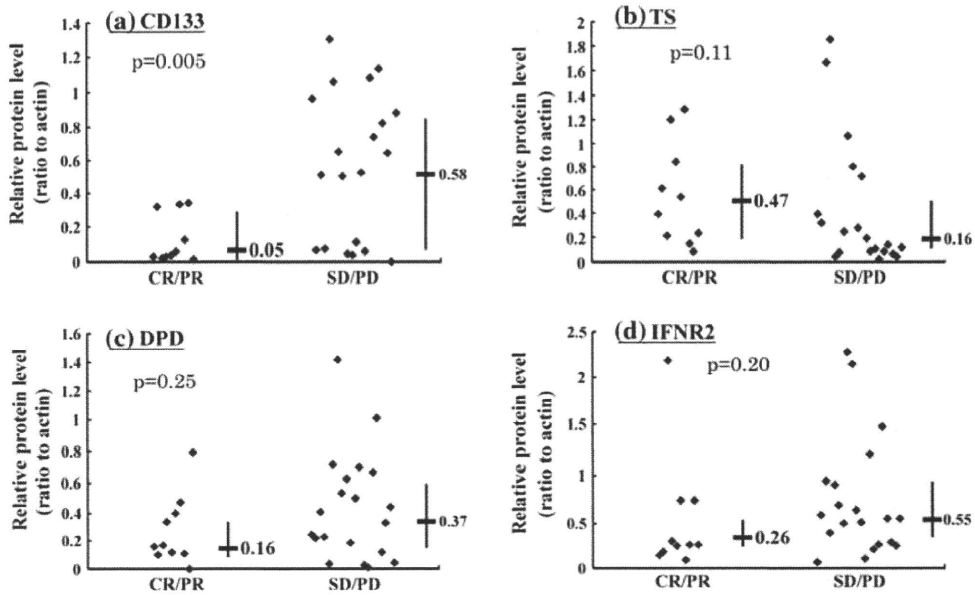

Fig. 2 Relationships between the expression levels of CD133 (**a**), TS (**b**), DPD (**c**), and IFNR2 (**d**) and the anticancer effect. *Vertical lines* on the right of these figures represent the quartile positions, and *horizontal lines* indicate the medians. The numbers of subjects with complete response (*CR*)/PR and stable disease (*SD*)/PD were ten and twenty, respectively

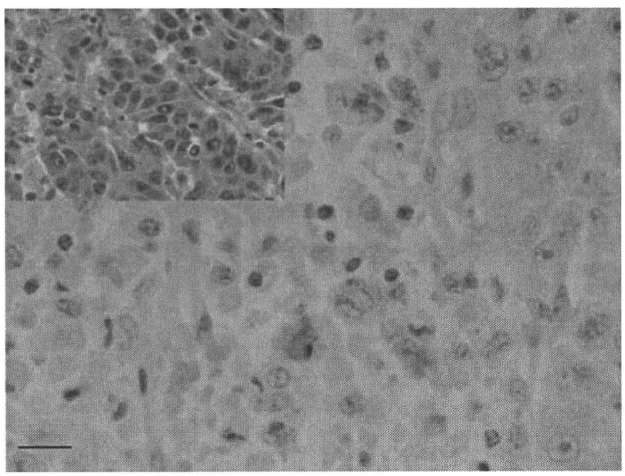

Fig. 3 Immunohistochemistry of CD133 in HCC tissue. This typical sample was intensely positive for CD133. Detection was facilitated using diaminobenzidine (DAB), which shows brown staining when positive. Magnification ×400. *Inset* image is H&E-stained specimen (magnification ×200)

The expression of CD133 was also studied in liver cancer tissues of all specimens using immunohistochemistry. Figure 3 presents specimens that showed intense staining for CD133. The presence or absence of an immunohistological signal was correlated with the CD133 protein expression level determined by Western blotting.

Comparison of the patient background and candidate factors predicting treatment effects with the final outcome (results of univariate analysis and multivariate analysis with a logistic regression model)

Univariate analysis showed a significantly higher CD133 level ($p < 0.01$) in the nonresponder (NR) than the responder group and a slightly higher TS level ($p = 0.11$) in the responder group (Table 3). Using CD133 and TS showing $p < 0.15$ on univariate analysis, multivariate analysis with a logistic regression model was performed. This analysis revealed that only CD133 was a significant factor (odds ratio 0.076, 95% CI 0.007–0.88, $p = 0.039$) (Table 4). Thus, irrespective of the TS level, CD133 was identified as an independent factor predicting the treatment effects.

Relationships of the CD133 protein expression level with the anticancer effect, PFS, and OS

The positive predictive value for nonresponders was 100% in patients whose CD133 expression level exceeded 0.4. Thus, we classified patients into high- and low-CD133 expression groups with a cutoff level of 0.4 (Fig. 2a). Thirteen patients were classified into the high-CD133 expression group and 18 patients into the low-CD133 expression group.

The relationship between the CD133 protein expression level and PFS is shown in Fig. 4. The median PFS was 1.6 months (95% CI 1.5–1.6 months) in the high-CD133 expression group and 7.2 months (95% CI 2–12.3 months) in the low-CD133 expression group. The log-rank test using the Kaplan–Meier method showed that the PFS was significantly prolonged in the low-level compared to the high-level CD133 expression group ($p = 0.036$).

The relationship between the CD133 protein expression level and overall survival (OS) is shown in Fig. 5. The median survival time was 3.1 months (95% CI 1.4–4.8 months) in the high-CD133 expression group, but 8.1 months (95% CI 0–19.3 months) in the low-CD133 expression group. The log-rank test using the Kaplan–Meier

Table 3 Comparison of patient characteristics according to the anticancer effect of the therapy

Variable	Responders (PR) ($n = 10$)	Nonresponders (SD + PD) ($n = 20$)	p value
Age (years)	64.5 (30–80)	65.5 (35–80)	0.85
Sex, no. (%)			0.25
Male	8 (80)	19 (95)	
Female	2 (20)	1 (5)	
Cause of disease, no. (%)			0.21
Hepatitis B	3 (30)	7 (35)	
Hepatitis C	3 (30)	11 (55)	
Non-B, non-C	4 (40)	2 (10)	
ECOG performance status, no. (%)			0.53
0	10 (100)	17 (85)	
1	0 (0)	3 (15)	
Tumor grade, no. (%)			0.21
Moderate	9 (90)	13 (65)	
Poor	1 (10)	7 (35)	
Vascular invasion, no. (%)	6 (60)	11 (55)	1
AFP (ng/ml), median (range)	409.5 (3–114,852)	560.5 (6–13,544)	1
PIVKA II (mAU/ml), median (range)	321.5 (16–61,319)	3,177.5 (16–116,140)	0.17
Previous therapy (last), no. (%)			0.83
OP	2 (20)	4 (20)	
TACE	2 (20)	7 (35)	
HAIC	2 (20)	2 (10)	
None	4 (40)	7 (35)	
CD133, median (range)	0.05 (0.01–0.34)	0.58 (0–1.30)	**<0.01**
IFNR2, median (range)	0.26 (0.09–2.19)	0.55 (0.06–2.27)	0.2
TS, median (range)	0.47 (0.08–1.29)	0.16 (0.03–1.85)	**0.11**
DPD, median (range)	0.16 (0–0.79)	0.37 (0–1.42)	0.25
p53 mutation, no. (%)	1 (10)	2 (10)	1

Values in bold are statistically significant

PR partial response, *SD* stable disease, *PD* progressive disease, *ECOG* Eastern Cooperative Oncology Group, *AFP* alpha-fetoprotein, *TACE* transcatheter arterial chemoembolization, *HAIC* hepatic arterial infusion chemotherapy using implanted port system, *IFNR2* interferon-receptor 2, *TS* thymidylate synthase, *DPD* dihydropyrimidine dehydrogenase, *PIVKA II* protein induced by vitamin K antagonist II

Table 4 Multivariate analysis with a logistic regression model

	Odds ratio	95% CI	p value
CD133 > 0.34	0.076	0.007–0.88	0.039
TS > 0.2	1.643	0.189–14.29	0.653

Cutoff value for each factor was determined by receiver operating characteristic curve (ROC) analysis

CI confidence interval

method showed that, in the low-level CD133 expression group, the OS was significantly prolonged compared to that in the high-level group ($p = 0.022$).

Relationship between p53 mutations and the anticancer effect

Mutant p53 was observed in 3 of the 31 patients. The response rate was 32.1% in the wild-type and 33.3% in the mutant specimens, with no significant difference. The disease control rate was 39.3% in the wild-type and 66.7% in the mutant specimens, with no significant difference.

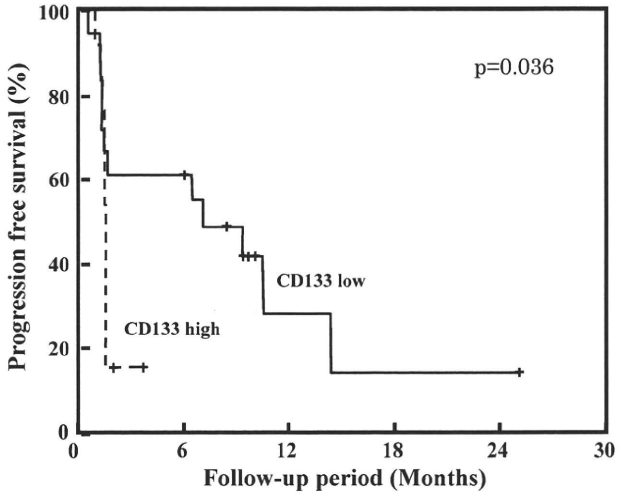

Fig. 4 Progression-free survival of patients who received combination therapy with S-1 and pegylated interferon (PEG-IFN) α-2b, stratified according to the CD133 expression level. Patients were divided into high- and low-CD133 expression groups, with a cutoff value of 0.4 (Fig. 2a). Thirteen and 18 patients belonged to the high- and low-expression groups, respectively

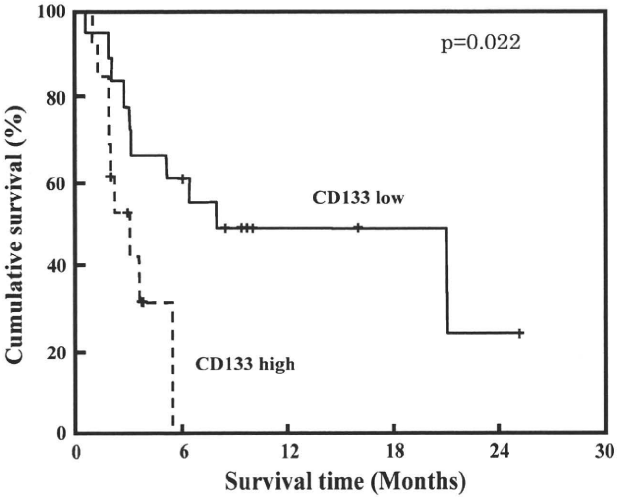

Fig. 5 Kaplan–Meier curve of overall survival in patients treated with combination therapy of S-1 and PEG-IFN α-2b, stratified according to the CD133 expression level

Toxicity

NCI-CTC grade 3 leukocytopenia, neutropenia, anemia, and thrombocytopenia were observed in 2 (6%), 2 (6%), 1 (3%), and 3 (10%) of the 31 patients, respectively. Grade 3 anorexia, stomatitis, rash, and fatigue were observed in 1 (3%), 1 (3%), 1 (3%), and 2 (6%) of the 31 patients, respectively. All adverse effects were alleviated when the treatment was discontinued, leading to no cases of mortality.

Discussion

Here we sought to identify factors predicting the therapeutic effect of S1+ PEG-IFN α-2b therapy in patients with advanced HCC. We collected pathological samples of HCC from all registered patients and studied proteins considered to be related to the therapeutic effect. The expression level of CD133 was significantly correlated with the therapeutic effect, but the expression levels of TS, DPD, and IFNR2, and the presence or absence of p53 mutations were not.

CD133 is a glycoprotein with five transmembrane regions and is a known blood stem-cell marker [28]. It also reportedly acts as a leukemia [15], brain [16], and colon cancer [17, 18] stem-cell marker. The characteristics of cancer stem cells include an ability to proliferate (self-replication capacity) and to differentiate into several cell types with different functions (multidifferentiation capacity), as well as a tumorigenic capacity, which was verified as tumor reproducibility in an experiment involving tumor implantation in an animal model [29–31]. In HCC, CD133-positive cells reportedly possess each of these cancer stem-cell characteristics. In 2007, Ma et al. [32] showed that 65–95% of cells in multiple HCC cell lines were CD133-positive, and Suetsugu et al. [33] reported that the HCC cell line Huh7 expressed CD133. In addition, Song et al. [34], who evaluated 60 patients with HCC, reported both significantly longer postoperative recurrence-free survival and total survival periods in a group with a low compared to that with a high CD133 level. There have been a few such reports on CD133 as a marker of postoperative recurrence.

In the present study, the CD133 protein expression level was significantly lower in the responder group than that in the nonresponder group. HCC showing high-level CD133 expression was resistant to the combination therapy used in this study. Several studies have suggested that the most cancer stem cells exist in the G_0 phase, and a reduced cell cycle velocity is involved in the drug resistance of cancer stem cells [35]. Furthermore, these cells are resistant to reactive oxygen-induced DNA damage because of their high radical-scavenging activities [36]. Furthermore, the drug resistance mechanism of cancer stem cells may also involve ATP binding cassette (ABC) transporters [16, 37]. The anti-apoptotic factors Akt/PKB and Bcl-2 are also activated in CD133-positive liver cancer cells by 5FU administration [38]. Because the anticancer effect of S1+ PEG-IFN α-2b therapy is primarily derived from apoptosis, the activation of Akt/PKB and Bcl-2 is considered to be directly related to the resistance to this therapy.

TS is a rate-regulating enzyme involved in the synthesis of deoxythymidine monophosphate, which is indispensable for DNA synthesis. Therefore, the anticancer effect of 5FU decreases when the TS expression level in the tumor is high, because the drug cannot sufficiently inhibit the enzyme [39]. In the present study, the TS expression level did not correlate with the therapeutic effect. Oie et al. [40] reported that TS expression was suppressed by IFN administration in all the HCC-derived cell lines they examined. Although we did not evaluate the TS expression level after IFN administration, the absence of a correlation between the TS expression level and the therapeutic effect may have been due to the inhibition of TS by IFN.

DPD is a 5FU-degrading enzyme present primarily in the liver. 5FU efficiency increases with low DPD expression in tumor cells [41]. In our study, no correlation between the DPD expression level and therapeutic effect was noted. This was an expected result, because S1 contains a DPD inhibitor.

IFNR, and particularly IFNR2, is considered to be the most important IFN-binding unit for IFN activity. IFNR2 is reportedly expressed in 61–77% of HCCs [42, 43], and its anticancer effect increases with its level of expression. In our study, IFNR2 expression did not correlate with the therapeutic effect. When multiple HCC cell lines were

treated with IFN-α in vitro, a relationship between IFNR2 expression and the anticancer effect was demonstrated [23]. Therefore, IFNR2 is undoubtedly important in the direct anticancer effect of IFN. However, indirect anticancer effects of IFN, such as the activation of natural killer cells and cytotoxic lymphocytes, must also be considered in regard to in vivo treatment [44–46]. Such indirect actions may be primarily responsible for the anticancer effects of IFN in some patients. Regardless of these findings, the IFNR2 expression level is not considered useful for the prediction of the therapeutic effect.

p53 is a typical tumor suppressor gene that may arrest the cell cycle and induce DNA repair or promote apoptosis, depending on the degree of DNA damage [47]. However, the mutation of p53 at some sites causes loss of its original function, allowing the initiation of tumor growth and acceleration of tumor proliferation [48]. In our study, no correlation was noted between the presence or absence of p53 mutations and the therapeutic effect. This may have been because there were only three patients with mutant p53, or because the mutations occurred at sites that do not affect p53 function.

In conclusion, S1+ PEG-IFN α-2b therapy can be a second-line treatment option for patients with advanced HCC, especially in those that show low CD133 expression. Further evaluation with a prospective randomized trial is necessary to confirm the results of the present study.

Acknowledgments The protocol of this study was approved by the Medical Committee of Kinki University of Medicine.

Conflict of interest None.

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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

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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

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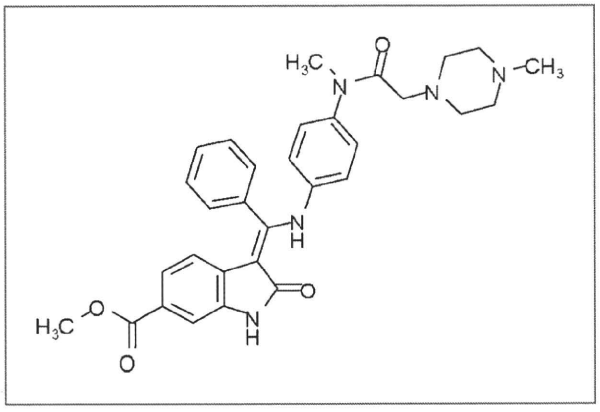

Figure 1. Structure of BIBF 1120.

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.

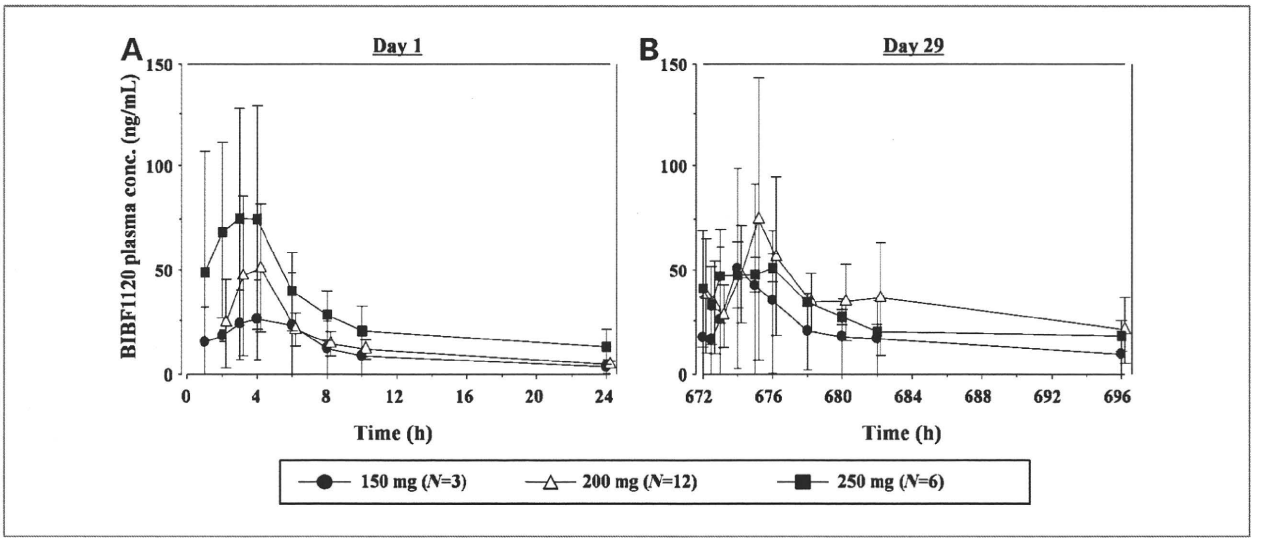

Figure 2. Mean (± SD) plasma concentration–time profiles of BIBF 1120 after single (A; day 1) and multiple (B; day 29) administration of 150, 200, and 250 mg BIBF 1120 twice daily.

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.

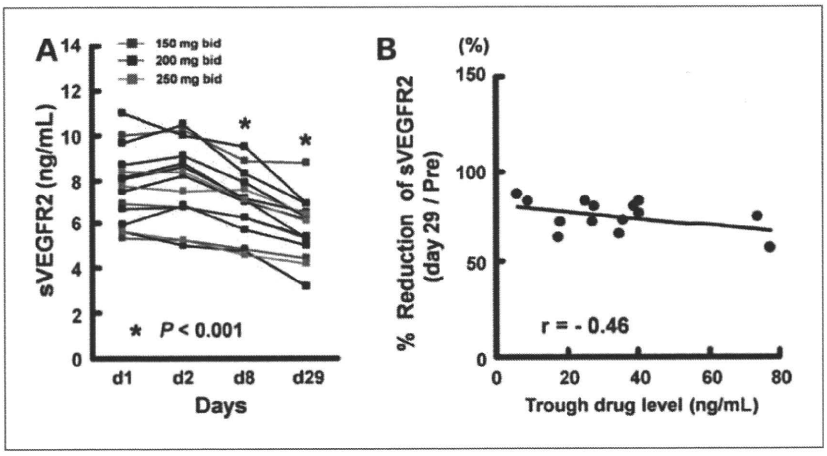

Figure 3. sVEGFR2 levels in plasma after BIBF 1120 treatment. A, plasma sVEGFR2 levels decreased during the 4-week treatment period. B, the decrease in sVEGFR2 at cycle 1, day 29 showed a modest inverse correlation with trough plasma drug levels of BIBF 1120 (r = −0.46).

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

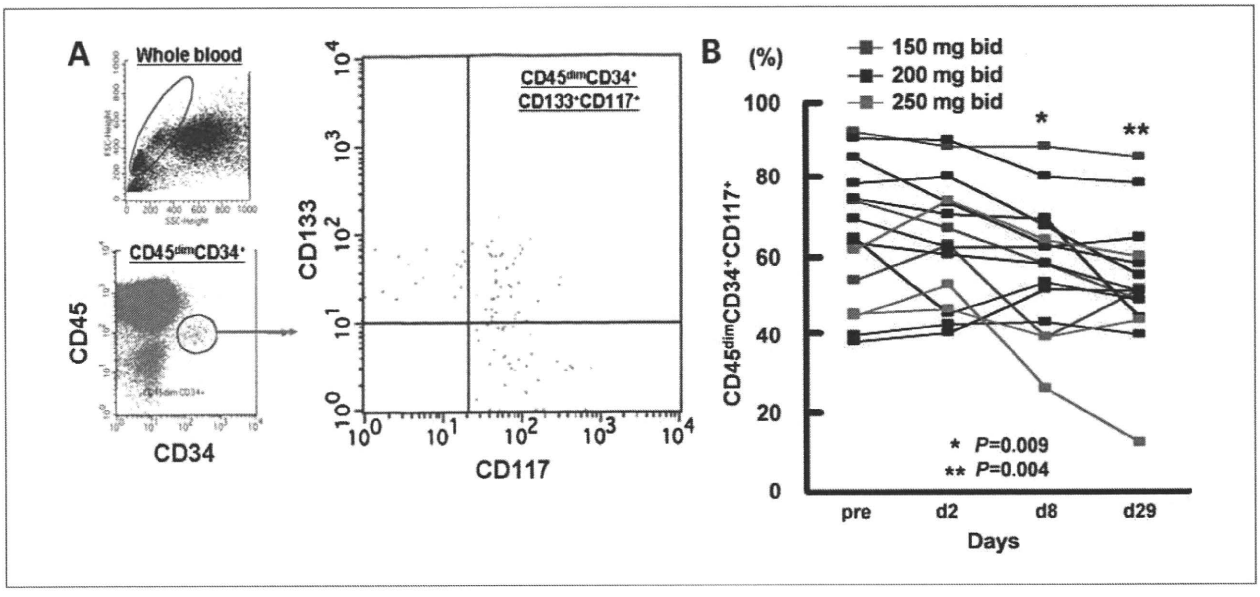

Figure 4. Levels of circulating CD117-BMD progenitor cells after BIBF 1120 treatment. A, representative flow cytometric analysis for determining the number of CD117-positive–BMD progenitor cells defined as $CD45^{dim}CD34^{+}CD117^{+}$. B, circulating levels of $CD45^{dim}CD34^{+}CD117^{+}$ cells decreased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

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Cisplatin and Etoposide as First-line Chemotherapy for Poorly Differentiated Neuroendocrine Carcinoma of the Hepatobiliary Tract and Pancreas

Satoru Iwasa[1], Chigusa Morizane[1]*, Takuji Okusaka[1], Hideki Ueno[1], Masafumi Ikeda[2], Shunsuke Kondo[1], Tsutomu Tanaka[1,3], Kohei Nakachi[2], Shuichi Mitsunaga[2], Yasushi Kojima[2], Atsushi Hagihara[1] and Nobuyoshi Hiraoka[4]

[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, [2]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital East, Chiba, [3]Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya and [4]Pathology Division, National Cancer Center Research Institute, Tokyo, Japan

*For reprints and all correspondence: Chigusa Morizane, Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: cmorizan@ncc.go.jp

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Objective: The combination chemotherapy consisting of cisplatin and etoposide, one of the standard regimens for small cell lung cancer, has been widely used to treat extrapulmonary poorly differentiated neuroendocrine carcinomas. However, there were no prior reports limited to the hepatobiliary tract and pancreas as the primary sites.

Methods: We reviewed the cases in our database from October 1995 to January 2009 and retrospectively examined the clinical data of patients, with unresectable or recurrent poorly differentiated neuroendocrine carcinoma arising from the hepatobiliary tract and pancreas, who received combination chemotherapy with cisplatin and etoposide as the first-line treatment. The chemotherapy regimen consisted of cisplatin 80 mg/m^2 given intravenously on day 1 and etoposide 100 mg/m^2 intravenously on days 1–3, repeated every 3–4 weeks.

Results: Twenty-one patients were treated with the above regimen of cisplatin and etoposide combination chemotherapy. The primary tumor site was the liver in 2 patients, gallbladder in 8 patients, pancreas in 10 patients and ampulla of Vater in 1 patient. Although no complete responses were obtained, three patients had partial responses, resulting in an overall response rate of 14%. Median progression-free survival was 1.8 months, and median overall survival was 5.8 months. The major adverse events were myelosuppression and gastrointestinal toxicities, with Grade 3 or 4 neutropenia (90%), nausea (33%) and anorexia (24%).

Conclusions: Cisplatin and etoposide combination as the first-line chemotherapy for hepatobiliary or pancreatic poorly differentiated neuroendocrine carcinoma had only marginal antitumor activity and relatively severe toxicity compared with previous studies on extrapulmonary poorly differentiated neuroendocrine carcinoma treated with the same regimen.

Key words: cisplatin – etoposide – neuroendocrine carcinoma – chemotherapy

INTRODUCTION

Neuroendocrine tumors are rare tumors that exhibit a variety of morphologic, functional and behavioral characteristics (1). The aggressiveness of these tumors varies greatly depending on the histological degree of differentiation, from well-differentiated neuroendocrine tumors to poorly differentiated neuroendocrine carcinomas (PD-NECs).

No standard treatment for unresectable extrapulmonary PD-NECs has been established yet. However, combined chemotherapy with cisplatin and etoposide, one of the standard regimens employed for the treatment of small cell lung cancer (SCLC), has been used widely for the treatment of extrapulmonary PD-NECs, because the genetic, pathological and clinical features of PD-NECs overlap with those of SCLC (2–6). The previous reports, in general, refer to a

wide variety of extrapulmonary sites of origin of the primary tumors, partly because the rarity of the disease precludes clinical studies devoted to each individual primary origin of the tumors. Thus, there have been no prior reports of treatment limited to neuroendocrine tumors arising from the hepatobiliary and pancreatic region as primary sites.

It is well established that adenocarcinomas arising from the hepatobiliary tract or pancreas have a worse prognosis when compared with that of gastric or colorectal adenocarcinomas, despite the histologies being similar. It remains to be determined whether these tumors of different primary origins can be included within the same group for treatment.

Therefore, it has not yet been clarified whether combined chemotherapy with cisplatin and etoposide might be as effective against hepatobiliary and pancreatic PD-NECs as it is for miscellaneous extrapulmonary PD-NECs. We report our experience of combined chemotherapy with cisplatin and etoposide as the first-line chemotherapy for patients with unresectable or recurrent PD-NECs, focusing on the tumors arising from the hepatobiliary tract and pancreas.

PATIENTS AND METHODS

Patients

Between October 1995 and January 2009, in total, 25 patients with PD-NEC arising from the hepatobiliary tract and pancreas were treated at the National Cancer Center Hospital, Tokyo, Japan. Of these 25 patients, 21 received the combination of cisplatin and etoposide as the first-line chemotherapy. Before the chemotherapy, tumor specimen obtained by a fine-needle biopsy or a surgical resection was pathologically diagnosed as PD-NECs according to the WHO classification (7,8). Typically, tumor tissue showed a dense proliferation of round or polygonal tumor cells with hyperchromatic nuclei and pale to eosinophilic granular cytoplasm, arranged in sheets, nests and cords. Extensive necrosis and mitotic figures were frequently observed. Immunohistochemically, the tumor cells expressed endocrine markers, such as chromogranin A, synaptophysin, neuron-specific enolase (NSE) and/or CD56. A Ki-67 proliferation index >15% was documented in the 21 patients receiving the cisplatin plus etoposide combination chemotherapy.

Treatment Schedule

Cisplatin, 80 mg/m^2, was administered intravenously (IV) over 2 h on the first day with adequate hydration. Etoposide, 100 mg/m^2/day, was administered IV over 2 h on days 1–3. This treatment was repeated every 3–4 weeks for a maximum of six cycles unless disease progression or unacceptable toxicity occurred. In two patients, a modified schedule with split-dose administration of cisplatin at a dose of 25 mg/m^2/day IV on days 1–3 and a reduced dose of etoposide 80 mg/m^2/day IV on days 1–3 was selected from the first cycle because of advanced age and poor performance status (9).

Antiemetic prophylaxis with 5-HT3 antagonists plus dexamethasone was used at the physician's discretion. Recombinant human granulocyte colony-stimulating factor was administered if patients developed febrile neutropenia.

Response and Toxicity Evaluations

Tumor assessments by computed tomographic (CT) scan of the abdomen were carried out at baseline and every cycle according to the Response Evaluation Criteria in Solid Tumors (RECIST). CT scan of the chest was carried out at the baseline and every cycle if a chest X-ray as a screening test detected lung metastases. Responses were to be confirmed by repeated assessments carried out no less than 4 weeks apart. In addition, tumor markers of carcinoembryonic antigen (CEA), cancer antigen (CA)19-9, NSE and progastrin-releasing peptide (ProGRP) were measured every cycle. All adverse events were reviewed based on medical records and evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Statistical Analysis

Overall survival was measured from the date of initial treatment to the date of death or the date of the last follow-up. Death from any cause was considered an event. Survival curves were constructed using the Kaplan–Meier method. Statistical analyses were performed using Dr. SPSS II (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Patient Characteristics

The characteristics of the 21 treated patients are listed in Table 1. The median age of the patients was 57 years, with an almost equal gender distribution. One patient (5%) had metastatic recurrent disease after surgery with curative intent, and 20 (95%) had unresectable metastatic disease at the initial diagnosis. Of the 21 patients, 20 (95%) had elevated serum NSE level and 4 (19%) had elevated serum ProGRP level. The primary tumor sites included the pancreas in 10 patients (48%), gallbladder in 8 (38%), liver in 2 (10%) and ampulla of Vater in 1 (5%). Two patients with multiple liver tumors without a definite primary site were classified as having a liver origin. The most common metastatic site was the liver. Other common sites were lymph nodes and the peritoneum.

Treatment

In total, 57 cycles were administered to the 21 patients with a median of 2 cycles per patient (range, 1–6 cycles). Eight

Table 1. Patient characteristics ($n = 21$)

Characteristics	n (%)
Age (years)	
Median	57
Range	30–70
Sex	
Male	11 (52)
Female	10 (48)
ECOG performance status	
0	9 (43)
1	10 (48)
2	2 (10)
Primary tumor site	
Liver	2 (10)
Gallbladder	8 (38)
Pancreas	10 (48)
Ampulla of Vater	1 (5)
Metastatic site	
Liver	17 (81)
Lung	2 (10)
Spleen	1 (5)
Bone	1 (5)
Adrenal gland	1 (5)
Pleural	1 (5)
Lymph node	11 (52)
Peritoneum/ascites	11 (52)
CEA	
Abnormal	13 (62)
Normal	8 (38)
CA19-9	
Normal	13 (62)
Abnormal	8 (38)
NSE (ng/ml)	
Median	143.1
Range	6–1930
ProGRP[a] (U/ml)	
Median	25.5
Range	11.9–63 090

Abnormal carcinoembryonic antigen (CEA) and CA19-9 represented ≥5 ng/ml and ≥37 U/ml, respectively. ECOG, Eastern Cooperative Oncology Group; NSE, neuron-specific enolase; ProGRP, pro-gastrin-releasing peptide.
[a]One patient did not have pre-treatment data examination.

patients (38%) required dose reductions during therapy. Of these patients, three required 20–25% dose reductions for both cisplatin and etoposide due to febrile neutropenia and renal dysfunction, three required a 20% dose reduction of etoposide alone due to febrile neutropenia and the remaining two required a 20% dose reduction of cisplatin alone due to serum creatinine level elevation. The median relative intensities of the doses of cisplatin and etoposide (calculated as the actual dose delivered divided by the intended dose of 3-week interval regimen) were 79% and 73%, respectively. The reasons for treatment discontinuation were radiological progressive disease in 15 patients, clinical progressive disease in 1 patient, unacceptable toxicities in 2 (gastrointestinal toxicity of prolonged Grade 2 nausea and anorexia in one, and renal toxicity as indicated by decreased creatinine clearance to <35 ml/min in the other), cytoreductive surgery in 1 and refusal of treatment by 1 (mental suffering). As for the patient who underwent cytoreductive surgery, she could not maintain response duration until the next course. In addition, she had multiple liver metastases with the maximum size of >13 cm produced abdominal discomfort.

After treatment discontinuation, eight patients received second-line chemotherapy: gemcitabine monotherapy was administered to four patients, irinotecan monotherapy to three, and combination chemotherapy with cisplatin, vincristine, doxorubicin and etoposide (CODE therapy) to one. Among them, one patient, who developed disease progression after one cycle of cisplatin and etoposide, achieved a partial response after two cycles of second-line chemotherapy with gemcitabine. Three patients were treated employing other therapeutic modalities, i.e. cytoreduction surgery, allogeneic peripheral blood stem cell transplantation and chemoembolization for liver metastases. The remaining nine patients received only supportive care.

Efficacy

At the time of analysis, 2 patients were alive with disease and 19 had died of their disease. All patients were assessable for tumor response. Although no patient achieved a complete response, two with gallbladder and one with pancreatic PD-NECs achieved a partial response, giving an overall response rate of 14% (95% confidence interval, 3–36%). Ten patients (48%) had shown stable disease and the remaining eight (38%) had progressive disease. The duration of the three objective responses were 2.4, 3.1 and 3.5 months. During treatment, the serum NSE level was reduced by >50% in 15 (75%) of 20 patients who had shown a pre-treatment level of ≥15 ng/ml. All patients were included in the survival assessment. Median progression-free survival, median overall survival and the 1-year survival rate were 1.8, 5.8 months and 5%, respectively (Fig. 1). Median progression-free survival and overall survival in the pancreas group ($n = 10$) were 1.5 and 6.2 months, whereas those in the hepatobiliary tract group ($n = 11$) were 3.0 and 5.8 months, although the differences between both groups did not appear to be statistically significant.

Adverse Events

All 21 patients were assessed for toxicities, as listed in Table 2. The most common toxicities were leukopenia and

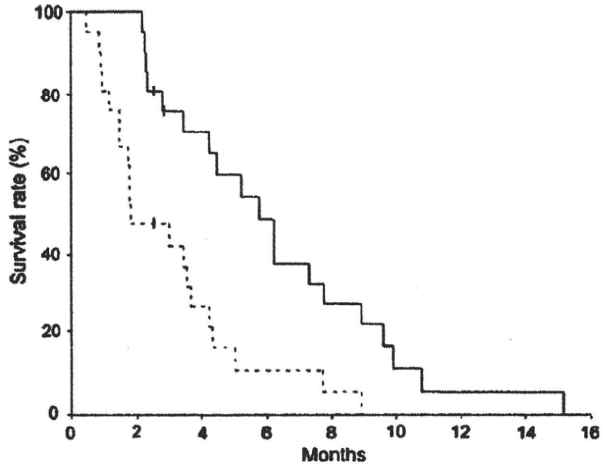

Figure 1. Overall survival (continuous line) and progression-free survival (dotted line) in the 21 patients.

neutropenia. Grade 3 or 4 leukopenia and neutropenia occurred in 15 (71%) and 19 (90%) patients, respectively, and febrile neutropenia in 8 (38%). As to non-hematological toxicities, vomiting of all grades was seen in 81% of the patients, whereas Grade 3 nausea and anorexia occurred in 33% and 24%, respectively. Although these gastrointestinal toxicities were frequently observed after cisplatin administration, most were manageable with appropriate medical treatment and only one patient needed to discontinue therapy due to gastrointestinal toxicity of prolonged Grade 2 nausea and anorexia. No other unexpected severe toxicities were observed during the treatment and there were no treatment-related deaths.

Table 2. Adverse events

	Grade				Grade 3/4, *n* (%)
	1	2	3	4	
Hematological toxicity					
Leukopenia	1	5	7	8	15 (71)
Neutropenia	1	1	2	17	19 (90)
Anemia	4	11	6	0	6 (29)
Thrombocytopenia	8	2	5	0	5 (24)
Non-hematological toxicity					
Bilirubin	3	1	3	1	4 (19)
AST	7	8	3	1	4 (19)
ALT	5	6	3	2	5 (24)
Creatinine	6	4	0	0	0
Fatigue	11	8	0	0	0
Anorexia	2	12	5	0	5 (24)
Nausea	4	9	7	0	7 (33)
Vomiting	7	10	0	0	0
Diarrhea	2	0	0	0	0
Mucositis	1	0	0	0	0
Alopecia	4	14			
Neurological sensory	1	0	0	0	0
Febrile neutropenia			8	0	8 (38)

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

DISCUSSION

In 1991, Moertel et al. (4) reported an objective response rate of 67% to combined chemotherapy with cisplatin and etoposide in 18 patients with anaplastic neuroendocrine tumors, which are analogous to the currently described extrapulmonary PD-NECs, with a median survival of 19 months. Mitry et al. (5) reported a response rate of 42% and median survival of 15 months in 41 patients with extrapulmonary PD-NECs treated with the same combination regimen. In these reports, not only tumors arising from the hepatobiliary and pancreatic regions, but also from the gastrointestinal, head and neck, and tracheal regions were included as extrapulmonary tumors. To the best of our knowledge, this is the first study of the efficacy of cisplatin plus etoposide focusing solely on tumors arising from the hepatobiliary and pancreatic regions.

In the current study, focusing on primary neuroendocrine tumors arising from the hepatobiliary and pancreatic regions, a response rate of 14% and median survival of 5.8 months were obtained in response to combined cisplatin plus etoposide therapy. Although the response rate and prognosis were extremely poor when compared with those reported by previous studies using the same combination of agents for extrapulmonary PD-NECs, when considering the finding that 75% of the patients showed a >50% decrease in the serum NSE levels, combined cisplatin plus etoposide may be considered to exert some degree of activity. However, whether this result may be comparable to that obtained with other treatment regimen for hepatobiliary and pancreatic PD-NECs is not yet clear, because few studies until date have reported on the efficacy of other regimens for this disease.

Malignant tumors arising from the hepatobiliary and pancreatic regions metastasize easily to the liver, becoming a typical cause of fatal visceral crisis; this anatomic nature may be one of the reasons for the relatively poor prognosis of these tumors. In fact, liver metastasis is a well-documented poor prognostic factor in patients with neuroendocrine tumors (10–14). The incidence of liver metastasis was 81% in the current study. Moreover, 52% had ascites as evidence of peritoneal dissemination, which is also generally recognized as a poor prognostic factor.

In the studies conducted to date, chemotherapeutic regimens for extrapulmonary PD-NECs have been patterned after those used for SCLC. However, these two entities, SCLC and extrapulmonary PD-NECs, may exhibit some differences at the molecular level. For example, Bcl-2 overexpression is observed at a high rate (75–95%) in SCLC

specimens, whereas only 33% of gastroenteropancreatic PD-NECs show this finding (15,16). Unlike SCLC, extrapulmonary PD-NECs show retention of both the short arms of chromosome 3, as revealed by restriction-fragment-length polymorphism studies and cytogenetic analyses (17). Since such cytogenetic differences between these tumors do exist, their clinical features and outcomes with the same treatment may also eventually diverge.

Neuroendocrine tumors also have other histological components in some cases (15,18–23). Such patients with PD-NECs arising from the gastric, colorectal and pancreatic regions generally have an adenocarcinoma component, whereas esophageal PD-NECs show a squamous cell carcinoma component. Thus, the nature of the non-neuroendocrine components in the PD-NECs also seems to depend on the primary site of the tumors. Two potential cells of origin of PD-NECs have been reported: pre-existing neuroectodermal cells and pluripotent epithelial stem cells, the latter appearing to be the more convincing at present (24–26). This cell of origin of the PD-NECs may explain the intermixing of adenocarcinoma or squamous cell carcinoma components in these tumors. It is well known that adenocarcinomas arising from the hepatobiliary tract and pancreas are less sensitive to chemotherapy and have a poor prognosis compared with adenocarcinomas arising from other organs. Likewise, the theory that PD-NECs arise from pluripotent epithelial stem cells may explain why hepatobiliary and pancreatic PD-NECs are less sensitive to chemotherapy and have a poor prognosis when compared with previous reports for miscellaneous extrapulmonary PD-NECs. In fact, it is interesting that elevated serum CEA and CA19-9 levels were confirmed in 38% of the patients in the current study, as both are widely used tumor markers of adenocarcinoma. In addition, one of these patients showed a partial response to gemcitabine monotherapy started after the detection of progressive disease in response to combined therapy with cisplatin and etoposide. Hence, there is a possibility that the tumor in this case showed a mixed histology consisting of neuroendocrine carcinoma and adenocarcinoma components, and that the adenocarcinoma component was refractory to the combination of cisplatin and etoposide and responsive to gemcitabine monotherapy. This may warrant the use of cytotoxic agents that are effective against both the PD-NEC component and the non-neuroendocrine carcinoma components, depending on the primary sites of the tumors.

In conclusion, the current study showed that the combination of cisplatin and etoposide exerted only marginal antitumor activity and relatively severe toxicity against PD-NECs of the hepatobiliary tract and pancreas, when compared with the treatment outcomes suggested by previous reports for extrapulmonary PD-NECs. The retrospective design of this study poses an inherent limitation. A prospective study is considered to be preferable to confirm the efficacy. Notwithstanding, because PD-NECs have an extremely poor prognosis and unsatisfactory treatment outcomes in response to combined chemotherapy with cisplatin plus etoposide, further development of novel treatment is necessary to improve the prognosis.

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Conflict of interest statement

None declared.

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