

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

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### 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 cyto-

static, 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  $\alpha$  and  $\beta$  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

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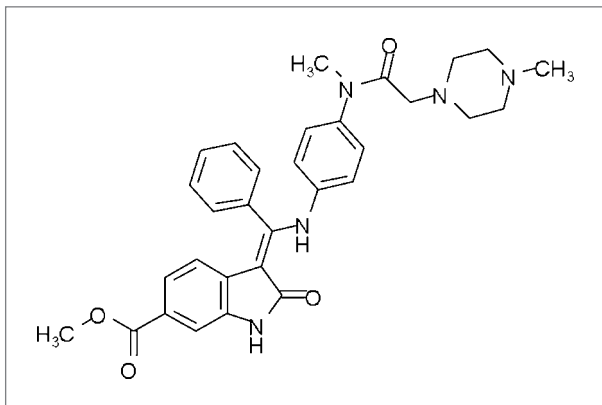


Figure 1. Structure of BIBF 1120.

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<sup>+</sup> BMD progenitors, immunophenotypically defined as CD45<sup>dim</sup>CD34<sup>+</sup>CD117<sup>+</sup> 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. Inpatient 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).

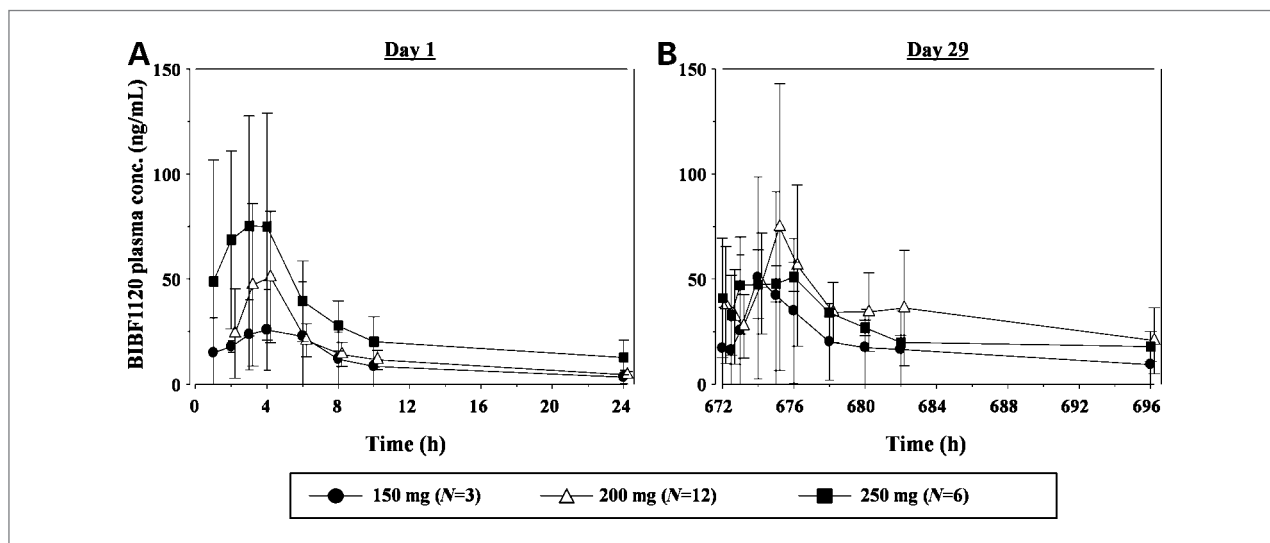
CD117/c-KIT-positive BMD progenitor cell subsets were measured with the use of flow cytometry. Peripheral blood was collected before starting, and after 2, 8, and 29 days of BIBF 1120 treatment. The 800 uL of whole blood was supplemented with 4.5 mL of 0.2% bovine serum albumin (BSA)-PBS and centrifuged 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  $\mu$ L of human  $\gamma$ -globulin. Antibodies (CD34-FITC, CD117-PE, and CD45-PerCP) were added and kept for 45 minutes

**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.

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



**Figure 2.** Mean ( $\pm$  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 $\gamma$ -GT increase; ALT increase; AST, ALT, and $\gamma$ -GT increase
250	6	3	AST and ALT increase; ALT increase; $\gamma$ -GT increase

Abbreviations: bid, twice daily;  $\gamma$ -GT,  $\gamma$ -glutamyl transferase.

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

#### Statistical analysis

Student's paired *t*-test was used to compare plasma sVEGFR2 levels or circulating CD45<sup>dim</sup>CD34<sup>+</sup>CD117<sup>+</sup> 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 ( $\geq 10\%$  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)	
	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
$\gamma$ -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;  $\gamma$ -GT,  $\gamma$ -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 increase in alanine aminotransferase (ALT) and  $\gamma$ -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  $\gamma$ -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  $\gamma$ -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  $\gamma$ -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  $\gamma$ -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) <sup>†</sup>
AUC <sub>0-12</sub> , 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) <sup>‡</sup>	23.8 (39.4) <sup>§</sup>
AUC <sub>ss</sub> , 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).

<sup>†</sup>N = 5.

<sup>‡</sup>N = 6.

<sup>§</sup>N = 2.



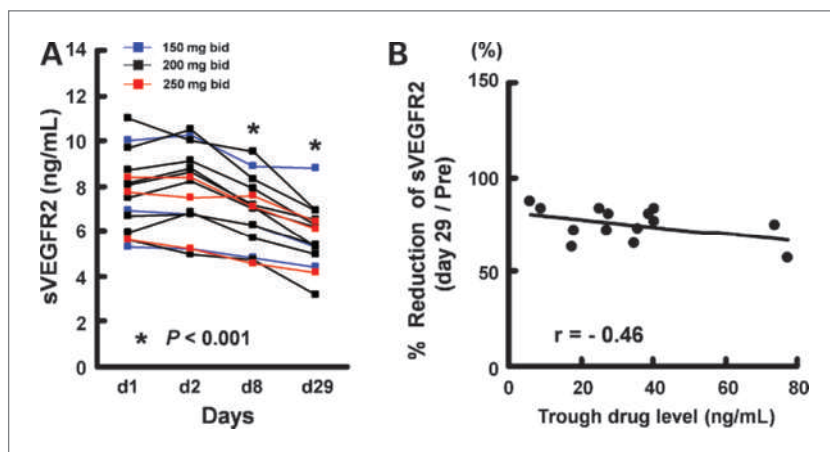


Figure 3. sVEGFR2 levels in plasma after BIBF 1120 treatment. A, plasma sVEGFR2 levels decreased during the 4-week treatment period. B, the decrease in sVEGFR2 at cycle 1, day 29 showed a modest inverse correlation with trough plasma drug levels of BIBF 1120 ( $r = -0.46$ ).

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 concentra-

tion 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

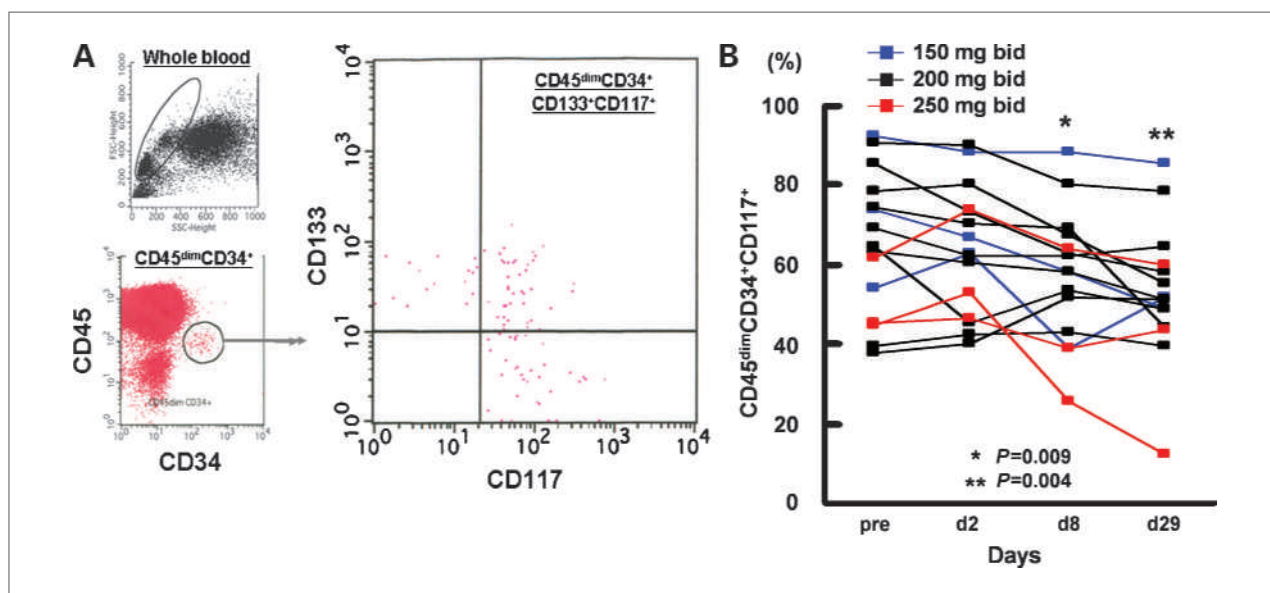


Figure 4. Levels of circulating CD117-BMD progenitor cells after BIBF 1120 treatment. A, representative flow cytometric analysis for determining the number of CD117-positive-BMD progenitor cells defined as  $CD45^{dim}CD34^{+}CD117^{+}$ . B, circulating levels of  $CD45^{dim}CD34^{+}CD117^{+}$  cells 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  $7.7 \pm 1.7$  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  $5.8 \pm 1.3$  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<sup>+</sup>-BMD progenitors during treatment with BIBF 1120

Subsets of CD117-positive-BMD progenitor cells were measured in progenitor-enriched (CD45<sup>dim</sup>CD34<sup>+</sup>) 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<sup>dim</sup>CD34<sup>+</sup> subset with a level of 60% to 80%, and representative data are shown in Fig. 4A. CD45<sup>dim</sup>CD34<sup>+</sup>CD117<sup>+</sup> 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<sup>dim</sup>CD34<sup>+</sup>CD117<sup>+</sup> 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  $\geq 150$  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  $\gamma$ -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  $\geq 50$  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<sup>+</sup> 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<sup>dim</sup>CD34<sup>+</sup>CD117<sup>+</sup>, 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-KIT<sup>+</sup> 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-KIT<sup>+</sup> 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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# 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

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