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A Phase I Dose–Escalation Study of Regorafenib (BAY 73–4506), an Inhibitor of Oncogenic, Angiogenic, and Stromal Kinases, in Patients with Advanced Solid Tumors

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Abstract

Purpose: Regorafenib is a novel oral multikinase inhibitor of angiogenic (VEGFR1-3, TIE2), stromal (PDGFR-β, FGFR), and oncogenic kinases (KIT, RET, and RAF). This first-in-man, phase I dose–escalation study assessed the safety, pharmacokinetic, pharmacodynamic, and efficacy profiles of regorafenib in patients with advanced solid tumors.

Patients and Methods: Patients aged 18 years or older with advanced solid tumors refractory to standard treatment were recruited. Regorafenib was administered orally for 21 days on/seven days off in repeating cycles, until discontinuation due to toxicity or tumor progression. Adverse events (AE) were assessed using National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. Pharmacokinetic profiles were measured after a single dose and on day 21. Pharmacodynamic and efficacy evaluations included tumor perfusion assessment using dynamic contrast-enhanced MRI, plasma cytokines, and tumor response using RECIST (v1.0).

Results: Fifty-three patients were enrolled into eight cohorts at dose levels from 10 to 220 mg daily. The recommended dose for future studies was determined to be 160 mg daily, with a treatment schedule of 21 days on/seven days off in repeating 28-day cycles. The most common drug-related grade 3 or 4 AEs were dermatologic AEs (hand–foot skin reaction, rash), hypertension, and diarrhea. Pharmacokinetic analysis revealed a similar exposure at steady state for the parent compound and two pharmacologically active metabolites. Tumor perfusion and plasma cytokine analysis showed biologic activity of regorafenib. Three of 47 evaluable patients achieved a partial response (renal cell carcinoma, colorectal carcinoma, and osteosarcoma).

Conclusion: Regorafenib showed an acceptable safety profile and preliminary evidence of antitumor activity in patients with solid tumors. *Clin Cancer Res; 18(9); 2658–67. ©2012 AACR.*

Introduction

Constitutive or enhanced receptor tyrosine kinase (RTK) activity is a commonly observed and causative feature of many key processes driving cancer pathogenesis. Activation of VEGF receptors (VEGFR) plays an important role in angiogenesis, a process long known to be essential for tumor growth and metastasis (1, 2). Meanwhile, other RTKs are involved in oncogenesis; for example, abnormal activation of the epidermal growth factor receptor family members occurs in many epithelial tumors and leads to enhanced cell proliferation and other tumor promoting activities (3). Signaling of many RTKs is mediated by downstream pathways, including the RAS/RAF/MEK/ERK mitogen-activated protein kinase pathway (4–12).

Inhibition of RTKs and/or their downstream effectors has been shown to have therapeutic benefits in some types of solid tumor. For example, several multikinase inhibitors (sunitinib, sorafenib, and pazopanib) and mTOR inhibitors (temsirolimus and everolimus) have shown significant activity in renal cell carcinoma (RCC) and are now approved agents for RCC (13). Similarly, agents targeting c-KIT such as imatinib and sunitinib have transformed the treatment paradigm for gastrointestinal stromal tumor (GIST; refs. 14, 15). However, despite the availability of several targeted kinase inhibitors for certain tumor types,

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Translational Relevance

Regorafenib is a novel multikinase inhibitor with a distinct kinase inhibition profile. In preclinical studies, regorafenib has shown potent antitumor activity in a broad range of xenograft tumor models. We report here the results of a phase I, first-in-human, dose–escalation study that evaluated regorafenib monotherapy in patients with advanced solid tumors. Regorafenib showed a safety profile that is in line with compounds of this drug class. Importantly, for this heavily pretreated patient population, objective partial responses were observed in 6% of patients and stable disease in 60%. These data provide evidence supporting the continued development of regorafenib in solid tumors. The dose and schedule of regorafenib monotherapy determined from this study is used in subsequent studies, including two ongoing phase III studies (in colorectal cancer and gastrointestinal stromal tumor, respectively) and a number of phase II studies.

drug resistance and subsequent disease relapse remain a problem, underscoring the need to develop new kinase inhibitors with favorable benefit/risk profile (16, 17).

Regorafenib (BAY 73–4506) is a novel oral diphenylurea-based multikinase inhibitor with distinct biochemical kinase inhibition profile and pharmacologic characteristics. Preclinical studies have shown that regorafenib is a potent inhibitor of several angiogenic and stromal RTKs, including VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, fibroblast growth factor receptor 1 (FGFR-1), and TIE2. In addition, regorafenib inhibits various oncogenic RTKs (c-KIT and RET) and intracellular signaling kinases (cRAF/RAF-1, B-RAF, and B-RAF V600E mutant; refs. 18, 19). In murine xenograft models, regorafenib has shown broad-spectrum antitumor efficacy (19). These preclinical data provide the rationale to investigate regorafenib in clinical trials. We report here the first-in-man phase I single-agent dose–escalation study that evaluated the safety, pharmacokinetic, pharmacodynamic, and efficacy profiles of regorafenib in patients with advanced solid tumors.

Patients and Methods

Patients

Men and women aged 18 years or older with histologically or cytologically confirmed advanced solid tumors that had progressed after standard therapies (as documented by CT or MRI scan at baseline) were eligible for inclusion in the study. Other key inclusion criteria included were an Eastern Cooperative Oncology Group performance status of 0–2; a life expectancy of 12 weeks or more; and adequate bone marrow, liver, and renal function. Exclusion criteria included were history of cardiac disease; uncontrolled hypertension; human immunodeficiency virus or active hepatitis B/C; clinically serious infection; serious nonhealing wound, ulcer, or bone fracture; symptomatic metastatic brain or meningeal tumors (unless the patient was 6 months or more from definitive therapy, had no evidence of tumor growth, and was clinically stable; patients with brain metastases must not be undergoing acute steroid therapy or steroid taper); any seizure disorder requiring anticonvulsant medication; history of organ allograft; pregnancy or breast-feeding; major surgery within 4 weeks of the start of study treatment; and evidence or history of coagulation disorders or thrombosis.

No other anticancer treatment was allowed during the study. Other excluded treatments were radiotherapy to the target lesions at any time from 3 weeks before first treatment to study end; autologous bone marrow transplant or stem cell rescue within 4 months before first treatment; and biologic response modifiers (e.g., granulocyte colony-stimulating factor) within 3 weeks before study entry. Chronic erythropoietin therapy was permitted provided that no dose adjustments were made within 2 months of first study treatment or during the study.

Study objectives and design

This was a phase I, open-label, nonrandomized, dose–escalating study conducted in one German center starting in July 2005 (sponsor study number 11650; EudraCT number 2005-001198-81). The primary objectives of the study were to define the safety profile, maximum-tolerated dose (MTD), and pharmacokinetics of oral regorafenib in patients with advanced solid tumors and to identify the recommended regorafenib dose for future studies. Secondary objectives included the evaluation of pharmacodynamic parameters and tumor responses in regorafenib-treated patients. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Approval by the national authority BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte) and appropriate ethics committees or institutional review boards was obtained. All patients gave written, informed consent before any study procedures.

At least 3 patients were recruited to each dose cohort. Dose escalation to the next cohort was decided following review of safety data collected during the first cycle of treatment in the previous cohort.

In cohort 1, patients received a single dose of 10 mg regorafenib oral solution on day 1 followed by 6 days off treatment (days 2–7), once daily dosing of regorafenib for 7 days (days 8–14), and 14 days off (days 15–28). Patients in cohort 1 were transferred to cohort 2 and continued to receive 10 mg regorafenib oral solution in repeating 28-day treatment cycles of 21 days on/seven days off. These 28-day repeating treatment cycles were also employed in all subsequent dose cohorts. In the first cycle of treatment in cohorts 2 and beyond, the first dosing day was followed by an off-treatment day (day 2) to allow single-dose pharmacokinetic assessments. From cohort 3 onwards, each patient remained in the same cohort for the duration of treatment, with no intrapatient dose increase. The planned dose of regorafenib in cohort 3 was 30 mg once daily oral

solution. Dose selection in subsequent cohorts was based solely on safety data collected from previous cohorts. If a dose-limiting toxicity (DLT; defined below) would have occurred in 2 of 3 or 2 of 6 patients, dose escalation was to be stopped and the dose at which this occurred declared the toxic dose.

Dosing cycles for individual patients continued until tumor progression, occurrence of unacceptable toxicity, or withdrawal of consent.

Study assessments

Planned safety assessments included adverse event (AE) monitoring and evaluation of DLTs and the MTD. AEs were graded according to National Cancer Institute Common Terminology Criteria (NCI-CTC) for Adverse Events v3.0. DLTs (hematologic or nonhematologic) were defined as the occurrence of any of the following during the first cycle of treatment: absolute neutrophil count (ANC) $<0.5 \times 10^9$/L for 7 days or more; febrile neutropenia with ANC $<0.5 \times 10^9$/L and fever of 38.5°C or more; platelets $<25 \times 10^9$/L or grade 3 or more thrombocytopenic bleeding; drug-related grade 3 or 4 nonhematologic toxicity, excluding nausea, and vomiting not refractory to antiemetics. As hypertension is a common side effect of VEGFR inhibitors, the onset of clinically manageable hypertension was not itself regarded as a DLT. The MTD was defined as the highest dose which could be given to 6 patients so that no more than one patient experienced a DLT. Interruptions in dosing and/or dose reductions were permitted if a patient experienced a DLT or an AE considered related to the study drug that was of grade 3 severity (NCI-CTC). Intrapatient dose escalation was not permitted.

Medical history assessment, physical examinations, and hematologic and biochemical laboratory evaluations were carried out at screening, on days 1, 7 (only cycle 1 and 2), 14 (only cohort 1, cycle 1), and 21 (cycles 1 to 3). Beginning from cycle 4, assessments were carried out on day 1 only.

Pharmacokinetics

Blood samples for determination of the plasma concentrations of regorafenib and its *N*-oxide metabolite (M-2) and *N*-oxide/*N*-desmethyl metabolite (M-5) were taken in cohort 1 on day 1 and day 14 at regular intervals up to 96 hours. In cohorts 2 and onwards, postdose samples up to 48 hours (day 1) and 72 hours (day 21) were taken during the first treatment cycle. Analyte concentrations were determined by high-performance liquid chromatography with tandem mass spectrometric detection with a lower limit of quantification of 2 μg/L using [$^2H_3$ ^{15}N]-labeled analogs as internal standards. The analyses were done in accordance with the U.S. Food and Drug Administration guideline on bioanalytical validation (20). Pharmacokinetic parameters were calculated using standard noncompartmental methods (WinNonlin Version 4.1; Pharsight Corporation) and summarized as geometric mean and geometric coefficient of variation. C_{max} and the times needed to reach these concentrations (t_{max}) were assessed by inspection of the concentration versus time plots. The area under the curve (AUC) was calculated from time zero to infinity ($AUC_{0-\infty}$) on day 1 and across the dosing interval (AUC_{0-24}) on day 14 and 21, respectively. The terminal half-life was calculated as $\ln(2)/\lambda_Z$, in which the terminal phase rate constant (λ_Z) was the slope of the log-linear regression of the last n data points (with $n \geq 3$). The accumulation ratios R_AC_{max} ($C_{max,md}/C_{max,sd}$) and R_ALin ($AUC_{0-24md}/AUC_{0-\infty sd}$) were calculated as ratios of multiple-dose (md) over single-dose (sd) parameters. An explorative ANOVA (including the factor treatment) was done on the log-transformed values of dose- and dose/body weight normalized C_{max} and AUC. The ratio R_ALin was evaluated across cohorts, separated by formulation and presented as geometric least square (LS) mean and explorative 90% confidence interval (CI).

An oral solution formulation of regorafenib was used in cohorts at dose levels of 10 to 120 mg. Subsequently, a coprecipitate tablet formulation was developed. To compare the relative bioavailability of the 2 formulations, an intrapatient cross-over substudy was conducted. For dose levels of 120 mg and higher, the coprecipitate tablet formulation was used.

Pharmacodynamics

Pharmacodynamic assessments included plasma concentrations of VEGF and sVEGFR-2. Blood samples were collected as follows: cohort 1, screening, predose, and 8 hours postdose on day 1 of cycle 1; cohorts 2 and onwards, screening, predose, and 8 hours postdose on days 1 and 21 of cycle 1, predose on days 1 and 21 of cycles 2 and 3, predose on day 1 of all subsequent cycles; and final study visit. These samples were analyzed using quantitative ELISA. Tumor perfusion properties before and during treatment were measured by noninvasive angiogenic imaging using dynamic contrast–enhanced MRI (DCE-MRI). Details of DCE-MRI methodology have been described previously (21). Regions of interest for DCE-MRI were selected after a review of staging radiographic studies done to establish patient eligibility. DCE-MRI was done at screening and on day 2 of cycle 1, day 14 of cycle 1 (cohort 1 only), day 21 of cycles 1 to 4, and every second cycle thereafter (cohorts 2 and onwards), and at the final visit. The area under the contrast agent concentration–time curve during the first 60 seconds after arrival of the contrast agent ($iAUC_{60}$) was used as the DCE-MRI end point (21). The MRI analyses were carried out by one experienced observer and independently controlled by a second one.

Tumor response

Tumor response and progression were assessed using Response Evaluation Criteria in Solid Tumors (RECIST, version 1.0). Tumor dimensions were measured at baseline, at the end of every second treatment cycle, and at the final visit. Tumor responses were confirmed by a second scan. University Hospital of Freiburg uses routine radiology services provided by another hospital.

Table 1. Dose cohort, treatment duration, and actual dose received

Dose cohort	Dose (mg)	No. patients	Median (range) treatment duration (d)[c]	Number (%) of patients who received actual dose as percentage of planned dose		
				≥90%	70%–90%	50%–70%
1	10[a]	3	77 (14–161)[d]	2 (67%)	0	1 (33%)
2	10[a]	3	77 (14–161)[d]	2 (67%)	0	1 (33%)
3	30[a]	5	20 (3–78)	3 (60%)	0	1 (20%)
4	60[a]	6	67.5 (21–1,239)	5 (83%)	1 (17%)	0
5	120[a]	8	49.5 (7–244)	3 (38%)	0	4 (50%)
6	120[b]	7	173 (51–581)	4 (57%)	2 (29%)	0
7	160[b]	12	119 (14–637)	8 (67%)	1 (8%)	3 (25%)
8	220[b]	12	92.5 (47–273)	2 (17%)	7 (58%)	3 (25%)
Total	10–220	53	78 (3–1,239)	27 (51%)	11 (21%)	12 (23%)

[a]Regorafenib formulation: solution.

[b]Regorafenib formulation: coprecipitate tablet.

[c]Duration of treatment = date of last day on study drug–date of first day on study drug + 1 (this includes planned and unplanned dose interruptions).

[d]For median treatment duration calculation, only treatments received in cohort 2 are considered.

Results

Patients, dose escalation, and treatments

A total of 53 patients were enrolled into this dose–escalation study. The median age was 60 years (range 20–77); all patients were Caucasian and the majority were male ($n = 30$, 57%). With regard to ECOG performance status, 26 patients (49%) had a score of 0, 25 (47%) of 1, and 2 (4%) had a score of 2. The most common tumor type was colorectal cancer ($n = 16$, 30%). The patients were heavily pretreated with a median of 3 different prior systemic anticancer regimens (range 0–9).

Dose escalation cohorts are listed in Table 1. Regorafenib was administered as oral solution for dose levels 10 to 120 mg. In a separate intrapatient cross-over study, the relative bioavailability of a coprecipitate tablet compared with oral solution was assessed as 70% (20 mg tablet) to 83% (100 mg tablet). Subsequently, coprecipitate tablets (20 mg or 100 mg in strength) were administered for dose levels of 120 mg (6×20 mg), 160 mg (8×20 mg), and 220 mg (2×100 mg and 1×20 mg). Patients in cohort 1 were able to tolerate the planned dose. All 3 patients in cohort 1 were transferred to cohort 2 and continued to receive 10 mg regorafenib solution in repeating 21 days on/seven days off schedule (no dosing on day 2 of cycle 1 for pharmacokinetic assessment). Dose escalation then proceeded with the same schedule. Eight dose cohorts were evaluated, with 220 mg once daily being the highest dose tested.

The median duration of treatment, which includes treatment off-days, is shown by cohort in Table 1. For all patients, the median duration of treatment was 78 days (range 3–1,239). Across all dose levels, 50 patients (94%) received 50% or more of the planned dose, with 38 patients (72%) receiving 70% or more of the planned dose (Table 1).

Fifty-one patients had discontinued treatment at the time of the data cut-off (June 29, 2009); 2 patients were still on active treatment, with treatment durations of 40.8 and 21.0 months, respectively. Reasons for discontinuation were disease progression ($n = 30$), AEs ($n = 17$), death due to disease progression ($n = 2$), and withdrawn consent ($n = 2$).

Safety

Overall, 44 (83%) patients experienced at least one treatment-related AE. As shown in Table 2, the most frequent treatment-related AEs occurring included voice changes, hand–foot skin reaction, mucositis, diarrhea, and hypertension. The most common grade 3 or 4 treatment-related AEs included hand–foot skin reaction, hypertension, diarrhea, and rash/desquamation. The frequency of treatment-related AEs increased with dose levels. There was no grade 5 treatment-related AE. Sixteen (30%) of patients experienced treatment-related serious AEs ($n = 3$: hypertension; $n = 2$: diarrhea, infection, abdominal pain; $n = 1$: allergic reaction, cardiac ischemia/infarction, fatigue, weight loss, dehydration, CNS hemorrhage with underlying brain metastases, lipase elevation, somnolence, pain, bronchospasm, hand–foot skin reaction, rash/desquamation, and urticaria).

Determination of maximum-tolerated dose

Treatment-related DLTs occurring in cycle 1 that led to dose reduction, a temporary interruption in treatment, or permanent discontinuation of treatment are shown in Table 3. For dose levels of 10 to 60 mg, none of the patients had an adverse event in cycle 1 leading to a change in dosing. At the 120-mg solution dose level, 2 patients had an infection with an unidentified pathogen, in whom a relationship to the study treatment could not be excluded.

Table 2. Treatment-emergent, drug-related adverse events occurring in 20% or more patients (all grades)

Incidence (%) by CTCAE term	10 mg ($n = 3$)[b]		30 mg ($n = 5$)[b]		60 mg ($n = 6$)[b]		120 mg ($n = 8$)[b]		120 mg ($n = 7$)[c]		160 mg ($n = 12$)[c]		220 mg ($n = 12$)[c]		Total ($n = 53$)	
CTCAE grade[a]	All	3–4	All	3–4	All	3–4	All	3–4	All	3–4	All	3–4	All	3–4	All	3–4
Any event	1 (33)	1 (33)	2 (40)	0 (0)	3 (50)	1 (17)	7 (88)	5 (63)	7 (100)	3 (43)	12 (100)	8 (67)	12 (100)	8 (67)	44 (83)	26 (49)
Voice changes			2 (40)		2 (33)		4 (50)		7 (100)		5 (42)		9 (75)	1 (8)	29 (55)	1 (2)
Hand-foot skin reaction					1 (17)		2 (25)	1 (13)	3 (43)	1 (14)	8 (67)	3 (25)	7 (58)	5 (42)	21 (40)	10 (19)
Mucositis (clinical examination, oral cavity)					1 (17)		2 (25)		5 (71)		3 (25)	1 (8)	8 (67)		19 (36)	1 (2)
Diarrhea							2 (25)		2 (29)	1 (14)	6 (50)	2 (17)	7 (58)	1 (8)	17 (32)	4 (8)
Hypertension	1 (33)		1 (20)		1 (17)	1 (17)	2 (25)				6 (50)	2 (17)	5 (42)	3 (25)	16 (30)	6 (11)
Fatigue							3 (38)	1 (13)	3 (43)		2 (17)		7 (58)	1 (8)	15 (28)	2 (4)
Anorexia							2 (25)		1 (14)		6 (50)	1 (8)	5 (42)		14 (26)	1 (2)
Rash/desquamation							1 (13)		2 (29)	1 (14)	6 (50)	1 (8)	3 (25)	1 (8)	12 (23)	3 (6)
Alopecia									4 (57)		4 (33)		3 (25)		11 (21)	

[a]Only CTCAE of grades 1 to 4 occurred.
[b]Regorafenib formulation: solution.
[c]Regorafenib formulation: coprecipitate tablet.

Retrospectively, after further conduct of the trial, these infection events were not considered as DLTs anymore. However, to ensure an adequate safety assessment, this cohort was expanded and overall eight patients were enrolled, one of whom experienced a dose-limiting hand–foot skin reaction in cycle 1. At that point, it was justified to continue the dose escalation at 120 mg using the coprecipitate tablet formulation. No patient had a DLT in the 120-mg tablet cohort and the dose escalation was continued at 160 mg, in which none of the initially enrolled patients had a DLT. At 220 mg, 2 of the initially enrolled patients had a dose-limiting hand–foot skin reaction necessitating dose reduction. As skin toxicity is considered a well-known toxicity for multikinase inhibitors such as sorafenib and sunitinib and is in general clinically manageable with remedial therapy or dose reductions, the study protocol was amended at that time, allowing this dose level to be expanded despite the occurrence of grade 3 skin toxicities exceeding the frequency of 1 of 6 subjects. For a more accurate tolerability assessment, 12 patients were enrolled at both 160 and 220 mg dose levels. Eventually, 2 of 12 patients at 160 mg had a dose-limiting adverse event in cycle 1 leading to a change in regorafenib dosing, compared with 5 of 12 patients at 220 mg (Table 3). Furthermore, for AEs occurring in cycle 1 or cycle 2, 3 of 12 patients at 160 mg had an AE leading to a change in regorafenib dosing, compared with 9 of 12 patients at 220 mg (data not shown). On the basis of these observations, 160 mg once daily administered as

Table 3. Incidence of treatment-related dose-limiting adverse events occurring in cycle 1, leading to dose reduction, interruption, or permanent discontinuation

Incidence (%) by CTCAE term	10–60 mg ($n = 14$)[a]	120 mg ($n = 8$)[a]	120 mg ($n = 7$)[b]	160 mg ($n = 12$)[b]	220 mg ($n = 12$)[b]	Total ($N = 53$)
Any event	0 (0)	3 (38)	0 (0)	2 (17)	5 (42)	10 (19)
Hand–foot skin reaction		1 (13)			2 (17)	3 (6)
Pruritus, rash/desquamation					1 (8)	1 (2)
Allergic reaction, rash/desquamation				1 (8)		1 (2)
Vomiting				1 (8)		1 (2)
Abdominal pain					1 (8)	1 (2)
Infection (other)		2 (25)				2 (4)
Asthma—bronchial					1 (8)	1 (2)

[a]Regorafenib formulation: solution.
[b]Regorafenib formulation: coprecipitate tablet.

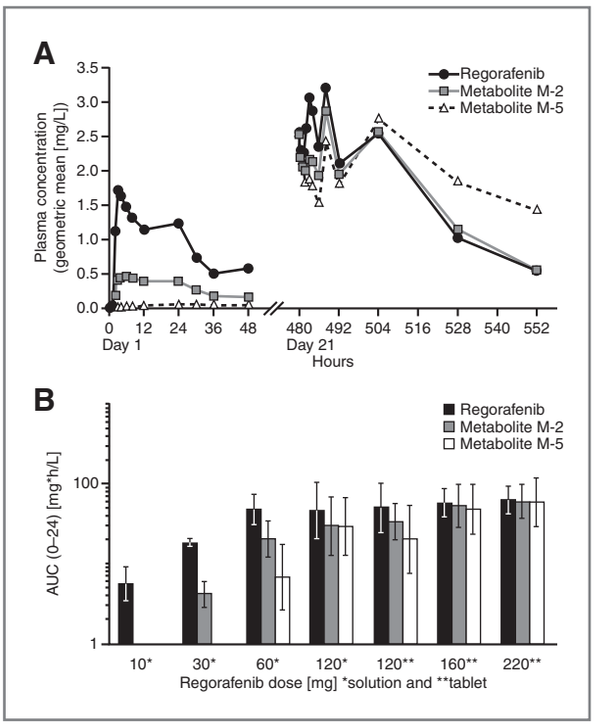

Figure 1. Pharmacokinetic evaluation of regorafenib. A, mean plasma concentration–time profiles of regorafenib, metabolite M-2, and metabolite M-5 in the 160-mg tablet dose cohort. B, regorafenib and metabolite AUC_{0-24} versus dose (day 14 of cycle 1 for 10-mg dose; day 21 of cycle 1 for all other doses; geometric mean and geometric SD).

coprecipitate tablets was determined as the maximum tolerated dose level for single-agent regorafenib treatment in a 21 days on/seven days off schedule.

Pharmacokinetics

The plasma concentration versus time profiles of regorafenib and its metabolites M-2 and M-5 at steady state were multipeak shaped with an initial maximum (t_{max}) of 1 to 6 hours, and secondary and tertiary maxima at about 6 to 8 and 24 hours (Fig. 1A). The peak-trough-fluctuation on day 21 was small, with about 1.4- to 2.8-fold differences between maximum and minimum mean plasma concentrations. Administration of oral solution (10–120 mg) resulted in dose-dependent increases in systemic exposure of parent drug up to 60 mg dose, although a further increase was not achieved when escalating to 120 mg. Less than dose proportional numeric increases in mean AUC_{0-24md} were observed with tablet doses of 120 to 220 mg (Table 4 and Fig. 1B). The terminal half-life of about 20 to 40 hours explains the accumulation of regorafenib in plasma after multiple doses, as evidenced by 2- to 4-fold increases in $C_{max,md,}$(multiple dose) compared with $C_{max,sd}$ (first dose). Across dose cohorts, the ratio of regorafenib AUC_{0-24md}/$AUC_{0-\infty sd}$ was 1.25 (0.94–1.68) for oral solution and 0.98 (0.79–1.21) for coprecipitate tablets [geometric LS mean (90% CI)]. The accumulation of regorafenib was predictable from plasma concentrations after the first dose, due to its time–linear pharmacokinetics. Unlike the parent drug, its metabolites M-2 and M-5 showed more than proportional increases in exposure at lower doses. For example, a 2-fold increase in dose (30–60 mg) resulted in a 5-fold increase in AUC_{0-24} of M-2 on day 21. At higher doses, better dose proportionality was observed. At the highest investigated doses (160 and 220 mg tablet), parent drug, M-2 and M-5 showed similar plasma exposure at steady state (Table 4 and Fig. 1B). The elimination half-life of M-2 was similar to parent drug. The elimination of M-5 appeared to be slower but a reliable estimate of half-life could not be obtained. The ratios of AUC_{0-24md}/$AUC_{0-\infty sd}$ for M-2 (1.9 for solution and 2.4 for tablet) and M-5 (4.3 for solution and 18.7 for tablet) showed greater accumulation of the metabolites after repeated administration compared with parent drug.

Pharmacodynamics

Pharmacodynamic assessments of plasma angiogenic cytokines and tumor perfusion analysis by DCE-MRI were used to support the dose findings. DCE-MRI assessments after 21 days of multiple dosing showed an average decrease of 40% or more for the initial AUC over 60 seconds of the gadolinium contrast agent for the dose levels of 120 mg (solution), 160 mg (tablet), and 220 mg (tablet; Fig. 2A). The reduction of plasma sVEGFR-2 concentration over various time points during cycles 1 to 3 was dose dependent (Fig. 2B). Plasma VEGF concentration increased over 21 days of multiple dosing and returned to baseline levels during the 7 days treatment break (Fig. 2C).

Efficacy

Tumor response according to RECIST was evaluable in 47 of 53 patients treated with regorafenib doses of 10 mg to 220 mg. Overall, disease control (i.e., partial response or stable disease) was achieved in 35 patients (66%): 32 patients (60%) showed stable disease and 3 (6%) showed partial response. Progressive disease was observed in 12 patients (23%) and 6 patients (11%) were not evaluable. The best change in tumor size from baseline for individual patients is shown in Supplementary Fig. S1. Three patients reached a partial response (RCC: 60 mg solution, time to progression 20.6 months; osteosarcoma: 120 mg solution, time to progression 8.3 months; CRC: 220 mg tablet, treatment ended after 5.3 months due to an AE). In addition, 2 patients reached tumor shrinkage of more than 30% at one tumor assessment, which could not be subsequently confirmed and was assessed as stable disease for overall best response (pancreatic carcinoma: ongoing at 21.0 months; alveolar soft tissue sarcoma: time to progression 8.9 months).

Discussion

This phase I dose–escalation trial was the first study of regorafenib in humans, with the primary objective of evaluating the safety, tolerability, and pharmacokinetics of

Table 4. Regorafenib and metabolite pharmacokinetic parameters at steady state [day 14 of cycle 1 for 10 mg dose (cohort 1); day 21 of cycle 1 for all other dose cohorts; geometric mean (%CV)]

Parameter	10 mg[a]	30 mg[a]	60 mg[a]	120 mg[a]	120 mg[b]	160 mg[b]	220 mg[b]
Regorafenib	($n = 3$)	($n = 3$)	($n = 6$)	($n = 6$)	($n = 7$)	($n = 10$)	($n = 10$)
AUC_{0-24} (mg*h/L)	5.668 (52.2)	18.64 (11.6)	48.34 (45.7)	45.97 (95.6)	50.93 (81.2)	58.27 (43.3)	63.67 (40.6)
C_{max} (mg/L)	0.5341 (53.8)	1.575 (38.1)	4.135 (28.4)	4.324 (51.6)	4.424 (74.1)	3.904 (43.8)	4.462 (41.9)
t_{max}[c] (h)	2.917 (2.917–3.167)	6.000 (2.000–6.000)	2.033 (0.5500–3.967)	3.092 (0.5167–5.833)	2.167 (1.900–3.467)	5.033 (0.5667–8.750)	3.050 (0.4167–8.000)
$t_{1/2}$ (h)	27.28 (6.7)	20.06 (23.7)	32.87 (69.1)[d]	41.73 (45.2)[e]	30.52 (55.1)[e]	22.23 (45.4)	35.43 (30.4)[e]
R_AC_{max} (%)	270.6 (75.7)	407.8 (34.3)	231.9 (19.3)	269.9 (46.0)	181.9 (91.2)	169.9 (40.9)	202.9 (89.4)
R_{Lin} (%)	100.7 (33.3)				92.81 (66.1)[f]	92.64 (17.2)[e]	
M-2		($n = 3$)	($n = 6$)	($n = 4$)	($n = 7$)	($n = 10$)	($n = 10$)
AUC_{0-24} (mg*h/L)		4.232 (38.9)	20.75 (56.3)	30.29 (101.6)	33.91 (57.1)	53.70 (69.3)	60.41 (51.5)
C_{max} (mg/L)		0.3404 (74.6)	1.671 (56.0)	2.690 (51.6)	2.509 (54.0)	3.337 (78.2)	3.864 (48.3)
t_{max}[c] (h)		6.000 (2.000–6.000)	2.567 (1.000–3.967)	2.042 (0.5167–23.50)	2.767 (1.133–4.050)	8.250 (0.5667–23.72)	4.933 (0.4167–23.75)
$t_{1/2}$ (h)		19.10 (34.8)	25.86 (40.7)[d]		25.96 (42.0)[e]	21.02 (28.2)[g]	31.82 (23.4)[i]
R_AC_{max} (%)		949.2 (64.6)	179.1 (44.9)	407.3 (36.3)	339.2 (63.4)	439.0 (47.8)	563.6 (115.2)
R_{Lin} (%)			128.3 (24.5)[d]		202.0 (66.8)[e]	263.5 (21.3)[h]	235.0 (82.5)[i]
M-5[j]			($n = 6$)	($n = 6$)	($n = 7$)	($n = 10$)	($n = 10$)
AUC_{0-24} (mg*h/L)			6.859 (121.2)	29.73 (99.9)	20.32 (127.2)	48.71 (82.9)	59.67 (80.6)
C_{max} (mg/L)			0.4704 (123.8)	1.956 (89.0)	1.288 (127.8)	2.929 (88.9)	3.737 (77.8)
t_{max}[c] (h)			2.100 (1.000–24.00)	12.79 (0.5167–23.50)	1.900 (0.6333–4.050)	10.38 (1.017–23.72)	2.925 (0.4167–23.75)
R_AC_{max} (%)			441.4 (109.2)	2,024 (107.9)	1,021 (55.5)	4,025 (66.5)	5,849 (146.0)

Abbreviations: AUC_{0-24}, area under the concentration–time curve from 0 to 24 hours; C_{max}, maximum plasma concentration; CV, coefficient of variation; R_AC_{max}, C_{max} accumulation ratio; R_{Lin}, AUC accumulation ratio; $t_{1/2}$, half-life; t_{max}, time of maximum concentration.

[a]Regorafenib formulation: solution.

[b]Regorafenib formulation: coprecipitate tablet.

[c]Median (range).

[d]$n = 3$.

[e]$n = 5$.

[f]$n = 4$.

[g]$n = 9$.

[h]$n = 7$.

[i]$n = 4$.

[j]$t_{1/2}$ (and R_{Lin}) could not be reliably estimated.

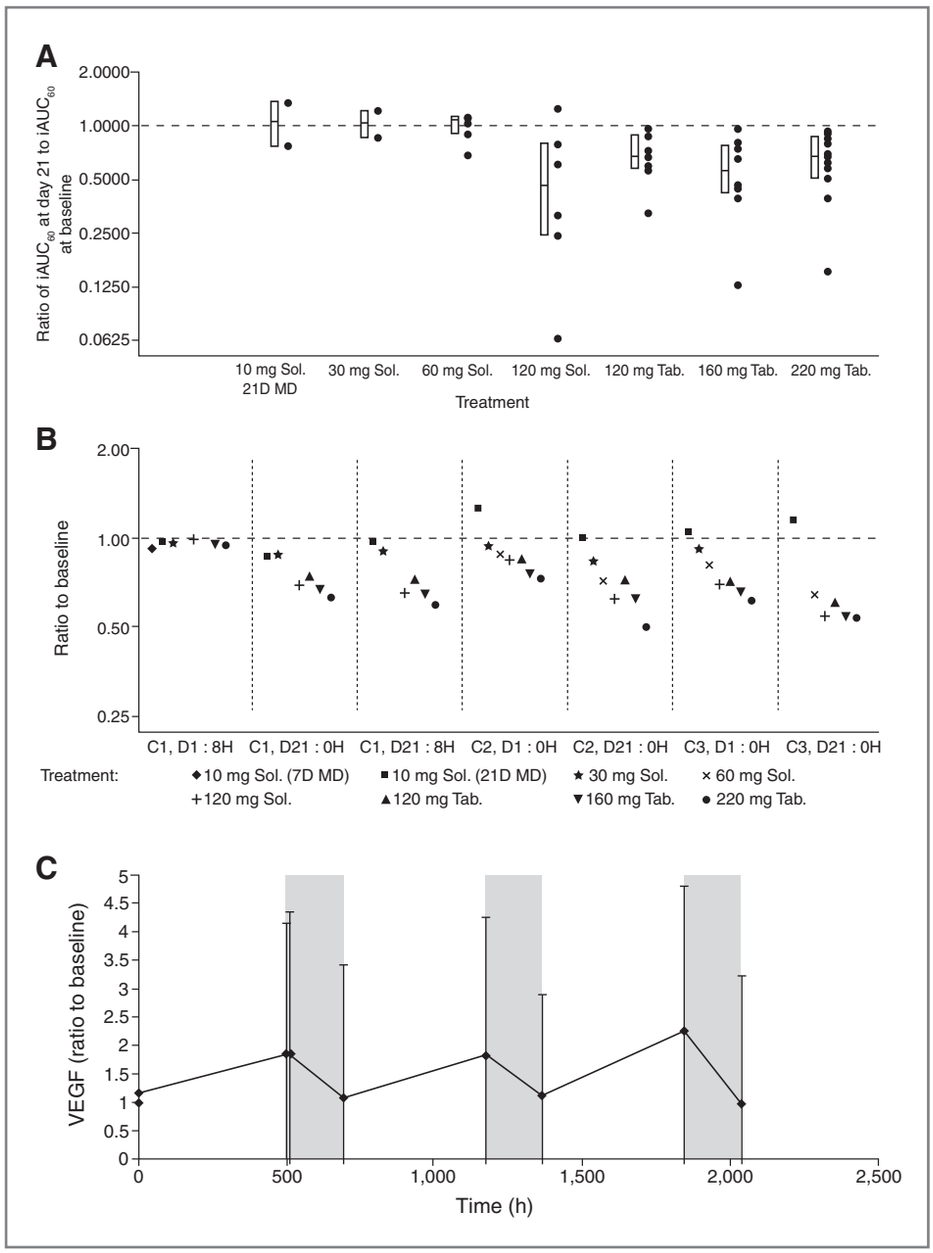

Figure 2. Pharmacodynamic evaluation of regorafenib treatment. A, DCE-MRI area under the contrast agent concentration–time curve during the first 60 seconds after arrival of the contrast agent ($iAUC_{60}$) on day 21. Results are presented as ratio (mean) to baseline. B, change in plasma sVEGFR-2 concentration over time. Results are presented as ratio (mean) to baseline. C, change in plasma VEGF concentration over time. Results are presented as ratio (mean values over all dose levels) to baseline. The shaded area is the off-treatment period. Tab, tablet; Sol, solution; 21D MD, 21 days of multiple dosing; 7D MD, 7 days of multiple dosing.

regorafenib. Initial dose selection for this study was based on the results of preclinical toxicity and pharmacokinetic assessments. These tests included a 4-week study in rats that showed that a 1 mg/kg dose of regorafenib was associated with an acceptable level of toxicity (Bayer, data on file). This dose was expected to be approximately equivalent to a 10 mg dose in humans, which was subsequently chosen as the starting dose for this trial. The observed human exposure (AUC) after the starting dose of 10 mg was consistent with the prediction based on preclinical data and scaling approaches.

The most common drug-related AEs occurring in 30% or more of patients were voice changes, hand–foot skin reaction, mucositis, diarrhea, and hypertension. This AE profile is consistent with the mechanism of action of regorafenib and is broadly similar to that observed in phase I studies involving other multikinase inhibitors in development (21–25). Overall, treatment emergent AEs were manageable, although dose adjustments were necessary. Seventeen patients permanently discontinued treatment due to AEs, the majority of which were grade 3 or less (Supplementary Table S1).

The observed terminal half-life of regorafenib was 20 to 40 hours, which supports the once daily dosing schedule and explains low peak/trough fluctuation at steady state, as well as the accumulation of regorafenib with repeated

dosing. Accumulation of major metabolites M-2 and M-5 were also observed. The systemic exposure of M-2 and M-5 was low after the first dose, but rose to a level approximately equivalent to that of the parent compound at steady state. There was no further accumulation once steady state was reached. Preclinical studies have shown that M-2 and M-5 metabolites are pharmacologically active (26). In this study, the unbound plasma concentration of the pharmacologically active species at the 160 mg dose level exceeded the IC_{50} of many target kinases, including mVEGFR2, RET, and c-KIT (19). It is therefore plausible that M-2 and M-5 may contribute to the clinical activity of regorafenib. Systemic exposure of regorafenib was dose proportional up to 60 mg dose level. We believe that limited solubility of regorafenib in gastrointestinal fluids is the main reason for lack of dose proportionality at doses more than 60 mg.

Pharmacodynamic assessments showed biologic activity of regorafenib that was consistent with the proposed antiangiogenic mechanism of action. DCE-MRI results showed a 40% or more decrease in tumor perfusion at dose levels of 120 mg or above. Such effects may be driven by decreases in permeable microvessels and subsequent reinstatement of the stromal barrier (27, 28). Although the DCE-MRI end point employed (iAUC) has not been fully validated, changes in this end point have been shown to be correlated with changes in VEGF-dependent perfusion (23, 24). Plasma concentration of VEGF increased steadily during the 21-day regorafenib treatment period but, interestingly, returned to almost baseline levels during the 7-day treatment break.

The recommended regorafenib dose (160 mg od coprecipitate tablets, 21 days on/7 days off) was determined primarily on the basis of tolerability and was also supported by pharmacokinetic and pharmacodynamic assessments. When treated with 220 mg od regorafenib, 5 of 12 patients had DLTs in cycle 1, compared with 2 of 12 at the 160 mg od dose. This difference was even more pronounced when considering AEs occurring in cycles 1 and 2. Only small increases in systemic exposure were observed when the dose was increased from 160 to 220 mg, accompanied by high interpatient variability. Pharmacodynamic assessments (DCE-MRI and plasma VEGF) showed pharmacologic activity for dose levels of 120 mg and above, but without significant difference across the dose levels between 120 to 220 mg. In this study, regorafenib was given in an intermittent dosing schedule. One potential disadvantage of intermittent dosing schedule might be tumor flare during the treatment break period. However, no obvious tumor flare was observed in this study, which in combination with the efficacy signal supports the intermittent schedule of regorafenib.

In this study, 66% of patients treated with regorafenib experienced disease control (partial response or stable disease). The efficacy is encouraging, and along with the acceptable safety profile, has prompted interest in further clinical investigation of regorafenib. In a phase I extension cohort study of patients with metastatic colorectal cancer, regorafenib showed antitumor efficacy and manageable AE profile (29). Two randomized controlled phase III studies have been initiated to investigate regorafenib in metastatic CRC and GIST (ClinicalTrials.Gov identifier: NCT01103323 and NCT01271712).

In conclusion, the multikinase inhibitor regorafenib showed acceptable safety profile and preliminary evidence of antitumor activity in this first-in-man dose–escalation study in patients with advanced solid tumors.

Disclosure of Potential Conflicts of Interest

O. Christensen is an employee of Bayer and O. Boix has an ownership interest in Bayer stock.

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