21. Tanaka K, Arao T, Maegawa M, et al. SRPX2 is overexpressed in gastric cancer and promotes cellular migration and adhesion. Int J Cancer 2009;124:1072–80.
22. United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR). Guidelines for the Welfare of Animals in Experimental Neoplasia (Second Edition). Br J Cancer 1998;77:1–10.
23. Guix M, Faber AC, Wang SE, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008;118:2609–19.
24. Turke AB, Zejnullahu K, Wu YL, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 2010; 17:77–88.
25. Tsao MS, Yang Y, Marcus A, Liu N, Mou L. Hepatocyte growth factor is predominantly expressed by the carcinoma cells in non-small-cell lung cancer. Hum Pathol 2001;32:57–65.
26. Onitsuka T, Uramoto H, Nose N, et al. Acquired resistance to gefitinib: the contribution of mechanisms other than the T790M, MET, HGF status. Lung Cancer 2010;68:198–203.
27. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature 2004;432:332–7.
28. Matsumoto K, Nakamura T. Hepatocyte growth factor and the Met system as a mediator of tumor-stromal interactions. Int J Cancer 2006;119:477–83.
29. Wang W, Li Q, Yamada T, et al. Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. Clin Cancer Res 2009;15:6630–8.

Molecular Cancer Therapeutics

Phase I Safety, Pharmacokinetic, and Biomarker Study of BIBF 1120, an Oral Triple Tyrosine Kinase Inhibitor in Patients with Advanced Solid Tumors

Isamu Okamoto[1], Hiroyasu Kaneda[1,2], Taroh Satoh[1], Wataru Okamoto[1], Masaki Miyazaki[1], Ryotaro Morinaga[1], Shinya Ueda[1], Masaaki Terashima[1], Asuka Tsuya[1], Akiko Sarashina[3], Koichi Konishi[3], Tokuzo Arao[2], Kazuto Nishio[2], Rolf Kaiser[4], and Kazuhiko Nakagawa[1]

Abstract

BIBF 1120 is an oral multitargeted tyrosine kinase inhibitor that blocks the activity of vascular endothelial growth factor (VEGF) and other growth factor receptors. We have done a phase I study to evaluate the safety, pharmacokinetics, and pharmacodynamic biomarkers of BIBF 1120. Patients with advanced refractory solid tumors were treated with BIBF 1120 at oral doses of 150 to 250 mg twice daily. Drug safety and pharmacokinetics were evaluated, as were baseline and post-treatment levels of circulating CD117-positive bone marrow–derived progenitor cells and plasma soluble VEGF receptor 2 as potential biomarkers for BIBF 1120. Twenty-one patients were treated at BIBF 1120 doses of 150 ($n = 3$), 200 ($n = 12$), or 250 mg twice daily ($n = 6$). Dose-limiting toxicities of reversible grade 3 or 4 elevations of liver enzymes occurred in 3 of 12 patients at 200 mg twice daily and 3 of 6 patients at 250 mg twice daily. Stable disease was achieved in 16 (76.2%) patients, and median progression-free survival was 113 days (95% confidence interval, 77-119 d). Pharmacokinetic analysis indicated that the maximum plasma concentration and area under the curve for BIBF 1120 increased with the dose within the dose range tested. Levels of CD117-positive bone marrow–derived progenitors and soluble VEGF receptor 2 decreased significantly during treatment over all BIBF 1120 dose cohorts. In conclusion, the maximum tolerated dose of BIBF 1120 in the current study was determined to be 200 mg twice daily, and our biomarker analysis indicated that this angiokinase inhibitor is biologically active. *Mol Cancer Ther; 9(10); 2825–33. ©2010 AACR.*

Introduction

Angiogenesis, defined as the formation of new blood vessels from a preexisting vasculature, is essential for tumor growth and the spread of metastases (1, 2). Tyrosine kinase receptors, including vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors, and fibroblast growth factor receptors, together with their corresponding ligands, play key roles in angiogenesis (1). Antiangiogenic therapy that targets signaling by these receptor-ligand systems represents an important advance in clinical oncology (3). Given that most angiogenesis inhibitors are cytostatic, however, it has been difficult to assess their biological effects in early clinical trials. Validated biomarkers that allow monitoring of the biological activity of these agents are thus urgently needed (4, 5). The most intuitive approach to measurement of the biological activity of such targeted agents is evaluation of their effects on tumor cells or the vasculature. However, this invasive approach raises practical and ethical concerns (6, 7). Noninvasive, blood-based biomarkers that allow repetitive sampling throughout treatment and follow-up are therefore preferred.

BIBF 1120 is an orally available triple tyrosine kinase inhibitor that predominantly blocks VEGFR1 to 3, fibroblast growth factor receptors 1 to 3, as well as platelet-derived growth factor receptors α and β tyrosine kinases at nanomolar concentrations (Fig. 1; refs. 8–10). In preclinical studies, BIBF 1120 has been shown to inhibit the growth of and to reduce vessel density in s.c. implanted human tumor xenografts in nude mice (8, 11). A previous phase I BIBF 1120 monotherapy study in patients with advanced and heavily pretreated malignancies showed encouraging antitumor activity and a tolerable safety profile. The maximum tolerated dose (MTD) was determined as 250 mg twice daily (12). A further phase I combination study showed that BIBF 1120 at 200 mg twice daily can be combined with standard doses

Authors' Affiliations: Departments of [1]Medical Oncology and [2]Genome Biology, Kinki University School of Medicine, Osaka-Sayama, Japan; [3]Nippon Boehringer Ingelheim Co. Ltd., Tokyo, Japan; and [4]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Isamu Okamoto, Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Phone: 81-72-366-0221; Fax: 81-72-360-5000. E-mail: chi-okamoto@dotd.med.kindai.ac.jp

doi: 10.1158/1535-7163.MCT-10-0379

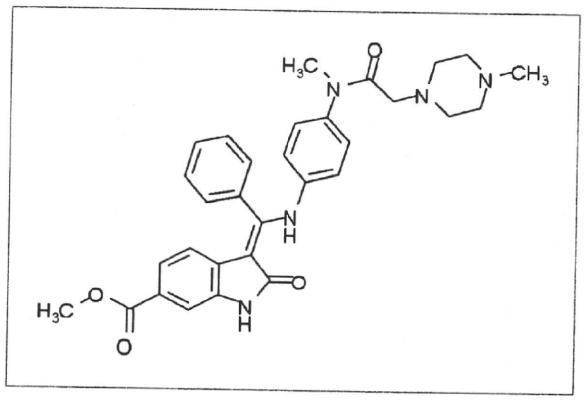

Figure 1. Structure of BIBF 1120.

of paclitaxel and carboplatin (13). Several phase II monotherapy trials have gone on to show promising signs of efficacy in patients with advanced non–small cell lung cancer and ovarian cancer (14, 15).

We have done a phase I dose-escalation study to determine the MTD, tolerability, basic pharmacokinetics, and antitumor effect of BIBF 1120 given p.o. on a twice daily schedule in Japanese patients with advanced refractory solid tumors. To identify biomarkers that reflect the pharmacodynamics and dose-response relation of BIBF 1120, we further evaluated baseline (before BIBF 1120 treatment) and post-treatment levels of circulating CD117 (c-KIT)-positive bone marrow–derived (BMD) progenitor cell subsets as well as of plasma soluble VEGFR2 (sVEGFR2). We show that a subset of $CD117^+$ BMD progenitors, immunophenotypically defined as $CD45^{dim}CD34^+CD117^+$ cells, is a potential biomarker for guidance of optimal therapy with BIBF 1120.

Patients and Methods

Patient eligibility

Eligible patients were 20 years of age or older with a confirmed diagnosis of advanced solid tumors who had not responded to conventional treatment or for whom no therapy of proven efficacy was available. They were required to have an Eastern Cooperative Oncology Group performance status of <2 and adequate organ function. Individuals were excluded if they had a brain tumor or brain metastases requiring therapy, gastrointestinal disorders that might interfere with absorption of the study drug, or serious illness or concomitant nononcologic disease that was difficult to control by medication. Patients were also excluded if they had a history of obvious pulmonary fibrosis or interstitial pneumonitis, autoimmune disease, serious drug hypersensitivity, cardiac infarction, or congestive heart failure. All subjects received information about the nature and purpose of the study, and they provided written informed consent in accordance with institutional guidelines.

Study design

This study was designed as a single-center, open-label, dose-escalation phase I trial. The primary objectives of this dose-escalation trial were to determine if BIBF 1120 doses from 150 to 250 mg given twice daily on a continuous daily schedule could be confirmed as safe and tolerable treatment, and to collect overall safety data. The secondary objectives included the determination of the MTD, pharmacokinetic variables, pharmacodynamics, and preliminary information about the antitumor activity and the efficacy on angiogenic peripheral blood biomarkers in this treatment population. The study was reviewed and approved by the Institutional Review Board.

Dose levels of BIBF 1120 were 150, 200, and 250 mg twice daily. Intrapatient dose escalation was not permitted. Each treatment course comprised 28 days of continuous daily treatment with BIBF 1120. If a patient experienced a drug-related dose-limiting toxicity (DLT), the treatment with BIBF 1120 had to be discontinued. If all DLTs were recovered to baseline or below grade 1 according to the Common Toxicity Criteria for Adverse Events version 3.0 within 14 days of stopping treatment with BIBF 1120, treatment could be resumed at one-dose lower level.

The dose escalation/reduction scheme was based on the occurrence of drug-related DLTs within the first treatment course. If a DLT was not observed in any of the first three patients, the dose was escalated to the next level. If a DLT was observed in one of the first three patients, three additional patients were recruited to that dose level. If a DLT occurred in only one of six patients, dose escalation was permitted. If two or more of six patients experienced a DLT, additional patients were recruited at one-dose lower level for a total of at least six patients. In addition to this dose escalation/reduction scheme, if the investigators and independent data monitoring committee agreed that additional patients were necessary to confirm the dose escalation/reduction decision in cases in which two or more patients experienced DLTs, which were not life-threatening, and were reversible and manageable with or without medication, entering additional patients at that dose level was allowed. The MTD was defined as the highest dose level at which <33% of the patients would experience a DLT during the first treatment course. Once the MTD had been determined, that cohort was expanded to at least 12 patients in total to more completely assess the safety and tolerability of the dose level.

Safety and efficacy assessments

The safety and tolerability of BIBF 1120 were assessed according to Common Toxicity Criteria for Adverse Events version 3.0. The following adverse events were defined as DLTs: drug-related adverse events involving hematologic or nonhematologic toxicity of Common Toxicity Criteria for Adverse Events grade 3 or 4 within the first treatment course with BIBF 1120. Objective

tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (16).

Pharmacokinetics

Blood samples (4 mL) were collected on days 1 and 2, and 29 and 30 before and 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hours after dosing. Predose blood samples to determine trough pharmacokinetic values and the attainment of a steady state of BIBF 1120 were collected on days 8, 15, 22, and 29 in the first treatment course. For pharmacokinetic reasons, BIBF 1120 was given only once daily on days 1 and 29 in the first treatment course. During repeated treatment courses (2–6), trough pharmacokinetic samples were taken on days 15 and 29. Plasma concentrations of BIBF 1120 were analyzed, and the pharmacokinetic variables were calculated in the same manner as the previously conducted phase I study (12).

Biomarker evaluation

The concentration of sVEGFR2 in plasma were measured by enzyme-linked immunosorbent assay on days 1, 2, 8, and 29 after BIBF 1120 treatment according to the manufacture's instructions (R&D System).

CD117/c-KIT–positive BMD progenitor cell subsets were measured with the use of flow cytometry. Peripheral blood was collected before starting, and after 2, 8, and 29 days of BIBF 1120 treatment. The 800 uL of whole blood was supplemented with 4.5 mL of 0.2% bovine serum albumin (BSA)-PBS and centrifuged for 5 minutes (1,500 rpm). After the removal of supernatant by aspiration, 4.5 mL of 0.2% BSA-PBS was added and centrifuged. Cell pellet was mixed with 50 μL of human γ-globulin. Antibodies (CD34-FITC, CD117-PE, and CD45-PerCP) were added and kept for 45 minutes at 4°C. Hemolytic agent (4.5 mL) was added and incubated for 10 minutes. After centrifugation (1,500 rpm, 5 min), supernatant was washed twice. Subsequently, 0.2% BSA-PBS (4.5 mL) was added, and supernatant was removed by centrifugation (1,500 rpm, 5 min). Cell pellet was filled up to 800 uL by BSA-PBS and

Table 1. Patient characteristics

Characteristic	No. of patients
Median (range) age (y)	62 (41-81)
Sex	
Male	11 (52%)
Female	10 (48%)
Performance status (ECOG)	
0	5 (24%)
1	16 (76%)
Previous therapy	
Surgery	18 (86%)
Chemotherapy	19 (91%)
Radiotherapy	6 (29%)
Tumor types	
Colorectal cancer	14 (67%)
Non–small cell lung cancer	1 (4.8%)
Small cell lung cancer	1 (4.8%)
Esophagus sarcoma	1 (4.8%)
Adrenal carcinoma	1 (4.8%)
Renal cell carcinoma	1 (4.8%)
Adenoid cystic carcinoma	1 (4.8%)
Unknown primary site	1 (4.8%)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

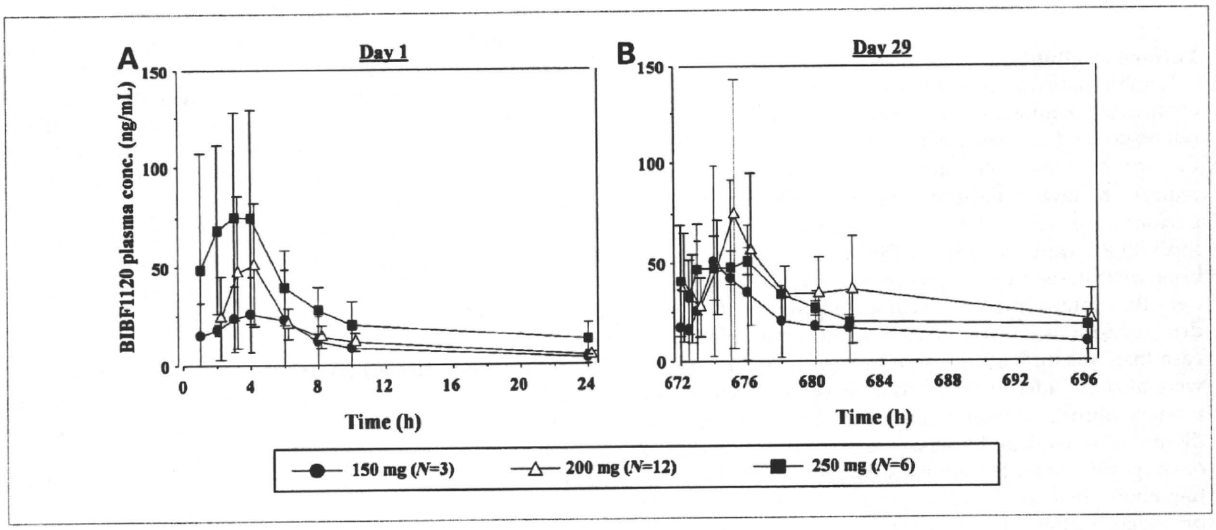

Figure 2. Mean (± SD) plasma concentration–time profiles of BIBF 1120 after single (A; day 1) and multiple (B; day 29) administration of 150, 200, and 250 mg BIBF 1120 twice daily.

Table 2. Dose-escalation scheme and DLT

BIBF 1120 dose (mg bid)	No. of patients		DLTs
	Total	DLT in first course	
150	3	0	
200	12	3	ALT and γ-GT increase; ALT increase; AST, ALT, and γ-GT increase
250	6	3	AST and ALT increase; ALT increase; γ-GT increase

Abbreviations: bid, twice daily; γ-GT, γ–glutamyl transferase.

analyzed by FACSCalibur flow cytometer (BD Biosciences). Cell surface markers of CD133 and CD117 were further identified from the $CD34^{+}CD45^{dim}$ cells in peripheral blood with the use of flow cytometry (Fig. 4A). The cell phenotype data of $CD133^{+/-}$ $CD117^{+/-}$ cells were calculated by the percentage of cell numbers of the target quadrant/those of all quadrants ($CD34^{+}CD45^{dim}$ cells).

Statistical analysis

Student's paired *t*-test was used to compare plasma sVEGFR2 levels or circulating $CD45^{dim}CD34^{+}CD117^{+}$ cell numbers between day 8 and before treatment, as well as between day 29 and before treatment, to evaluate the significance of changes induced by BIBF 1120 treatment (Microsoft Excel). A *P*-value of <0.05 was considered statistically significant.

Results

Patient demographics

Twenty-one patients with advanced refractory solid tumors were recruited between June 2006 and July 2007. The demographic and clinical characteristics of the patients are listed in Table 1. The median number of cycles given per patient was three (range, 1-7 cycles), and 10 patients received at least 4 cycles.

Table 3. Adverse events (≥10% incidence) related to BIBF 1120 in all treatment courses

BIBF 1120 dose	150 bid (*N* = 3)		200 bid (*N* = 12)		250 bid (*N* = 6)		Total (*N* = 21)	
CTCAE grade	1/2	3/4	1/2	3/4	1/2	3/4	All	
	N	N	N	N	N	N	N	(%)
ALT increased	0	0	4	4	3	2	13	61.9
AST increased	0	0	6	2	3	1	12	57.1
γ-GT increased	0	0	4	4	2	2	12	57.1
Vomiting	1	0	9	0	2	0	12	57.1
Anorexia	1	0	8	0	2	0	11	52.4
Fatigue	2	0	6	0	2	1	11	52.4
ALP increased	0	0	5	1	3	0	9	42.9
Nausea	1	0	5	0	2	0	8	38.1
Diarrhea	0	0	5	0	2	0	7	33.3
Hemoptysis	1	0	3	0	0	0	4	19.0
Upper abdominal pain	1	0	1	0	2	0	4	19.0
Weight decreased	0	0	4	0	0	0	4	19.0
Abdominal pain	1	0	2	0	0	0	3	14.3
Hypertension	1	1	1	0	0	0	3	14.3
Rash	0	0	2	0	1	0	3	14.3
Proteinuria	1	0	2	0	0	0	3	14.3
LDH increased	0	0	2	0	1	0	3	14.3

NOTE: Presented is the highest ever reached CTCAE grade. One patient may have experienced >1 event.
Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; bid, twice daily; γ-GT, γ–glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

Dose escalation and MTD

No DLT was observed at the starting dose of 150 mg twice daily in the first three patients (Table 2), so the dose was escalated to the second dose level of 200 mg twice daily. Because one of the first three patients experienced a DLT of grade 3, an increasein alanine aminotransferase (ALT) and γ–glutamyl transpeptidase levels at 200 mg twice daily, three patients were additionally treated at this dose according to the protocol definition. Among the first six patients treated at 200 mg twice daily, two patients experienced a DLT of grade 3 (ALT and γ–glutamyl transpeptidase increases in one patient, ALT increase in one patient). Given that these increases in hepatic enzyme levels were fully reversible, the investigators and independent data monitoring committee agreed to add four more patients to confirm the judgment of dose escalation/reduction of the dose level. The four additional patients did not experience a DLT, and overall, 2 of 10 patients at this dose level experienced a DLT; therefore, dose escalation proceeded to 250 mg twice daily. At this dose level, three of six patients showed DLTs [aspartate aminotransferase (AST) and ALT elevations of grade 3 in one patient, ALT elevation of grade 3 in one patient, and γ–glutamyl transpeptidase elevation of grade 3 in one patient], and the MTD had been exceeded. The next lower dose of 200 mg twice daily was therefore identified as the MTD. According to the protocol definition, two additional patients were further evaluated at the MTD cohort. Among the total of 12 patients who received 200 mg twice daily, 3 patients experienced a reversible grade 3 or 4 AST, ALT, and γ–glutamyl transpeptidase elevation, which correspond to DLT, and 200 mg twice daily BIBF 1120 was thus confirmed as the MTD.

Safety

Twenty-one patients received at least one dose of study treatment and were evaluated for safety. As shown in Table 3, the most frequent BIBF 1120–related side effects were increased hepatic enzymes [ALT (61.9% of patients), AST (57.1%), and γ–glutamyl transpeptidase (57.1%)], vomiting (57.1%), anorexia (52.4%), fatigue (52.4%), alkaline phosphatase increase (42.9%), nausea (38.1%), and diarrhea (33.3%). Most of these events were of mild-to-moderate intensity and of Common Toxicity Criteria for Adverse Events grade 1 or 2, fully reversible and clinically manageable over all doses. The predominant Common Toxicity Criteria for Adverse Events grades 3 and 4 adverse events were reversible liver enzyme elevations occurring at BIBF 1120 at 200 mg twice daily and BIBF 1120 at 250 mg twice daily in a total of eight patients. Except for one patient with combined grade 4 AST and ALT elevations, all elevations were of grade 3 intensity. One patient in the BIBF 1120 150 mg twice daily cohort reported grade 3 hypertension, and another patient in the BIBF 1120 250 mg twice daily cohort reported grade 3 fatigue. Drug-related increases in hepatic enzymes occurred within the 1st week after treatment initiation and were fully reversible on

Table 4. Pharmacokinetic variables of BIBF 1120 after a single dose (day 1) and multiple dosing for 29 days

Single dose	BIBF 1120 dose (mg)		
	150 (*N* = 3)	200 (*N* = 12)	250 (*N* = 6)
C_{max}, ng/mL	28.9 (61.5)	52.0 (64.3)	99.8 (70.3)
t_{max}*, h	2.00 (1.00-6.00)	2.98 (1.98-4.00)	2.98 (1.00-4.07)
$t_{1/2}$, h	10.3 (15.8)	10.2 (30.4)	9.53 (10.8)†
AUC_{0-12}, ng·h/mL	145 (88.3)	233 (40.9)	399 (64.9)
Multiple dosing	**150 (*N* = 3)**	**200 (*N* = 7)**	**250 (*N* = 3)**
$C_{max,ss}$, ng/mL	38.8 (107)	67.6 (74.3)	62.9 (14.4)
$t_{max,ss}$, h	2.00 (1.98-4.00)	2.97 (1.98-3.98)	2.00 (1.00-4.00)
$t_{1/2,ss}$, h	20.4 (55.3)	19.9 (75.5)‡	23.8 (39.4)§
AUC_{ss}, ng·h/mL	207 (135)	423 (66.2)	411 (9.15)
Rac	1.42 (35.4)	1.70 (40.9)	1.50 (79.0)

NOTE: Geometric mean (geometric coefficient of variation %).
Abbreviations: $t_{max,ss}$, time to reach maximum plasma concentrations at steady state; AUC, area under the curve.
*Median (range).
†*N* = 5.
‡*N* = 6.
§*N* = 2.

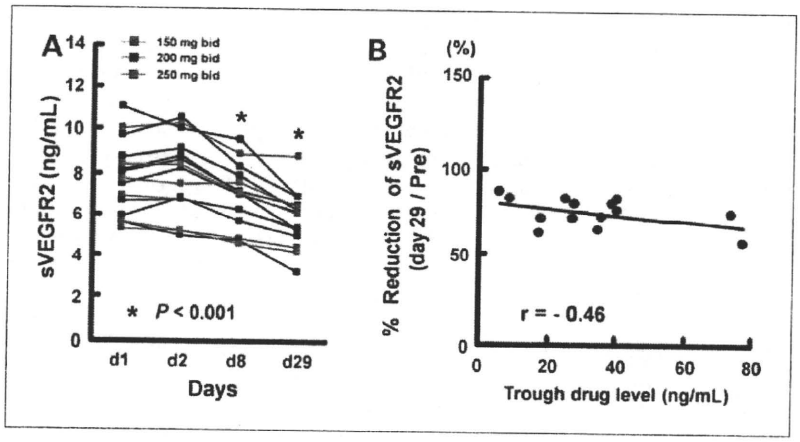

Figure 3. sVEGFR2 levels in plasma after BIBF 1120 treatment. A, plasma sVEGFR2 levels decreased during the 4-week treatment period. B, the decrease in sVEGFR2 at cycle 1, day 29 showed a modest inverse correlation with trough plasma drug levels of BIBF 1120 ($r = -0.46$).

cessation of treatment. There were no bleeding events or clinically relevant hematologic toxicities during all treatment courses throughout the study. Due to adverse events or DLTs, four patients in the BIBF 1120 200 mg twice daily and three patients in the BIBF 1120 250 mg twice daily dose cohorts required dose reduction.

Pharmacokinetics

The pharmacokinetic variables after a single oral dose and multiple oral doses of BIBF 1120 (150-250 mg twice daily) are shown in Table 4. Maximum plasma concentrations [$C_{max,(ss)}$] were reached at 2 to 3 hours after dosing after single and multiple dosing of BIBF 1120 (Fig. 2A and B; Table 4). After attaining C_{max}, the plasma concentration declined in an apparent biexponential manner with the terminal half-life of ~10 hours. Of note, the terminal half-life of BIBF 1120 was calculated from samples obtained during the first 24 hours post dose. After multiple dosing of BIBF 1120, C_{max} were reached at 2 to 3 hours after dosing (Fig. 2B; Table 4). The accumulation ratio (Rac) values based on area under the curve were 1.42 to 1.7, and accumulation was consistent with the terminal half-life observed after single doses. Steady-state plasma concentrations were attained at least on day 8 of repeated twice daily oral dosing based on visual inspection of the trough plasma concentration. In general, C_{max} and area under the curve were increased with increasing dose. Trough plasma concentrations of BIBF 1120 during repeated treatment courses were

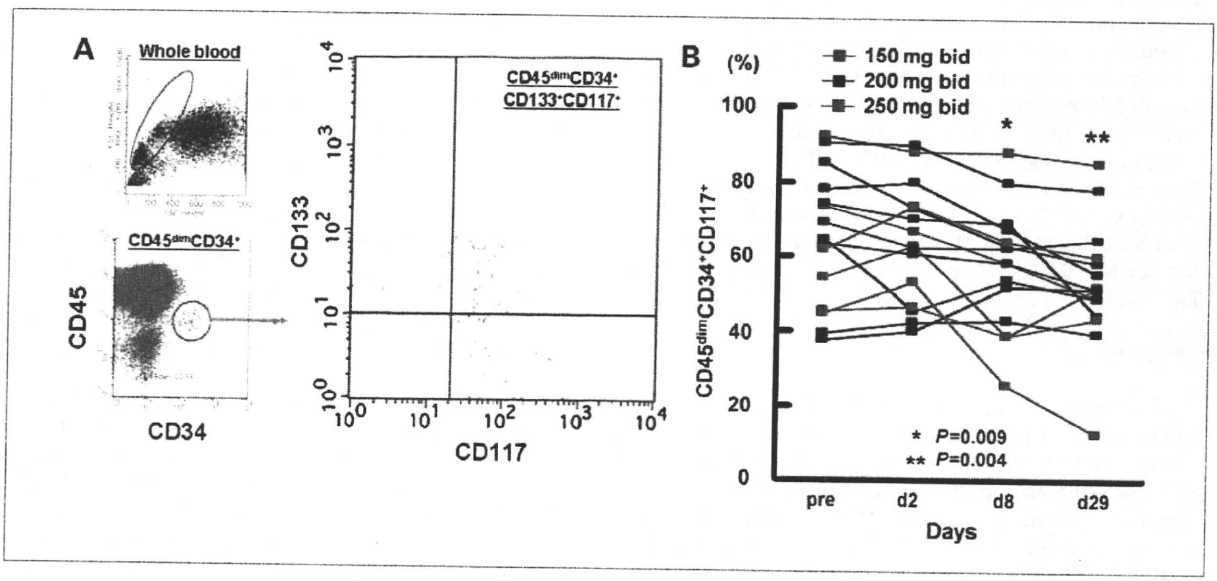

Figure 4. Levels of circulating CD117-BMD progenitor cells after BIBF 1120 treatment. A, representative flow cytometric analysis for determining the number of CD117-positive–BMD progenitor cells defined as $CD45^{dim}CD34^{+}CD117^{+}$. B, circulating levels of $CD45^{dim}CD34^{+}CD117^{+}$ cells decreased during the 4-week treatment period.

almost at the same level within each dose group. The range of the geometric mean of the trough concentration was 14.4 to 38.4 nmol/L for the 150 mg twice daily group and 28.2 to 84.6 nmol/L for the 200 mg twice daily group. In the 250 mg twice daily group, the number of trough concentrations collected during repeated treatment courses was very limited due to the occurrence of dose reduction in this group.

Tumor response

Twenty patients were evaluated for tumor response. Although no complete or partial responses were observed, 16 (76.2%) patients had stable disease for at least two treatment courses (56 d). The disease stabilization was observed across all the tested doses: BIBF 1120 150 mg, all patients (100%) of 3; 200 mg, 9 (75%) of 12; 250 mg, 4 (67%) of 6. Median progression-free survival for all patients was 113 days (95% confidence interval, 77-119 d).

Plasma levels of sVEGFR2 during treatment with BIBF 1120

At baseline, the mean plasma level of sVEGFR2 obtained from 15 patients [150 mg twice daily (n = 3), 200 mg twice daily (n = 9), and 250 mg twice daily (n = 3)] was 7.7 ± 1.7 ng/mL (range, 5.3-11.0 ng/mL). Plasma concentrations of sVEGFR2 decreased significantly over the first 4 weeks of treatment to a level of 5.8 ± 1.3 ng/mL (range, 3.2-8.8; $P < 0.001$, t-test; Fig. 3A). The decreases in sVEGFR2 levels were seen across all doses tested. As shown in Fig. 3B, the decrease in sVEGFR2 showed an inverse linear correlation with the trough plasma drug levels of BIBF 1120 ($r = -0.46$).

Levels of circulating CD117/C-KIT$^+$–BMD progenitors during treatment with BIBF 1120

Subsets of CD117-positive–BMD progenitor cells were measured in progenitor-enriched ($CD45^{dim}CD34^+$) whole blood of 15 patients [150 mg twice daily (n = 3), 200 mg twice daily (n = 9), and 250 mg twice daily (n = 3)]. CD117 was expressed in the $CD45^{dim}CD34^+$ subset with a level of 60% to 80%, and representative data are shown in Fig. 4A. $CD45^{dim}CD34^+CD117^+$ cells significantly decreased over all BIBF 1120 dose cohorts during the 1st cycle of therapy ($P = 0.009$ on day 8 and $P = 0.004$ on day 29, t-test; Fig. 4B).

Discussion

This phase I study showed that BIBF 1120 can be safely given to Japanese patients with advanced solid tumors, and the MTD was determined as 200 mg twice daily, which was one dose lower than in Caucasian patients (12). Biomarker investigations revealed that the plasma concentration levels of the sVEGFR2 and the $CD45^{dim}CD34^+CD117^+$ cells significantly decreased over the first 4 weeks of treatment with BIBF 1120.

As has been observed in previous phase I and phase II studies with BIBF 1120, gastrointestinal side effects, such as vomiting, fatigue, nausea, and diarrhea, were the most frequent adverse events (12, 15) and have also been observed with other VEGFR inhibitors, such as sorafenib or sunitinib (4, 5, 17). These side effects of mostly mild or moderate intensity occurred predominantly at the MTD of BIBF 1120 or at higher doses, and were easy to monitor and manageable with standard supportive treatment. Hypertension has also been reported with several other VEGF and VEGFR inhibitors (4, 5), and was observed in three patients in this study. All cases were controllable with appropriate antihypertensive treatment.

The pharmacokinetic analysis revealed that there was a dose linear increase for C_{max} and area under the curve. C_{max} values were reached within 3 hours after administration, and steady state was reached at least on day 8. All pharmacokinetic variables displayed a moderate-to-high variability as expected for an oral compound. In addition, different patients with various anticancer pretreatments have been enrolled in this study; thus, differences in pretreatment and other intrinsic factors, such as age and status, might have influenced the variability of these variables, too. Overall, there was no difference in the pharmacokinetic behavior of BIBF 1120 between Japanese and Caucasian patients (12, 18). Based on the trough plasma concentrations for BIBF 1120 at dose levels ≥150 mg twice daily, sufficient exposure has been reached to block the target structures of the molecule according to the IC_{50} values (8, 11).

All DLTs observed in this study were liver enzyme elevations (grade 3 or 4 ALT, AST, and γ–glutamyl transpeptidase). These liver enzyme elevations were fully reversible, responded within 2 weeks to treatment discontinuation or dose reduction, indicating reversible liver side effects, and were not accompanied by an increase of bilirubin. However, at 200 mg twice daily of BIBF 1120 in Caucasian patients, no such liver enzyme elevations were observed in a previous phase I study (12). We cannot exclude the possibility of ethnic differences, although there were no pharmacokinetic differences between Japanese and Caucasian patients. From the exploratory data evaluation, the body weight of all three patients who experienced DLTs at 200 mg twice daily as MTD was below 50 kg, whereas that of the remaining nine patients treated without DLTs was ≥50 kg. This finding suggested that body size, such as body weight or body surface area, might confer liver enzyme elevations on BIBF 1120, with further investigation of possible dose dependency being warranted.

Evaluation of novel targeted agents, such as VEGF signaling inhibitors, may be supported by the identification of suitable biomarkers of biological activity. The most intuitive method to measure the effect of any anticancer drug is to evaluate the tumor tissue. Tumor biopsy strategies provide a way to thoroughly characterize tumor histology and molecular processes with immunohistochemistry, DNA microarray, and proteomics analyses. Indeed, several considerable biomarkers of angiogenesis, such as microvessel density or tumor VEGF expression,

have been extensively investigated with the use of tumor tissue specimens. On the other hand, identifying circulating biomarkers of angiogenesis would have the advantage of being minimally invasive, allowing repetitive sampling throughout treatment without the ethical and technical complications of multiple biopsy. Circulating levels of sVEGFR2 were previously found to be decreased by other VEGFR2 inhibitors that directly target this receptor, such as AZD2171 (8) and SU11248 (9), although the mechanism behind the consistent decrease in sVEGFR2 levels is not entirely understood (4, 5, 19–21). In the present study, plasma sVEGFR2 levels showed time-dependent decrease at all dose levels studied, and the changes in sVEGFR2 were inversely associated with trough plasma concentration of BIBF 1120, suggesting that sVEGFR2 is a useful pharmacodynamic marker of drug exposure, with similar findings reported for other agents.

Circulating endothelial cells have emerged as a potentially useful surrogate marker of antiangiogenic drug activity (4, 10, 19–21). They comprise two distinct populations: mature circulating endothelial cells, which originate from vessel walls and have a limited growth capability, and BMD circulating endothelial cells, which are responsible for most endothelial proliferative potential. Circulating BMD endothelial progenitors have been reported to contribute to tumor vasculogenesis in animal models as well as in humans (18, 21–23). However, the variable degrees of incorporation of circulating endothelial cells shown in different tumor models have led to controversy about the extent of their actual involvement in tumor vascularization. The identification of circulating endothelial cells is highly complex and has been hampered by the overlapping antigenic similarities, with a lack of consensus about the definition of these endothelial cells (4, 24). The pan-hematopoietic marker CD45 has been widely used to first exclude hematopoietic cells (22). CD34 was chosen as a colabel because it is reported to be present on endothelial progenitors, and $CD34^+$ cells alone can repopulate bone marrow *in vivo* (23). This present study reported the first quantitative analysis of subsets of circulating CD117-BMD progenitor cells, characterized as $CD45^{dim}CD34^+CD117^+$, after treatment with BIBF 1120. Results show that levels of circulating CD117-BMD progenitor cells were significantly decreased after BIBF 1120 treatment in time-dependent fashion. One possible explanation for the BIBF 1120–induced decrease in CD117-BMD progenitor cells is that $CD117/C\text{-}KIT^+$ is one of the target receptors of BIBF 1120 as well as many other VEGFR tyrosine kinase inhibitors, resulting in the impaired growth of $CD117/C\text{-}KIT^+$ cells or inhibitory effects of differentiation/mobilization on peripheral blood. This study further showed that the patients who responded (stable disease) to BIBF 1120 had a larger decrease in CD117-BMD progenitor cells after the initial 4 weeks of the study treatment compared with patients who did not (progressive disease; Supplementary Fig. S1) although, given the sample size, there was limited power to detect a significant difference. This observation suggests that a reduction in CD117-BMD progenitor cells would be associated with a higher degree of target inhibition and greater clinical efficacy after BIBF 1120 treatment. This is the first study to show evidence of decreased levels of circulating CD117-BMD progenitor cells during treatment with antiangiogenic agents. Meanwhile, the main limitations in evaluating the circulating endothelial progenitor cells for surrogate biomarkers are "nonstandardized protocols" or "labor-intensiveness." Further investigation to validate whether it will be useful for monitoring the response to antiangiogenic therapy is warranted.

In conclusion, BIBF 1120 shows an acceptable profile for Japanese patients suffering from advanced solid tumors at doses up to 200 mg twice daily. The preliminary evaluation of biological activity of BIBF 1120 with the use of plasma (sVEGFR2) and cellular (CD117-BMD progenitor cells) markers, and disease stabilization data show that this agent is biologically active. BIBF 1120 is currently being investigated in a range of tumor types, and recruitment to a series of randomized, double-blind phase II and III trials is ongoing.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received 04/22/2010; revised 07/24/2010; accepted 07/29/2010; published OnlineFirst 08/05/2010.

References

1. Taylor M, Rossler J, Geoerger B, et al. High levels of circulating VEGFR2+ bone marrow-derived progenitor cells correlate with metastatic disease in patients with pediatric solid malignancies. Clin Cancer Res 2009;15:4561–71.
2. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer 2002;2:727–39.
3. Morabito A, De Maio E, Di Maio M, Normanno N, Perrone F. Tyrosine kinase inhibitors of vascular endothelial growth factor receptors in clinical trials: current status and future directions. Oncologist 2006;11:753–64.
4. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. Nat Rev Cancer 2006;6:835–45.
5. Brown AP, Citrin DE, Camphausen KA. Clinical biomarkers of angiogenesis inhibition. Cancer Metastasis Rev 2008;27:415–34.
6. Agulnik M, Oza AM, Pond GR, Siu LL. Impact and perceptions of mandatory tumor biopsies for correlative studies in clinical trials of novel anticancer agents. J Clin Oncol 2006;24:4801–7.
7. Helft PR, Daugherty CK. Are we taking without giving in return? The ethics of research-related biopsies and the benefits of clinical trial participation. J Clin Oncol 2006;24:4793–5.
8. Drevs J, Siegert P, Medinger M, et al. Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. J Clin Oncol 2007;25:3045–54.
9. Norden-Zfoni A, Desai J, Manola J, et al. Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. Clin Cancer Res 2007;13:2643–50.
10. Roth GJ, Heckel A, Colbatzky F, et al. Design, synthesis, and evaluation

of indolinones as triple angiokinase inhibitors and the discovery of a highly specific 6-methoxycarbonyl-substituted indolinone (BIBF 1120). J Med Chem 2009;52:4466–80.

11. Hilberg F, Roth GJ, Krssak M, et al. BIBF1120: triple angiokinase inhibitor with sustained receptor blockade and good anti-tumor efficacy. Cancer Res 2008;68:4774–82.
12. Mross K, Stefanic M, Gmehling D, et al. Phase I study of the angiogenesis inhibitor BIBF 1120 in patients with advanced solid tumors. Clin Cancer Res 2010;16:311–9.
13. du Bois A, Huober J, Stopfer P, et al. A phase I open-label dose-escalation study of oral BIBF 1120 combined with standard paclitaxel and carboplatin in patients with advanced gynecological malignancies. Ann Oncol 2010;21:370–5.
14. Ledermann JA, Rustin GJ, Hackshaw A, et al. A randomized phase II placebo-controlled trial using maintenance therapy to evaluate the vascular targeting agent BIBF 1120 following treatment of relapsed ovarian cancer (OC). J Clin Oncol 2009;27:15s (abstr 5501).
15. von Pawel J, Kaiser R, Eschbach C, et al. Efficacy, safety and pharmacokinetic (PK) results of a phase II study with the triple angiokinase inhibitor BIBF 1120 in patients suffering from advanced non small cell lung cancer (NSCLC). J Thorac Oncol 2008;3:S61.
16. 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.
17. Faivre S, Djelloul S, Raymond E. New paradigms in anticancer therapy: targeting multiple signaling pathways with kinase inhibitors. Semin Oncol 2006;33:407–20.
18. Beaudry P, Force J, Naumov GN, et al. Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. Clin Cancer Res 2005;11:3514–22.
19. Beerepoot LV, Mehra N, Vermaat JS, Zonnenberg BA, Gebbink MF, Voest EE. Increased levels of viable circulating endothelial cells are an indicator of progressive disease in cancer patients. Ann Oncol 2004;15:139–45.
20. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood 2001;97:3658–61.
21. Stamper IJ, Byrne HM, Owen MR, Maini PK. Modelling the role of angiogenesis and vasculogenesis in solid tumour growth. Bull Math Biol 2007;69:2737–72.
22. Summer R, Kotton DN, Sun X, Fitzsimmons K, Fine A. Translational physiology: origin and phenotype of lung side population cells. Am J Physiol Lung Cell Mol Physiol 2004;287:L477–83.
23. Blair A, Sutherland HJ. Primitive acute myeloid leukemia cells with long-term proliferative ability *in vitro* and *in vivo* lack surface expression of c-kit (CD117). Exp Hematol 2000;28:660–71.
24. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 2002;109:337–46.

Int J Clin Oncol (2010) 15:497–499
DOI 10.1007/s10147-010-0064-7

CASE REPORT

Retreatment of recurrent malignant pleural mesothelioma with cisplatin and pemetrexed

Hidetoshi Hayashi · Isamu Okamoto · Yasuko Ichikawa · Masaki Miyazaki · Hiroshige Yoshioka · Kei Kunimasa · Kazuhiko Nakagawa

Received: 28 December 2009 / Accepted: 4 February 2010 / Published online: 12 March 2010

Abstract Combination chemotherapy with cisplatin and pemetrexed is the most active first-line regimen for malignant pleural mesothelioma (MPM). However, no drugs have been approved for second-line treatment of MPM, with effective regimens remaining to be identified for patients in relapse. We have now evaluated the combination of cisplatin and pemetrexed for retreatment of patients with recurrent MPM. Four men with MPM, all of whom received initial treatment with cisplatin and pemetrexed, underwent retreatment with this drug combination. Two of the patients achieved an objective response to the first-line chemotherapy with no evidence of disease progression for 6.4 or 11.4 months, respectively. The other two patients had stable disease with a duration of 7.8 or 5.0 months, respectively. The two patients who showed an objective response to first-line chemotherapy showed a partial response to retreatment, with a time to progression of 5.0 or 8.2 months, whereas the other two patients had progressive disease with a time to progression of 1.0 or 1.4 months, respectively. Retreatment with cisplatin plus pemetrexed was generally well tolerated. Retreatment with cisplatin and pemetrexed is a potential therapeutic option for certain patients with recurrent epithelioid MPM, possibly including those who show tumor regression with a time to progression of 6 months or more after the initial chemotherapy. Further studies are warranted to evaluate the efficacy of such retreatment and to clarify the criteria for patient selection.

Keywords Malignant pleural mesothelioma · Retreatment · Pemetrexed · Cisplatin

H. Hayashi · I. Okamoto (✉) · Y. Ichikawa · M. Miyazaki · K. Nakagawa
Department of Medical Oncology,
Kinki University School of Medicine,
377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan
e-mail: chi-okamoto@dotd.med.kindai.ac.jp

H. Yoshioka · K. Kunimasa
Department of Respiratory Medicine,
Kurashiki Central Hospital, 1-1-1 Miwa,
Kurashiki, Okayama 710-0047, Japan

Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm. Treatment of MPM patients with the combination of cisplatin and pemetrexed has been associated with an increased survival time (12.1 vs. 9.3 months), longer time to progression (5.7 vs. 3.9 months), and greater response rate (41.3% vs. 16.7%), as well as with improved pulmonary function and symptom control compared with treatment with cisplatin alone [1, 2]. However, most patients eventually manifest disease progression after the initial response to such combination chemotherapy. Although a previous study has suggested that second-line chemotherapy after initial treatment with cisplatin and pemetrexed has a positive impact on the survival of MPM patients [3], no drugs have been approved for second-line treatment of MPM. Effective chemotherapy is thus needed for the treatment of patients with MPM who relapse after first-line chemotherapy. We now present a report of four patients with recurrent MPM who received an initial course of treatment with pemetrexed and cisplatin and who subsequently underwent retreatment with this drug combination.

Case report

Four patients with MPM underwent retreatment with cisplatin and pemetrexed. Treatment response was evaluated

according to the modified RECIST criteria for MPM proposed by Byrne and Nowak [4]. Time to progression was defined as the period from the start of treatment to the date of disease progression or death, whichever occurred first. Overall survival was defined as the time from the initial visit until death from any cause. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 3).

Patient characteristics are summarized in Table 1. The patients were men aged 65, 60, 53, or 69 years. Tumor histology was epithelial in three patients and biphasic in one patient. All patients received four cycles of treatment with cisplatin and pemetrexed as the first-line chemotherapy. Patient 1 achieved a partial response (Fig. 1) and patient 2 achieved a complete response, with no evidence of disease progression for 6.4 and 11.4 months, respectively. Patients 3 and 4 had stable disease for a duration of 7.8 and 5.0 months, respectively.

At the time of this analysis, all four patients were no longer undergoing retreatment with cisplatin and pemetrexed. Patients 1 and 2 showed a partial response to retreatment, with a time to progression of 5.0 and 8.2 months, respectively (Fig. 1, Table 1). Patients 3 and 4 manifested progressive disease, with a time to progression of only 1.0 and 1.4 months, respectively. With the exception of hyponatremia of grade 3 observed in one patient (patient 1), no toxicities of grade 3 or 4 were apparent during retreatment.

Discussion

We have presented four patients who were retreated with the same chemotherapy regimen on progression of their MPM after initial treatment with cisplatin plus pemetrexed and durable tumor control. Two of the four patients achieved an objective response after four cycles of retreatment with acceptable toxicity.

For many types of malignant neoplasm, the standard treatment options for disease progression after first-line chemotherapy are chemotherapeutic regimens that differ from the initial treatment. However, in the case of MPM, no drugs have been approved for second-line treatment. We elected to retreat the present patients after disease recurrence with the same regimen as that used for the initial chemotherapy, given that all four individuals manifested disease control (one a partial response, one a complete response, and two stable disease) after the first-line treatment. Retreatment of ovarian cancer patients with the combination of carboplatin and paclitaxel is established for individuals who show sensitive relapse, defined as disease that responds to first-line chemotherapy but which relapses more than 6 months after the last dose of the first-line treatment [5]. A previous report presented four patients with relapsed MPM who achieved long-lasting tumor control with the combination of platinum and pemetrexed for retreatment [6]. The time to progression after initial platinum–pemetrexed chemotherapy was unusually long in these patients, ranging from 23 to 73 months. In the present report, the time to progression after the initial chemotherapy was 6.4 or 11.4 months for the two patients who achieved a second response, times that are substantially shorter than those in the previous study [6]. Our findings suggest that patients who show a time to progression of 6 months or more after initial chemotherapy with cisplatin plus pemetrexed may show a response on retreatment. The histological subtype of the two patients who responded to the retreatment was epithelioid histology, consistent with

Table 1 Patient characteristics and response to first-line chemotherapy and retreatment

	Patient 1	Patient 2	Patient 3	Patient 4
Age, years	65	60	53	69
Sex	Male	Male	Male	Male
Histology	Epithelial	Epithelial	Epithelial	Biphasic
Dose of first-line chemotherapy[a]	P500 + C60	P500 + C75	P500 + C75	P500 + C75
No. of cycles of first-line chemotherapy	4	4	4	4
Time to progression (months) after first-line chemotherapy	6.4	11.4	7.8	5.0
Response to first-line chemotherapy)	Partial response	Complete response	Stable disease	Stable disease
Dose of retreatment[a]	P500 + C60	P500 + C75	P500 + C75	P500 + C60
No. of cycles of retreatment	4	4	1	1
Time to progression (months) after retreatment	5.0	8.2	1.0	1.4
Response to retreatment	Partial response	Partial response	Progression disease	Progression disease
Overall survival[b] (months)	19.0	26+	10.0	9.3

[a] Doses for pemetrexed (P) and cisplatin (C) are given in milligrams per square meter (mg/m^2)

[b] Overall survival was defined as the time from the initial visit until death from any cause

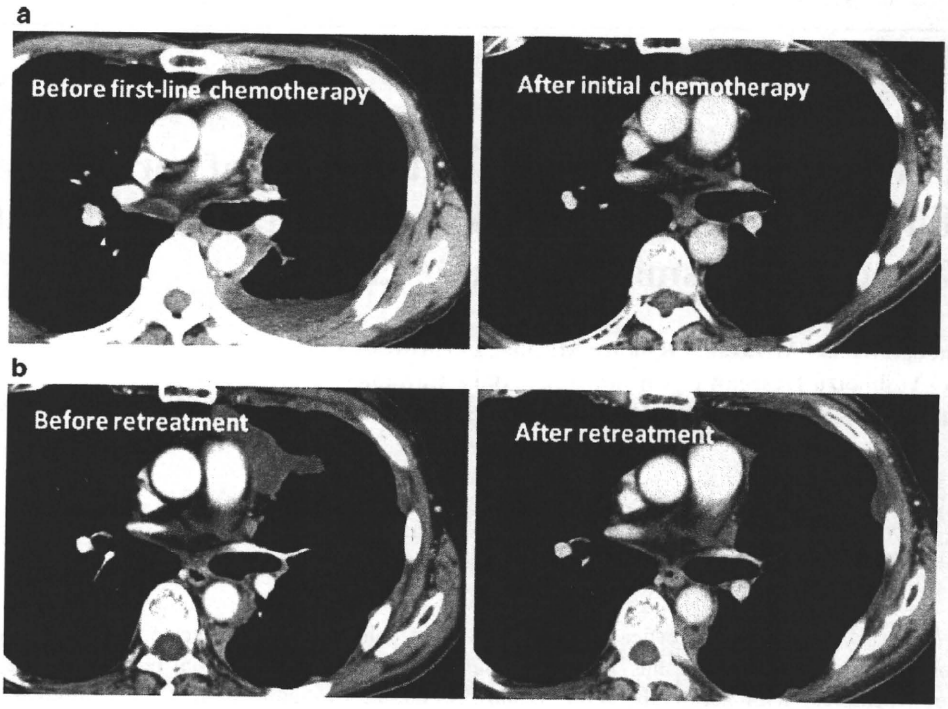

Fig. 1 Responses of patient 1 to first- and second-line chemotherapy with cisplatin plus pemetrexed. Computed tomography scans revealed that pleural nodules of patient 1 showed a partial response to first-line chemotherapy (**a**) and that a second response was obtained after retreatment with cisplatin plus pemetrexed (**b**)

the previous report [6]. These findings suggest that epithelioid histological subtype may also define a response on retreatment.

Our cohort included two patients who developed progressive disease with retreatment. These two patients did not develop an objective response to initial chemotherapy with cisplatin–pemetrexed, instead manifesting stable disease, whereas the two patients who achieved a response to retreatment showed a partial or complete response to first-line treatment. This observation suggests that failure to respond to initial chemotherapy may be a negative predictive factor for the effectiveness of retreatment.

The overall survival of the two patients who achieved a second response was 19 and more than 26 months, respectively, suggesting that successful retreatment with cisplatin plus pemetrexed can prolong survival time. Our observations thus suggest that retreatment with cisplatin plus pemetrexed may yield clinical benefits in patients who show a partial or complete response of long duration (>6 months) to the initial combination chemotherapy. Further studies are warranted to evaluate the efficacy of such second-line treatment and to clarify the criteria for selection of patients likely to respond to retreatment with cisplatin plus pemetrexed.

Conflict of interest statement I. Okamoto and K. Nakagawa received honoraria from Boehringer Ingelheim. The other authors have no conflict of interest.

References

1. Vogelzang NJ, Rusthoven JJ, Symanowski J et al (2003) Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol 21:2636–2644
2. Paoletti P, Pistolesi M, Rusthoven JJ et al (2003) Correlation of pulmonary function tests with best tumor response status: results from the phase III study of pemetrexed + cisplatin vs. cisplatin in malignant pleural mesothelioma. Proc Am Soc Clin Oncol 22:659 (Abstr 2651)
3. Manegold C, Symanowski J, Gatzemeier U et al (2005) Second-line (post-study) chemotherapy received by patients treated in the phase III trial of pemetrexed plus cisplatin versus cisplatin alone in malignant pleural mesothelioma. Ann Oncol 16:923–927
4. Byrne MJ, Nowak AK (2004) Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Ann Oncol 15:257–260
5. Pfisterer J, Ledermann JA (2006) Management of platinum-sensitive recurrent ovarian cancer. Semin Oncol 33:S12–S16
6. Razak AR, Chatten KJ, Hughes AN (2008) Retreatment with pemetrexed-based chemotherapy in malignant pleural mesothelioma (MPM): a second line treatment option. Lung Cancer 60:294–297

Invest New Drugs (2010) 28:844–853
DOI 10.1007/s10637-009-9321-x

PHASE I STUDIES

Phase I clinical and pharmacokinetic study of sorafenib in combination with carboplatin and paclitaxel in patients with advanced non–small cell lung cancer

Isamu Okamoto · Masaki Miyazaki · Ryotaro Morinaga · Hiroyasu Kaneda · Shinya Ueda · Yoshikazu Hasegawa · Taroh Satoh · Akira Kawada · Masahiro Fukuoka · Koichi Fukino · Takahiko Tanigawa · Kazuhiko Nakagawa

Received: 7 August 2009 / Accepted: 2 September 2009 / Published online: 18 September 2009

Summary *Objectives* Unsatisfactory efficacy of current treatments for advanced lung cancer has prompted the search for new therapies, with sorafenib, a multikinase inhibitor, being one candidate drug. This phase I trial was conducted to evaluate drug safety and pharmacokinetics as well as tumor response of sorafenib in combination with paclitaxel and carboplatin in patients with advanced non-small cell lung cancer (NSCLC). *Methods* Eligible patients received paclitaxel (200 mg/m^2) and carboplatin (area under the curve [AUC]of 6 mg min mL^{-1}) on day 1 and sorafenib (400 mg, twice daily) on days 2 through 19 of a 21-day cycle. *Results* Four of the initial six patients (cohort 1) experienced dose-limiting toxicities (DLTs), resulting in amendment of the treatment protocol. An additional seven patients (cohort 2) were enrolled, two of whom developed DLTs. DLTs included erythema multiforme, hand-foot skin reaction, and elevated plasma alanine aminotransferase in cohort 1 as well as gastrointestinal perforation at a site of metastasis and pneumonia in cohort 2. Most adverse events were manageable. One complete and six partial responses were observed among the 12 evaluable patients. Coadministration of the three drugs had no impact on their respective pharmacokinetics. *Conclusion* The present study confirmed that sorafenib at 400 mg once daily in combination with carboplatin AUC 5 mg min mL^{-1} and paclitaxel 200 mg/m^2 is feasible in Japanese patients with advanced NSCLC. The results of this study also showed that this combination therapy had encouraging antitumor activity and was not associated with relevant pharmacokinetic interaction in Japanese NSCLC patients.

Keywords Carboplatin · Lung cancer · Paclitaxel · Pharmacokinetics · Safety · Sorafenib

I. Okamoto (✉) · M. Miyazaki · R. Morinaga · H. Kaneda · S. Ueda · Y. Hasegawa · T. Satoh · M. Fukuoka · K. Nakagawa
Department of Medical Oncology,
Kinki University School of Medicine,
377-2 Ohno-higashi,
Osaka-Sayama, Osaka 589-8511, Japan
e-mail: chi-okamoto@dotd.med.kindai.ac.jp

A. Kawada
Department of Dermatology,
Kinki University School of Medicine,
Osaka-Sayama, Osaka, Japan

K. Fukino · T. Tanigawa
Bayer Yakuhin Ltd.,
Kita-ku, Osaka, Japan

Introduction

Non–small cell lung cancer (NSCLC) accounts for ~75% of all lung cancers and is the most common cause of cancer-related deaths worldwide [1]. Individuals with metastatic NSCLC are candidates for palliative systemic chemotherapy that confers only a limited survival benefit [2, 3]. The dismal outlook for patients with advanced NSCLC who receive currently available therapies has prompted a search for new, more effective chemotherapeutic agents and combination regimens. Target-based therapies are therefore being pursued as potential treatment alternatives.

Sorafenib (BAY 43-9006; Nexavar; Bayer HealthCare, Montville, NJ; Onyx Pharmaceuticals, Emeryville, CA), is an oral multikinase inhibitor that inhibits Raf serine-threonine kinases and several receptor tyrosine kinases

that function in tumor growth and angiogenesis [4]. The Ras-Raf-MEK-ERK signaling pathway plays a pivotal role in the regulation of tumor cell growth by relaying signals from the cell surface to the nucleus, with the components of this pathway, including Raf, thus representing potential targets for anticancer treatment [5, 6]. Sorafenib also targets the vascular endothelial growth factor (VEGF) receptors VEGFR-2 and VEGFR-3 as well as platelet-derived growth factor receptor–β (PDGFR-β), the ligands for which (VEGF and PDGF) are pro-angiogenic factors essential for tumor growth and metastasis [4]. Sorafenib has recently been approved for treatment of advanced renal cell carcinoma and hepatocellular carcinoma in the United States, Europe, and several other countries. Furthermore, sorafenib is currently undergoing clinical evaluation for a variety of additional cancers, including NSCLC.

Although several phase I clinical trials of sorafenib alone or in combination with other drugs have been conducted [7–19], no such phase I study for a specific type of lung cancer has been performed. The aim of the present phase I study was to evaluate the safety and pharmacokinetics of sorafenib in combination with carboplatin and paclitaxel in patients with advanced NSCLC.

Patients and methods

Patient selection

Eligible patients were 18 years of age or older with unresectable NSCLC, as confirmed histologically or cytologically, and with a life expectancy of at least 12 weeks. They were required to be naïve to chemotherapy and to have an Eastern Cooperative Oncology Group performance status of 0 or 1. The eligibility criteria also included adequate bone marrow, hepatic, and renal function as well as normal blood coagulation parameters. Individuals were excluded if they had previous or concurrent cancer distinct in primary site or histology from NSCLC or any cancer curatively treated >3 years prior to study entry; clinically active or significant cardiovascular disease; human immunodeficiency virus infection, chronic hepatitis B or C, or other serious infections; a seizure disorder requiring medication; a history of organ allograft, substance abuse, or medical, psychological, or social conditions that might interfere with participation in the study; or allergy to the study treatment. Pregnant or breast-feeding patients were also excluded. All patients received information regarding the nature and purpose of the study, and they provided written informed consent in accordance with institutional guidelines. The study protocol was approved by the Institutional Review Board of Kinki University Hospital.

Study design

The study was designed as a single-center, open-label, non–placebo-controlled phase I trial to define the safety, tolerability, pharmacokinetics, and tumor response profile of sorafenib administered according to a dosing schedule of 18 days on and 3 days off and in combination with paclitaxel and carboplatin chemotherapy in chemonaïve patients with advanced NSCLC. The other phase I trial of sorafenib in combination with paclitaxel and carboplatin had already confirmed the safety of sorafenib 400 mg twice daily in combination with paclitaxel at 225 mg/m^2 and carboplatin at area under the curve [AUC] of 6 mg min mL^{-1} in a dose-escalation manner [16]. Based on this result, the starting doses of the present study were decided as follows; Paclitaxel (200 mg/m^2, infused over 3 h) and carboplatin (AUC 6 mg min mL^{-1} during infusion for 30 min) were administered consecutively on day 1, and sorafenib (400 mg, twice daily) was administered for 18 days starting on day 2. There was a concern that sorafenib may inhibit cytochrome P450 enzymes responsible for the clearance of paclitaxel. Based on this possible pharmacokinetic interaction and antagonistic effects, sorafenib administration was discontinued for two days (days 20 and 21) before the next administration of paclitaxel in both the present study and the other phase I trial [16]. This treatment cycle was repeated every 21 days until unacceptable toxicity, tumor progression, or death occurred. Carboplatin-paclitaxel chemotherapy was not allowed to exceed six cycles, after which sorafenib administration could continue until the occurrence of intolerable toxicity, disease progression. If fewer than two of the first six patients experienced dose limiting toxicity (DLT) in the first cycle, the dose level was to be recommended for subsequent clinical trials and an additional six patients were to be enrolled to the cohort.

Patient evaluation

All observations pertinent to the safety of sorafenib were recorded, including results of physical examinations, vital signs, adverse events, use of concomitant medications, and laboratory test data. Patients were routinely monitored for adverse events, which were recorded with severity and relation to study medication according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Assessment of the chest and abdomen for tumors was performed radiologically (computed tomography or magnetic resonance imaging) according to the Response Evaluation Criteria in Solid Tumors (RECIST) [20]. The same radiological method was performed to maintain consistency of evaluation. Patients for whom antitumor efficacy (complete or partial response)

was observed or who had stable disease were continuously treated according to the study protocol. Measurements were repeated in patients with a complete or partial response at a time more than 4 weeks after the response criteria were first met in order to confirm tumor response according to RECIST.

Pharmacokinetics

To investigate the effect of paclitaxel-carboplatin on the pharmacokinetics of sorafenib, we collected blood samples on days 2 and 19 of treatment cycle 1 for cohort 1 and determined the plasma concentration of sorafenib. On both days, samples were collected at 0 h (pre–morning dose of sorafenib); at 0.5, 1, 3, 6, and 12 h (pre–evening dose); and at 24 h (pre–morning dose on day 3). After dosing on day 19, additional samples were collected at 48 and 72 h (before infusion of paclitaxel in cycle 2). The evening dose of sorafenib was not administered on day 19 of cycle 1 for the purpose of pharmacokinetic sampling. As a result of amendment to the treatment protocol for cohort 2, a modified schedule of blood sampling was adopted. For determination of the plasma concentration of sorafenib, blood samples were collected at the same time points in cycle 2 as in cycle 1, with the exception that the blood sample obtained at 12 h after the morning administration of sorafenib on day 2 was collected before the evening dose on day 2 in cohort 2. The concentration of sorafenib in plasma samples was determined with the use of a validated high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay.

To investigate the effects of sorafenib on the pharmacokinetics of paclitaxel and carboplatin, we collected blood samples on day 1 of cycle 1 for cohort 1 and determined the plasma concentrations of carboplatin, paclitaxel, and the paclitaxel metabolite 6-hydroxy-paclitaxel. Samples were collected at 0 h, 1.5 h (during paclitaxel infusion), 3 h (within 5 min before completion of paclitaxel infusion), 3.5 h (within 5 min before completion of carboplatin infusion), as well as 4, 5, 7, 11, 24, and 48 h. The amended treatment protocol for cohort 2 was accommodated by collection of blood samples immediately before, 1.5 h after the start of, within 5 min before completion of, as well as 0.5, 1, 2, 4, 8, 21, and 45 h after completion of paclitaxel infusion on day 1 of cycles 1, 2, and 3 for paclitaxel, and immediately before, within 5 min before completion of, as well as 0.5, 1, 3, 7, 20, 31, and 44 h after completion of carboplatin infusion on day 1 of cycles 1, 2, and 3 for carboplatin. The plasma concentrations of free (unbound) platinum derived from carboplatin, of paclitaxel, and of 6-hydroxy-paclitaxel were measured with the use of atomic absorption spectrophotometry and were validated by LC-MS/MS assays.

Pharmacokinetic parameters, including the AUC, maximum concentration (C_{max}), and elimination half-life ($t_{1/2}$), for sorafenib, paclitaxel, and carboplatin were calculated by noncompartment analysis as previously described [17].

Results

Patient demographics

A total of 13 chemonaïve patients with advanced NSCLC was enrolled in the study, six in cohort 1 and seven in cohort 2. The baseline demographics for all patients are shown in Table 1. Histological diagnosis revealed that the most common histology was adenocarcinoma (eight patients, or 61.5%), followed by large cell carcinoma and squamous cell carcinoma (each with two patients, or 15.4%).

DLT

Table 2 summarizes the dosing regimens for evaluated cohorts together with DLTs. The first six patients enrolled in cohort 1 were treated with 400 mg of sorafenib twice daily (days 2 to 19) combined with paclitaxel at 200 mg/m^2 and carboplatin at an AUC of 6 mg min mL^{-1} (30-min infusion). Four of these six patients experienced DLTs during the first cycle of treatment (two with erythema

Table 1 Patient demographics

	No. of patients
Total enrolled	13
Cohort 1	6
Cohort 2	7
Age (years)	
Median	66
Range	41–76
Sex	
Male	9
Female	4
ECOG performance status	
0	4
1	9
Disease stage	
IV	13
Histology	
Adenocarcinoma	8
Large cell carcinoma	2
Squamous cell carcinoma	2
Undifferentiated carcinoma	1

ECOG Eastern Cooperative Oncology Group

Table 2 Observed DLTs according to dose level

Cohort	Paclitaxel (mg/m^2)	Carboplatin ($mgminmL^{-1}$)	Sorafenib (mg)	No. of patients	No. of patients with DLTs	DLTs
1	200	6	400 twice daily	6	4	Erythema multiforme, grade 3 ($n=2$)
						Hand-foot skin reaction, grade 3 ($n=1$)
						ALT elevation, grade 3 ($n=1$)
2 (cycle 1)	200	5	400 once daily	7	0	None
2 (cycle 2)	200	5	400 twice daily	7	2	Perforation, GI, small bowel NOS, grade 3 ($n=1$)
						Infection-lung (pneumonia) of grade 3 with neutrophil of grade 4 ($n=1$)

DLTs dose-limiting toxicities, *ALT* alanine aminotransferase, *GI* gastrointestinal, *NOS* not otherwise specified

multiforme of grade 3, one with a hand and foot skin reaction of grade 3, and one with elevation of plasma alanine aminotransferase [ALT] of grade 3). One of the patients diagnosed with erythema multiforme developed a rash of grade 1 on the arms, thigh, and hip on day 5; by day 15, the rash had spread to the entire body with development of pruritus (grade 3), and histopathologic analysis of skin biopsy specimens revealed superficial dermal vasodilation as well as perivascular lymphocyte and plasma cell infiltration, consistent with erythema multiforme (Fig. 1a, b). The second patient also developed a localized rash of grade 1 that appeared in the right lower part of the abdomen on day 5 and had spread to the entire body with the development of a high fever on day 12; histopathologic analysis of skin biopsy specimens again supported a diagnosis of erythema multiforme. Both patients responded well to steroid therapy and improved.

Given that the incidence of DLT at the adopted dose level exceeded that predefined for the maximum tolerated dose, a modified dose level consisting of 400 mg of sorafenib once daily (days 2 to 19) combined with paclitaxel at 200 mg/m^2 and carboplatin at an AUC of 5 mg min mL^{-1} (60-min infusion) was evaluated for the seven additional patients of cohort 2. None of these seven patients experienced DLT during cycle 1. Intrapatient escalation of sorafenib dose was allowed if the patient did not experience DLT in cycle 1 of cohort 2; the dose of sorafenib was thus increased to 400 mg twice daily from day 2 to day 19 in subsequent courses. Among the seven patients who received sorafenib at 400 mg twice daily combined with paclitaxel (200 mg/m^2) and carboplatin (AUC of 5 mg min mL^{-1}), two individuals developed DLT: one a perforation of the small bowel of grade 4 and one pneumonia of grade 3. The patient with gastrointestinal perforation, who had metastases in the left adrenal gland and small intestine, developed abdominal pain, fever, and peritonitis 26 days after initiation of sorafenib at 400 mg twice daily and required emergency surgery. He recovered after surgery, and pathological examination of the surgical specimen confirmed the presence of tumor cells at the site of perforation. Given the marked tumor response of the patient on radiographic examination, the perforation event was likely associated with the antitumor effect of the study treatment.

Safety

All 13 enrolled patients were evaluable for safety analysis. Treatment-emergent adverse events (Table 3) occurred in all patients, the most common being hematologic or dermatologic in nature, sensory neuropathy, anorexia, and nausea. Neutropenia of grade 4 occurred in nine (69%) patients (four in cohort 1 and five in cohort 2). Hand-foot skin reaction occurred in five patients (three in cohort 1 and two in cohort 2), hypertension in four patients (two in cohort 1 and two in cohort 2), elevated plasma lipase in four patients (three in cohort 1 and one in cohort 2), and erythema multiforme in three patients (two in cohort 1 and one in cohort 2).

Antitumor activity

Tumor response was evaluated in 12 of the 13 patients (Fig. 2), with the remaining patient in cohort 2 not being available for assessment of such response. One patient in cohort 1 had a confirmed complete response, and six patients (three in each cohort) had a confirmed partial response; the overall response rate was thus 58% (95% confidence interval of 28 to 85%). Five patients, two in cohort 1 and three in cohort 2, had stable disease. Cavitation of lung lesions was observed in one patient (Fig. 1c, d). The median time to disease progression was 5.7 months (95% confidence interval of 4.3 to 20.1 months).

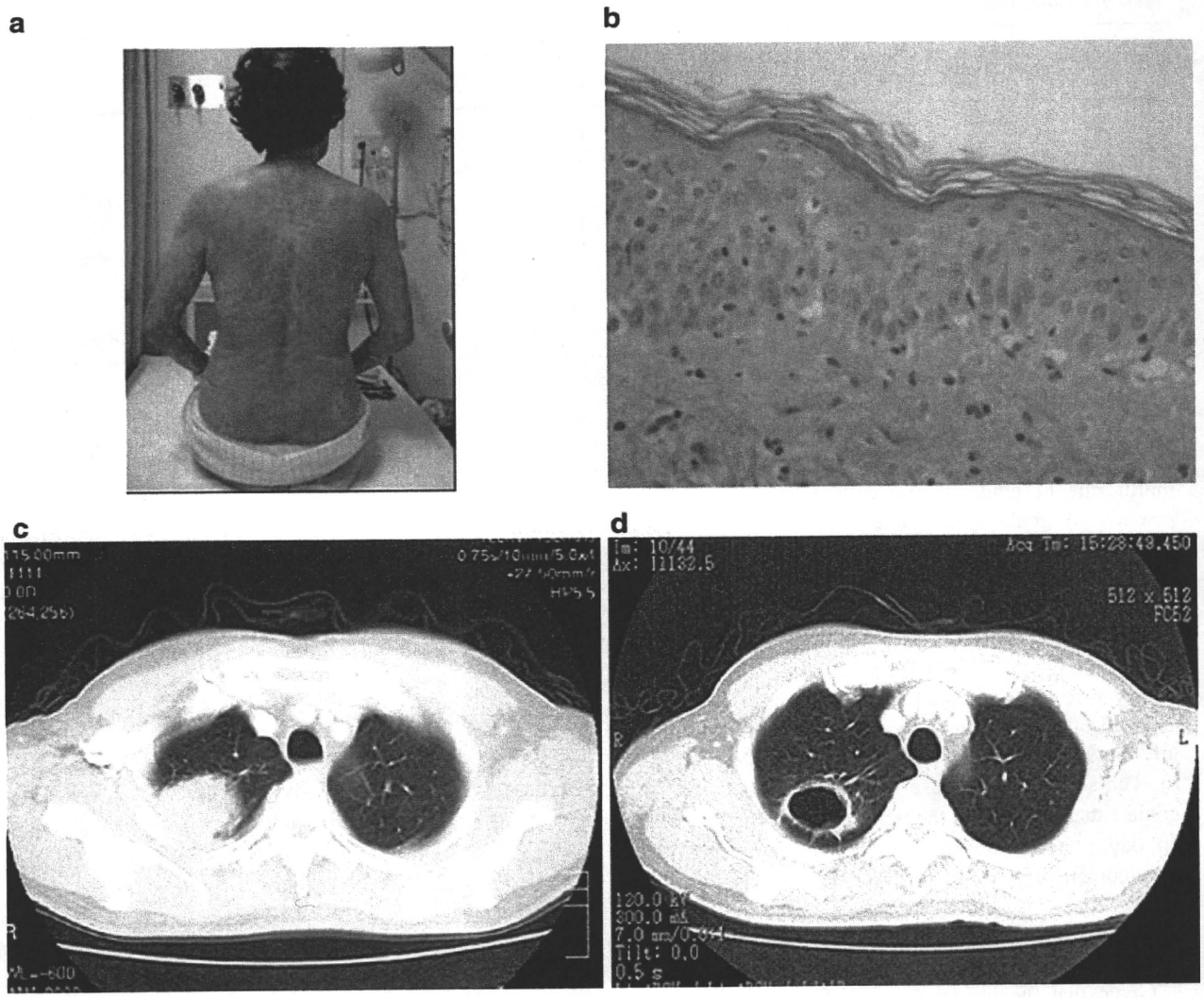

Fig. 1 Development of erythema multiforme and tumor cavitation in patients with advanced NSCLC treated with sorafenib in combination with carboplatin-paclitaxel. **a** A rash, initially localized to the arms, thigh, and hip, spread to the entire body. **b** Hematoxylin-eosin staining of a skin lesion from the patient shown in (**a**) revealed infiltration of inflammatory cells, mostly lymphocytes, around superficial dermal blood vessels and the epidermal-dermal junction. Liquefaction degeneration in basal epidermal layers and cavernous transformation in part of the epidermal squamous cell layer were also observed. **c**, **d** Computed tomography revealed a solid tumor without cavitation in the right lung of a patient at baseline (**c**), whereas the same tumor showed marked central cavitation on day 19 of cycle 1 (**d**)

Pharmacokinetics

Pharmacokinetic analysis for sorafenib in the presence of paclitaxel and carboplatin (Table 4) was based on the patients in cohort 1 (cycle 1) and cohort 2 (cycles 1 and 2) after administration of a single dose (day 2) or multiple doses (day 19). The increases in mean C_{max} from days 2 to 19 were consistent with those in mean AUC_{0-12}, likely reflecting the long mean $t_{1/2}$ (20.4 to 26.8 h on day 19). In cohort 2, the increases in the mean values of AUC_{0-12} and C_{max} in cycle 2 (400 mg, twice daily) compared with those in cycle 1 (400 mg, once daily) were consistent with the increase in sorafenib dosing. At steady state, after multiple administrations of sorafenib at 400 mg twice daily together with paclitaxel and carboplatin, the mean values of AUC_{0-12} and C_{max} in cohort 1 (cycle 1, day 19) were 31.3 mg h L^{-1} and 4.6 mg/L, respectively, and those in cohort 2 (cycle 2, day 19) were 39.1 mg h L^{-1} and 5.9 mg/L, respectively.

Given that treatment was discontinued after cycle 1 in four of the six patients in cohort 1, the effects of multiple doses of sorafenib on the pharmacokinetics of paclitaxel and carboplatin were evaluated in cohort 2. Pharmacokinetic analysis for paclitaxel and carboplatin was performed during cycle 1 before sorafenib administration and during cycles 2 and 3 after sorafenib administration (Table 4). Small increases in the mean AUC and C_{max} values for

Table 3 Numbers of patients with treatment-emergent adverse events including those with a CTCAE worst grade of 3 or 4

Event category	CTCAE term	Cohort 1 (n=6)			Cohort 2 (n=7)		
		CTCAE grade			CTCAE grade		
		Any	3	4	Any	3	4
Allergy/immunology	Allergic reaction	2			2		1
Blood/bone marrow	Hemoglobin	2			5	3	
	Leukocytes	5	4		6	3	1
	Lymphopenia	2	2		5	3	1
	Neutrophils	5		4	7		5
	Platelets	3			5	2	1
Cardiac, general	Hypertension	2			2	1	
Constitutional symptoms	Weight loss	1			4	1	
Dermatology/skin	Erythema multiforme	2	2		1		
	Hand-foot skin reaction	3	1		2		
	Rash/desquamation	4			5		
Gastrointestinal	Anorexia	5			6	3	
	Dehydration				2	1	
	Nausea	4			5	1	
	Perforation, GI, small bowel NOS				1		1
Infection	Febrile neutropenia	1	1				
	Infection with G4 neutrophils, lung (pneumonia)				1	1	
Metabolic/laboratory	ALT	3	1	1	1		
	AST	2	1		1		
	Hypokalemia				1	1	
	Hyponatremia				2	2	
	Hypophosphatemia	4	2				
	Lipase	3	2		1		
Neurology	Neuropathy, motor				1	1	
	Neuropathy, sensory	4			6	2	
Pulmonary/upper respiratory	Dyspnea	1			1	1	

CTCAE Common Terminology Criteria for Adverse Events, *GI* gastrointestinal, *NOS* not otherwise specified, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

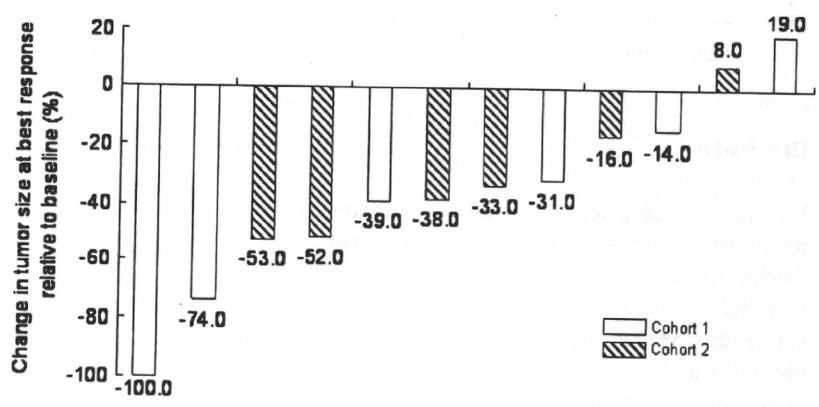

Fig. 2 Tumor response. Ten of the 12 evaluable patients showed tumor shrinkage, with one individual manifesting a complete response (−100%)

Table 4 Pharmacokinetic analysis

Sorafenib	Cohort 1		Cohort 2			
	Cycle 1		Cycle 1		Cycle 2	
	Day 2 400 mg sd (n=6)	Day 19 400 mg bid (n=3)	Day 2 400 mg sd (n=7)	Day 19 400 mg od (n=7)	Day 2 400 mg sd (n=6)	Day 19 400 mg bid (n=4)
AUC_{0-12} (mg h L^{-1})	18.2 (74)	31.3 (32)	9.0 (82)	24.4 (25)	14.6 (25)	39.1 (51)
C_{max} (mg/L)	2.5 (96)	4.6 (36)	1.2 (93)	3.2 (22)	2.0 (21)	5.9 (38)
$t_{1/2}$ (h)		20.4 (18)		26.8 (41)		23.9 (29)

Paclitaxel	Cohort 2		
	Cycle 1 (n=7)	Cycle 2 (n=6)	Cycle 3 (n=4)
AUC (mg h L^{-1})	27889.1 (36)	29538.6 (23)	34712.8 (51)
Ratio [90% CI]		1.05 [0.88–1.25]	1.26 [1.02–1.55]
C_{max} (mg/L)	8016.5 (53)	10076.4 (18)	11218.8 (65)
Ratio [90% CI]		1.19 [0.80–1.77]	1.39 [0.88–2.21]
$t_{1/2}$ (h)	10.7 (10)	11.1 (6)	11.4 (3)

Free platinum	Cohort 2		
	Cycle 1 (n=7)	Cycle 2 (n=6)	Cycle 3 (n=4)
AUC (mg h L^{-1})	44.9 (23)	44.4 (25)	38.5 (10)
Ratio [90% CI]		1.00 [0.91–1.10]	0.90 [0.80–1.00]
C_{max} (mg/L)	17.5 (36)	17.4 (34)	17.5 (9)
Ratio [90% CI]		0.92 [0.82–1.02]	0.97 [0.85–1.11]

Pharmacokinetic parameters are presented as geometric means (% coefficient of variation). Ratios for AUC and C_{max} values of paclitaxel and free platinum are dose-adjusted ratios in cycles 2 or 3 relative to those in cycle 1

sd single dose, *od* once daily, *bid* twice daily, *CI* confidence interval

paclitaxel were observed with progress of the cycles; however, these changes were not significant based on the inclusion of 1.00 in the 90% confidence interval for the ratio of AUC or C_{max} in cycles 2 or 3 to the corresponding value in cycle 1. Similar results were obtained for 6-hydroxy-paclitaxel (data not shown). There were also no significant differences in the mean AUC or C_{max} values of free platinum when standard chemotherapy was administered with or without sorafenib.

Discussion

We have investigated the effects of sorafenib, an oral multikinase inhibitor, in combination with standard chemotherapy (paclitaxel and carboplatin) in chemonaïve individuals with advanced NSCLC. Our results show that sorafenib can be integrated with the combination of paclitaxel and carboplatin. In the present study, the dose of carboplatin had to be capped one dose level lower (AUC of 5 mg min mL^{-1}) than is typical for administration of paclitaxel and carboplatin alone, because four out of six patients developed DLTs in cohort 1.

Two of the patients with DLTs in cohort 1 experienced erythema multiforme of grade 3. Previous studies have reported that most patients receiving sorafenib as monotherapy manifested dermatologic toxicities, mostly of grade 1 or 2, including rash or desquamation (18 to 66%), hand-foot syndrome (25 to 62%), and alopecia (18 to 53%) [15, 21, 22]. Erythema multiforme was reported to occur in only 0.1 to <1% of patients [22, 23]. In the two cases of erythema multiforme in the present study, skin rashes occurred within a week after initiation of sorafenib treatment and spread to the entire body without organ dysfunction. Histopathologic examination of skin specimens supported the diagnosis of erythema multiforme. Steroid treatment and discontinuation of sorafenib resulted in marked improvement of the patients within days. A drug lymphocyte stimulation test was performed for both patients, with the results being positive for sorafenib and