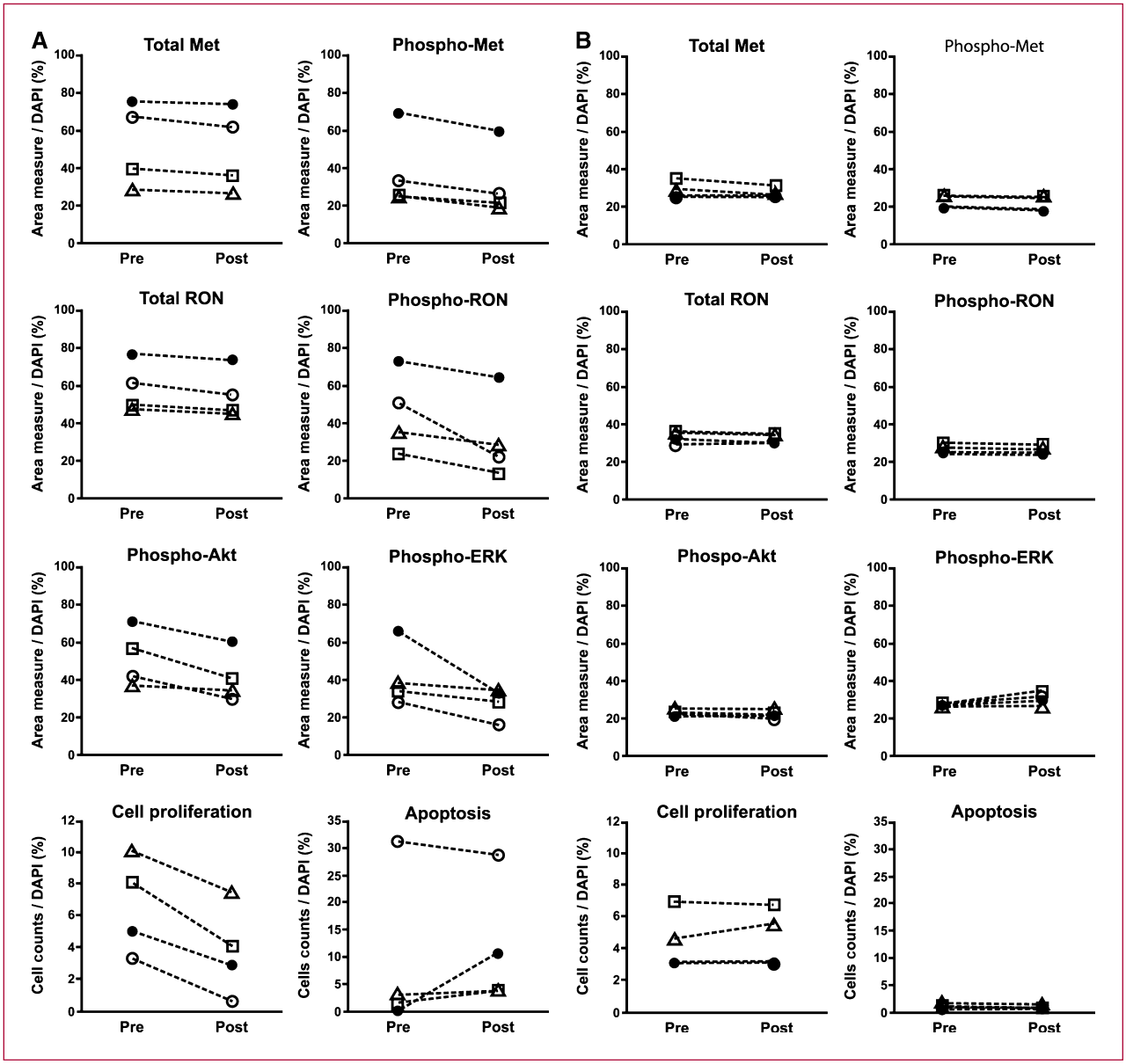

Fig. 2. Foretinib effects on biochemical targets, cell proliferation, and apoptosis in tumor biopsies. Tumors were sampled before and 4 hours after dosing and were analyzed by immunohistochemistry or TUNEL staining. Melanoma, cycle 1 (●) and cycle 6 (○); breast, cycle 1 (pre) and cycle 3 (post; △); thyroid, cycle 1 (□).

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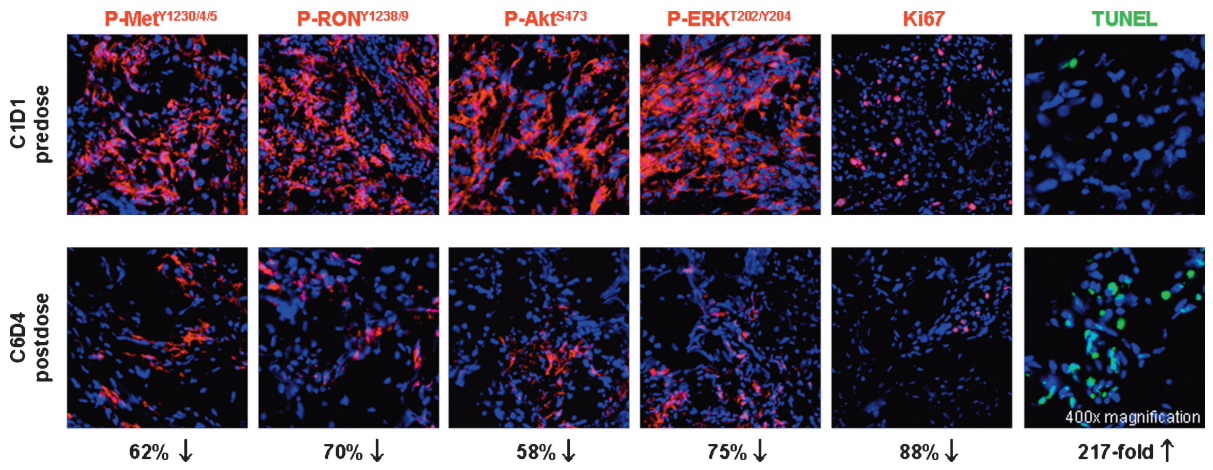

Fig. 3. Immunohistochemistry analysis of serial tumor biopsies from a patient with melanoma. Images are shown at 200× magnification, except for TUNEL (400×). Changes in intensity are calculated with MetaMorph and are expressed relative to DAPI counterstain.

cycle may be warranted. The recommended phase II dose of foretinib by this schedule is 240 mg. Although patients were dosed by body weight in this study, analysis of data did not indicate that plasma pharmacokinetics were affected by age, sex, or weight, and clinical practicality of multiple capsules of different strengths would be an unneeded burden on patients in clinical trials. In the expanded cohort of 3.6 mg/kg/d (the recommended phase II dose), the average dose that patients actually received was 240 mg.

Serial biopsy results in three patients provided preliminary evidence of sustained target and downstream pathway modulation, with resultant antiproliferative and proapoptotic effects in the tumors. There is evidence of Met receptor inhibition, with decreased Met and RON phosphorylation, and reduced downstream signaling of pERK and pAkt in tumor biopsies with a corresponding decrease in the Ki67-positive cell fraction and an increase in the TUNEL staining fraction of tumor cells. These findings were observed in tumor cells as evidence of Met inhibition at doses that did not produce clinical evidence of VEGF axis inhibition such as hypertension and proteinuria.

The observation of three partial responses and 22 cases of stable disease underscores that foretinib exposure produces clinical and immunohistochemical activity in human tumors. Four patients with sporadic papillary renal carcinoma were treated in this trial. The first patient, treated at 0.2 mg/kg (1/18th the maximum tolerated dose), continues in a partial response condition 4 years later. A second patient, with an amplification of chromosomes 7 and 17, suggesting Met rearrangement, achieved a partial response lasting 12 months (21). Activity was seen in both papillary and medullary thyroid cancer, melanoma, and other solid tumors where alterations in Met are not a characteristic finding, suggesting that foretinib may be working through multiple target effects (22).

HGF-Met activation acts as an independent angiogenic factor and one that may interact with the angiogenic proliferation and survival signals of VEGF, angiopoietin-1,

Table 4. Quantification of immunohistochemistry readouts of serial tumor biopsies

	Inhibition of IHC signal intensity postdose vs predose (%)							
	Total-MET	P-MET	Total-RON	P-RON	P-Akt	P-ERK	Ki67	TUNEL
Melanoma cycle 1	2	14	4	12	15	50	42	–8,077
Melanoma cycles 1–6	18	62	29	70	58	75	88	–22,054
Metastatic breast cancer cycle 1 (pre) to cycle 3 (post)	6	24	5	20	8	11	26	–33
Medullary thyroid carcinoma cycle 1	9	16	6	44	28	17	49	–201

NOTE: Quantitative MetaMorph data were analyzed for signaling molecules as area measures relative to DAPI, and for Ki67 and TUNEL as cell counts relative to DAPI. Inhibition of signaling by foretinib is expressed in percent.
Abbreviation: IHC, immunohistochemistry.

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and hypoxia-inducible factor-1α (4, 5, 23). HGF-Met signaling increases VEGF levels in tumor and endothelial cells (24, 25). Increased HGF-Met signaling decreases thrombospondin-1, the major endogenous inhibitor of angiogenesis (5). Combined HGF-Met and VEGF signaling cooperate to increase the expression of VEGF-regulated genes and to express novel transcripts in endothelial cells (26). Combined VEGF and HGF-Met signaling prevents endothelial cell apoptosis, forms capillaries *in vivo*, and increases the microvessel density within tumors (5, 25). Recent reports implicate increased expression of Met and HGF protein at the invasive edge of VEGF inhibitor–targeted therapy as the mechanism by which therapy directed solely at the VEGF axis may lead to increased tumor invasion and metastasis, thus producing therapeutic failure (12). Therefore, targeting inhibition of HGF-Met both enhances VEGF/VEGFR axis–mediated inhibition of angiogenesis at the time of initial therapy and provides a solution to the expected hypoxic response, which is a cause of therapeutic resistance.

Foretinib inhibits its intended targets in a balanced manner at clinically tolerable doses. The responses in papillary renal carcinoma, where Met is an established oncogene, clinically support the immunohistochemistry findings of Met inhibition in tumor biopsies (21). The dose-limiting vascular effects are indicative of VEGF axis inhibition, a potential additional mechanism of action in tumors where HGF-Met is not a major impetus to tumor proliferation. The clinical activity in this phase I trial supports plans to investigate alternative schedules and to explore the mechanism by which Met or angiogenesis drives tumors. An additional study with daily dosing of foretinib has been completed. Phase II studies with the intermittent schedule described here have been completed in papillary renal carcinoma and refractory gastric cancer. Phase II trials with the daily schedule are under way in several tumor types, including hepatocellular carcinoma and lung cancer. The activity observed in this trial further supports additional investigation into agents with extended target spectrums of activity, selected for potentially significant interdependency in the neoplastic progression found in refractory solid tumor malignancies.

Disclosure of Potential Conflicts of Interest

J.P. Eder: employment, AstraZeneca LP; A.X. Zhu: consultant/advisory board, Genentech and Bayer; D. Miles: stock ownership, Exelixis; H. Keer: employment, Five Prime Therapeutics, stock ownership, Exelixis; B. Cancilla: stock ownership, Exelixis; L. Sherman and S. McCallum: stock ownership, GlaxoSmithKline; E. Heath: commercial research grant: GlaxoSmithKline; P.M. LoRusso: extensive consultant, advisory board, honoraria, or speaker positions.

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Dedicated to Carolyn L. Tanzola, RN, who passed away from cancer on January 1, 2010. Her steadfast and cheerful contributions to this and many other studies will be dearly missed.

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References

1. Trusolino L, Comoglio PM. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nat Rev Cancer 2002;2:289–300.
2. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442–54.
3. Derksen PW, de Gorter HP, et al. The hepatocyte growth factor/Met pathway controls proliferation and apoptosis in multiple myeloma. Leukemia 2003;17:764–74.
4. Zhang YW, Vande Woude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. J Cell Biochem 2003;88: 408–17.
5. Zhang YW, Su Y, Volpert OV, Vande Woude GF. Hepatocyte growth factor/scatter factor mediates angiogenesis through positive VEGF and negative thrombospondin 1 regulation. Proc Natl Acad Sci U S A 2003;100:12718–23.
6. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol 2003;4:915–25.
7. Yamashita J, Ogawa M, Yamashita S, et al. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. Cancer Res 1994;54: 1630–3.
8. Ghoussoub RA, Dillon DA, D'Aquila T, Rimm EB, Fearon ER, Rimm DL. Expression of c-met is a strong independent prognostic factor in breast carcinoma. Cancer 1998;82:1513–20.
9. Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. Hepatology 1997;25: 619–23.
10. Daveau M, Scotte M, Francois A, et al. Hepatocyte growth factor, transforming growth factor α, and their receptors as combined markers of prognosis in hepatocellular carcinoma. Mol Carcinog 2003; 36:130–41.
11. Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. Nat Rev Cancer 2006;6: 637–45.
12. Sennino B, Naylor RM, Tabruyn SP, You W-K, Aftab DT, McDonald D. Reduction of tumor invasiveness and metastasis and prolongation of survival of RIP-TAG2 mice after inhibition of VEGFR plus C-MET by XL 184. Mol Cancer Ther 2009;8, Abstract 13.
13. Qian F, Engst S, Yamaguchi K, et al. Inhibition of tumor cell growth, invasion, and metastasis by EXEL-2880 (XL880, GSK1363089), a novel inhibitor of HGF and VEGF receptor tyrosine kinases. Cancer Res 2009;69:8009–16.
14. Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. Nat Rev Drug Discov 2006; 5:730–9.
15. Food and Drug Administration. General guideline for starting dose

Published OnlineFirst May 14, 2010; DOI:10.1158/1078-0432.CCR-10-0574

selection for a cytotoxic agent in cancer patients. Available from: http://www.fda.gov/OHRMS/DOCKETS/AC/06/briefing/2006_4203b1_02_FDA.Backgrounder.pdf.
16. National Cancer Institute. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Available from: http://ctep.cancer.gov/reporting/ctc.html (last accessed 21 March 2009).
17. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
18. van Heeckeren WJ, Ortiz J, Cooney MM, Remick SC. Hypertension, proteinuria, and antagonism of vascular endothelial growth factor signaling: clinical toxicity, therapeutic target, or novel biomarker? J Clin Oncol 2007;25:2993–5.
19. Eder J, Zuckerman D. Endpoints for the determination of efficacy of antiangiogenic agents in clinical trials. In: Teicher B, Ellis L, editors. Antiangiogenic agents in cancer therapy. 2nd edition Totowa, NJ: Humana Press; 2008, p. 509–24.
20. Lorusso PM, Eder JP. Therapeutic potential of novel selective-spectrum kinase inhibitors in oncology. Expert Opin Investig Drugs 2008;17:1013–28.
21. Schmidt L, Duh FM, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 1997;16:68–73.
22. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov 2008;7:504–16.
23. Sullivan R, Graham CH. Hypoxia-driven selection of the metastatic phenotype. Cancer Metastasis Rev 2007;26:319–31.
24. Bottaro DP, Liotta LA. Cancer: out of air is not out of action. Nature 2003;423:593–5.
25. Xin X, Yang S, Ingle G, et al. Hepatocyte growth factor enhances vascular endothelial growth factor-induced angiogenesis *in vitro* and *in vivo*. Am J Pathol 2001;158:1111–20.
26. Gerritsen ME, Tomlinson JE, Zlot C, Ziman M, Hwang S. Using gene expression profiling to identify the molecular basis of the synergistic actions of hepatocyte growth factor and vascular endothelial growth factor in human endothelial cells. Br J Pharmacol 2003;140:595–610.