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

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

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

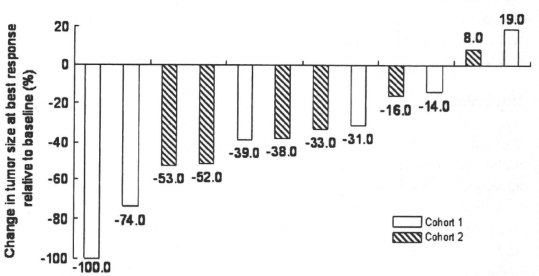

Fig. 2 Tumor response. Ten of the 12 evaluable patients showed tumor shrinkage, with one individual manifesting a complete response (−100%)

Table 4 Pharmacokinetic analysis

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

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

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

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

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

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

Discussion

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

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

negative for both paclitaxel and carboplatin, suggesting that the exanthematous rashes were caused by drug allergy to sorafenib rather than by dose-dependent toxicity. Indeed, serious erythema multiforme was not observed in any of the seven patients in cohort 2, for whom sorafenib was administered at 400 mg twice daily in cycle 2 and subsequent cycles. The only differences between the treatment regimen in cohort 1 and that of cycle 2 and subsequent cycles in cohort 2 were the dose (AUC) and infusion time of carboplatin, which were 6 mg min mL^{-1} over 30 min and 5 mg min mL^{-1} over 60 min, respectively, and pharmacokinetic analysis revealed that the triplet regimen had no significant effects on the pharmacokinetics of the individual agents. These data thus suggest that the sorafenib-related erythema multiforme observed in cohort 1 was likely the result of classic skin hypersensitivity to the drug.

Two additional DLTs (hand-foot skin reaction and elevation of ALT, both of grade 3) were observed in cohort 1, both of which were manageable and resolved by treatment interruption and remedial therapy. Although the study treatment was discontinued after the first cycle in the four patients with DLTs in cohort 1, one patient showing a partial response received three cycles of carboplatin-paclitaxel-sorafenib and an additional 13 cycles of sorafenib maintenance monotherapy, and another patient showing a complete response received four cycles of the combination therapy and an additional 23 cycles of sorafenib monotherapy. A previous phase I study of sorafenib combined with paclitaxel and carboplatin for advanced solid tumors (mostly malignant melanoma) recommended doses for future trials of sorafenib at 400 mg twice daily, carboplatin at an AUC of 6 mg min mL^{-1}, and paclitaxel at 225 mg/m^2. In a recently completed randomized phase III study of advanced NSCLC, patients were randomly assigned to treatment either with sorafenib at 400 mg twice daily plus carboplatin (AUC of 6 mg min mL^{-1}) and paclitaxel (200 mg/m^2) or with carboplatin and paclitaxel alone [24]. The present study suggests that the dose of sorafenib tolerated by Japanese patients is likely to be lower than that tolerated by Western patients when this agent is combined with standard doses of carboplatin and paclitaxel.

We examined the pharmacokinetics of paclitaxel, carboplatin, and sorafenib in order to detect any relevant drug-drug interactions. The pharmacokinetics of sorafenib in the present combination study were similar to those described in previous monotherapy [7, 17] and combination [16] trials, in which there was no evidence of drug-drug interactions. Neither of the carboplatin doses administered in the present study (AUC of 5 or 6 mg min mL^{-1}) appeared to affect the pharmacokinetics of sorafenib. Furthermore, we have shown for the first time that administration of sorafenib at 400 mg twice daily had no effect on the pharmacokinetics of carboplatin. Whereas small increases in the AUC and C_{max} values of paclitaxel and 6-hydroxy-paclitaxel were observed after sorafenib administration at 400 mg twice daily, these increases were not statistically significant. Paclitaxel is primarily metabolized in the liver by the CYP2C8 pathway to 6-hydroxy-paclitaxel and is also metabolized by CYP3A4 [25]. Although we are not able to exclude possible inhibition by sorafenib of the metabolic clearance of paclitaxel, the observed increase in paclitaxel exposure was not associated with increased clinical toxicity. Together, our pharmacokinetic results suggest that concomitant administration of sorafenib, carboplatin, and paclitaxel had no significant impact on the pharmacokinetics of any of these three drugs in this treatment schedule, although our finding on pharmacokinetics will need to be reproduced in larger cohort of patients treated with this combination.

Although tumor evaluation was not the primary objective of our study, the combination treatment yielded promising results, with one complete response and six partial responses observed among the 12 evaluable patients. Despite this substantial antitumor activity observed in the present study, a phase III trial (ESCAPE: Evaluation of Sorafenib, Carboplatin, and Paclitaxel Efficacy) of 926 patients with advanced NSCLC receiving first-line therapy with paclitaxel and carboplatin in the absence or presence of sorafenib failed to show an improvement in efficacy with the addition of sorafenib to the standard combination chemotherapy [24]. Indeed, a subset analysis of the 219 patients with squamous histology was suggestive of a detrimental effect of sorafenib inclusion. The complete response and all partial responses in our phase I study occurred in patients with non-squamous NSCLC. Although the biological basis for a possible ethnic difference in sorafenib efficacy and toxicity remains unknown, further investigation are warranted to identify the patients who are more likely to benefit from this agent.

In conclusion, in combination with carboplatin AUC 5 mg min mL^{-1} and paclitaxel 200 mg/m^2, administration of sorafenib at 400 mg once daily was confirmed to be feasible in Japanese patients with advanced NSCLC. There was no relevant pharmacokinetic interaction and the observed antitumor activity was encouraging in this study.

Funding This research was sponsored by Bayer Yakuhin Ltd.

Conflicts of interest Two of the co-authors, Koichi Fukino and Takahiko Tanigawa, are employees of Bayer Yakuhin Ltd.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008) Cancer statistics, 2008. CA Cancer J Clin 58:71–96. doi:10.3322/CA.2007.0010
2. The American Society of Clinical Oncology (1997) Clinical practice guidelines for the treatment of unresectable non-small-cell lung cancer. Adopted on May 16, 1997 by the American Society of Clinical Oncology. J Clin Oncol 15:2996–3018
3. Socinski MA, Crowell R, Hensing TE, Langer CJ, Lilenbaum R, Sandler AB, Morris D, American College of Chest Physicians (2007) Treatment of non-small cell lung cancer, stage IV: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 132(3 Suppl):277S–289S. doi:10.1378/chest.07-1381
4. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA (2004) BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64:7099–7109. doi:10.1158/0008-5472.CAN-04-1443
5. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3:11–22. doi:10.1038/nrc969
6. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA (2007) Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 1773:1263–1284. doi:10.1016/j.bbamcr.2006.10.001
7. Strumberg D, Richly H, Hilger RA, Schleucher N, Korfee S, Tewes M, Faghih M, Brendel E, Voliotis D, Haase CG, Schwartz B, Awada A, Voigtmann R, Scheulen ME, Seeber S (2005) Phase I clinical and pharmacokinetic study of the novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. J Clin Oncol 23:965–972. doi:10.1200/JCO.2005.06.124
8. Clark JW, Eder JP, Ryan D, Lathia C, Lenz HJ (2005) Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43–9006, in patients with advanced, refractory solid tumors. Clin Cancer Res 11:5472–5480. doi:10.1158/1078-0432.CCR-04-2658
9. Awada A, Hendlisz A, Gil T, Bartholomeus S, Mano M, de Valeriola D, Strumberg D, Brendel E, Haase CG, Schwartz B, Piccart M (2005) Phase I safety and pharmacokinetics of BAY 43–9006 administered for 21 days on/7 days off in patients with advanced, refractory solid tumours. Br J Cancer 92:1855–1861. doi:10.1038/sj.bjc.6602584
10. Moore M, Hirte HW, Siu L, Oza A, Hotte SJ, Petrenciuc O, Cihon F, Lathia C, Schwartz B (2005) Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43–9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. Ann Oncol 16:1688–1694. doi:10.1093/annonc/mdi310
11. Kupsch P, Henning BF, Passarge K, Richly H, Wiesemann K, Hilger RA, Scheulen ME, Christensen O, Brendel E, Schwartz B, Hofstra E, Voigtmann R, Seeber S, Strumberg D (2005) Results of a phase I trial of sorafenib (BAY 43–9006) in combination with oxaliplatin in patients with refractory solid tumors, including colorectal cancer. Clin Colorectal Cancer 5:188–196. doi:10.3816/CCC.2005.n.030
12. Richly H, Henning BF, Kupsch P, Passarge K, Grubert M, Hilger RA, Christensen O, Brendel E, Schwartz B, Ludwig M, Flashar C, Voigtmann R, Scheulen ME, Seeber S, Strumberg D (2006) Results of a Phase I trial of sorafenib (BAY 43–9006) in combination with doxorubicin in patients with refractory solid tumors. Ann Oncol 17:866–873. doi:10.1093/annonc/mdl017
13. Siu LL, Awada A, Takimoto CH, Piccart M, Schwartz B, Giannaris T, Lathia C, Petrenciuc O, Moore MJ (2006) Phase I trial of sorafenib and gemcitabine in advanced solid tumors with an expanded cohort in advanced pancreatic cancer. Clin Cancer Res 12:144–151. doi:10.1158/1078-0432.CCR-05-1571
14. Escudier B, Lassau N, Angevin E, Soria JC, Chami L, Lamuraglia M, Zafarana E, Landreau V, Schwartz B, Brendel E, Armand JP, Robert C (2007) Phase I trial of sorafenib in combination with IFN alpha-2a in patients with unresectable and/or metastatic renal cell carcinoma or malignant melanoma. Clin Cancer Res 13:1801–1809. doi:10.1158/1078-0432.CCR-06-1432
15. Strumberg D, Clark JW, Awada A, Moore MJ, Richly H, Hendlisz A, Hirte HW, Eder JP, Lenz HJ, Schwartz B (2007) Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. Oncologist 12:426–437. doi:10.1634/theoncologist.12-4-426
16. Flaherty KT, Schiller J, Schuchter LM, Liu G, Tuveson DA, Redlinger M, Lathia C, Xia C, Petrenciuc O, Hingorani SR, Jacobetz MA, Van Belle PA, Elder D, Brose MS, Weber BL, Albertini MR, O'Dwyer PJ (2008) A phase I trial of the oral, multikinase inhibitor sorafenib in combination with carboplatin and paclitaxel. Clin Cancer Res 14:4836–4842. doi:10.1158/1078-0432.CCR-07-4123
17. Minami H, Kawada K, Ebi H, Kitagawa K, Kim YI, Araki K, Mukai H, Tahara M, Nakajima H, Nakajima K (2008) Phase I and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors. Cancer Sci 99:1492–1498. doi:10.1111/j.1349-7006.2008.00837.x
18. Furuse J, Ishii H, Nakachi K, Suzuki E, Shimizu S, Nakajima K (2008) Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. Cancer Sci 99:159–165. doi:10.1111/j.1349-7006.2007.00648.x
19. Takimoto CH, Awada A (2008) Safety and anti-tumor activity of sorafenib (Nexavar) in combination with other anti-cancer agents: a review of clinical trials. Cancer Chemother Pharmacol 61:535–548. doi:10.1007/s00280-007-0639-9
20. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205–216. doi:10.1093/jnci/92.3.205
21. Strumberg D, Awada A, Hirte H, Clark JW, Seeber S, Piccart P, Hofstra E, Voliotis D, Christensen O, Brueckner A, Schwartz B (2006) Pooled safety analysis of BAY 43–9006 (sorafenib) monotherapy in patients with advanced solid tumours: Is rash associated with treatment outcome? Eur J Cancer 42:548–556. doi:10.1016/j.ejca.2005.11.014

22. Robert C, Mateus C, Spatz A, Wechsler J, Escudier B (2009) Dermatologic symptoms associated with the multikinase inhibitor sorafenib. J Am Acad Dermatol 60:299–305. doi:10.1016/j.jaad.2008.06.034
23. MacGregor JL, Silvers DN, Grossman ME, Sherman WH (2007) Sorafenib-induced erythema multiforme. J Am Acad Dermatol 56:527–528. doi:10.1016/j.jaad.2006.10.981
24. Scagliotti G, von Pawel J, Reck M, Cupit L, Cihon F, DiMatteo S, O'Leary J, Hanna N (2008) Sorafenib plus carboplatin/paclitaxel in chemonaive patients with stage IIIB-IV non-small cell lung cancer: Interim analysis results from the phase III, randomized, double-blind, placebo-controlled, ESCAPE (Evaluation of Sorafenib, Carboplatin, and Paclitaxel efficacy in NSCLC) trial. J Thoracic Oncol 3(Suppl 1):S97–S98
25. Sonnichsen DS, Liu Q, Schuetz EG, Schuetz JD, Pappo A, Relling MV (1995) Variability in human cytochrome P450 paclitaxel metabolism. J Pharmacol Exp Ther 275:566–575

Lung Cancer 70 (2010) 168–173

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Dose-escalation study of pemetrexed in combination with carboplatin followed by pemetrexed maintenance therapy for advanced non-small cell lung cancer

Isamu Okamoto[a,*], Koji Takeda[b], Haruko Daga[b], Masaki Miyazaki[a], Kimio Yonesaka[a], Hidemi Kiyota[a], Junji Tsurutani[a], Shinya Ueda[a], Yasuko Ichikawa[a], Masayuki Takeda[a], Risa Sekiguchi[c], Kiyomi Tominaga[c], Sotaro Enatsu[c], Yoshihiro Nambu[c], Kazuhiko Nakagawa[a]

[a] Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan
[b] Department of Clinical Oncology, Osaka City General Hospital, Osaka, Japan
[c] Lilly Research Laboratories, Eli Lilly Japan K.K., Japan

ARTICLE INFO

Article history:
Received 26 December 2009
Received in revised form 16 February 2010
Accepted 18 February 2010

Keywords:
Pemetrexed
Carboplatin
Dose-escalation
First-line therapy
Maintenance therapy
NSCLC

ABSTRACT

Introduction: The primary objectives of this study were to determine the recommended dose of pemetrexed and carboplatin in patients with chemo-naive advanced non-small cell lung cancer (NSCLC).
Methods: Patients received escalated doses of carboplatin area under the concentration–time curve (AUC) of 5 (cohort 1) or 6 (cohort 2) and pemetrexed 500 mg/m^2 every 3 weeks for six cycles. For patients with objective response and stable disease, pemetrexed were continued until disease progression or unacceptable toxicity.
Results: In cohort 1, a dose-limiting toxicity (DLT) was observed in one of the six patients: grade 4 thrombocytopenia. No DLTs were seen in the first 6 patients of cohort 2, and thus the combination of pemetrexed 500 mg/m^2 plus carboplatin at AUC 6 was determined as the recommended dose. Among a total of 20 patients, 8 patients received a median of four cycles of pemetrexed monotherapy in a maintenance setting without unexpected or cumulative toxicities. No complete responses and 12 partial responses were observed, giving an overall response rate of 60.0% [95% confidence interval (CI), 36.1–80.9%]. Median progression-free survival time for all patients was 7.6 months (95% CI: 4.8–8.0 months).
Conclusions: Pemetrexed 500 mg/m^2 plus carboplatin AUC 6 combination therapy followed by pemetrexed maintenance therapy, is generally tolerable, and shows encouraging antitumor activity in chemotherapy-naive patients with advanced NSCLC.

1. Introduction

Lung cancer is the most common cancer in the world and the leading cause of cancer-related mortality. Approximately 85% of lung cancers are non-small cell, and approximately 70% of patients with NSCLC present with inoperable, locally advanced (Stage IIIB) or metastatic (Stage IV) disease. Platinum-based chemotherapy is the standard first-line treatment for advanced NSCLC on the basis of moderate improvement in survival and quality of life it confers compared with best supportive care alone [1–3]. The poor outlook even for patients with advanced NSCLC who receive such treatment has prompted a search for new chemotherapeutic agents and combination regimens.

Pemetrexed is a multi-targeted antifolate cytotoxic agent. Randomized phase III clinical studies have demonstrated that pemetrexed is efficacious as a single agent in second-line treatment of NSCLC [4], and in combination with cisplatin for first-line treatment of nonsquamous NSCLC [5]. The latter phase III study reported noninferior efficacy and better tolerability for cisplatin/pemetrexed than for cisplatin/gemcitabine in the first-line setting [5]. In addition, overall survival was statistically superior for cisplatin/pemetrexed versus cisplatin/gemcitabine in patients with nonsquamous NSCLC [6]. Another phase III clinical study demonstrated superior overall survival (OS) when pemetrexed was used in a maintenance setting following 4 cycles of non-pemetrexed induction therapy containing a platinum doublet [7]. These study results indicate that pemetrexed-based induction therapy followed by pemetrexed maintenance therapy would be a possible treatment option for patients with nonsquamous NSCLC.

Because carboplatin-based regimens have been shown to be less toxic, convenient, and capable of being administered on an outpatient basis, they have been widely used as a substitute for cisplatin regimens in clinical practice. In wake of the above results, there has been interest in studying the substitution of carboplatin for

* Corresponding author. Tel.: +81 72 366 0221; fax: +81 72 360 5000.
E-mail address: chi-okamoto@dotd.med.kindai.ac.jp (I. Okamoto).

0169-5002/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2010.02.007

cisplatin in cisplatin plus pemetrexed regimens for NSCLC [8–11]. In previous studies of pemetrexed and carboplatin combination therapy in a first-line setting, different carboplatin doses (AUC 5 and 6) were used. To date, carboplatin dose escalation has not been evaluated in chemotherapy-naive patients with advanced NSCLC. Therefore, we conducted this study to determine the recommended dose of carboplatin combined with pemetrexed and assess the feasibility of pemetrexed monotherapy in a maintenance setting.

2. Materials and methods

2.1. Study design

This phase I dose-escalation study was conducted to examine the safety of the pemetrexed and carboplatin combination in chemotherapy-naive patients with advanced NSCLC. The primary objectives of the study were to evaluate incidence, type and severity of adverse events and to determine the recommended dose of carboplatin used in combination with pemetrexed.

Patients were divided into 2 cohorts. In the first cohort, 3 patients received pemetrexed 500 mg/m^2 plus carboplatin at AUC 5 according to the Calvert formula [12] (cohort 1). If dose-limiting toxicity (DLT) was not observed in any of three patients, an escalated dose of carboplatin (AUC 6) was administered to the first 6 patients in cohort 2. If DLT was observed in 1 out of the first 3 patients, additional 3 patients were enrolled to assess the tolerability of this dose level. If DLT occurred in only 1 out of the 6 patients in the cohort 1, dose escalation of carboplatin (AUC 6) was made. If DLT was observed in two or more of first three patients, a reduced dose of carboplatin (AUC 4) was administered. Dose escalation was decided by the toxic data only in the first cycle of chemotherapy.

The recommended dose was determined based on these initial results from cohorts 1 and 2. Following this and additional number of patients, up to maximum of 20 patients, were enrolled to receive the recommended dose of study treatment.

2.2. Eligibility

Patients with histologically or cytologically confirmed advanced NSCLC were eligible for the study. Each patient was required to meet the following criteria: (1) clinical stage IIIB, IV, or post-operative recurrent disease; (2) lesion not amendable for curative radiation; (3) no prior chemotherapy; (4) aged 20–75 years old; (5) ECOG PS 0 or 1; (6) adequate function of major organs (lung: $SpO_2 \geq 90\%$, heart: normal 12 lead ECG, bone marrow: hemoglobin ≥ 9.0 g/dL, neutrophil $\geq 1500/mm^3$, platelet $\geq 100{,}000/mm^3$, liver: AST (GOT)/ALT(GPT) ≤ 2.5 times upper limit of normal, total bilirubin ≤ 1.5 mg/dL, kidney: serum creatinine ≤ 1.2 mg/dL, predicted creatinine clearance or 24-h creatinine clearance ≥ 45 mL/min as estimated by the Cockcroft and Gault formula [13]); (7) life expectancy of at least 12 weeks.

This study followed the ethical principles in the Declaration of Helsinki, and the study protocol was approved by the institutional review board at each participating center. All patients provided written informed consent before study-related procedures were performed.

2.3. Study treatment

All patients received pemetrexed 500 mg/m^2 by 10 min intravenous infusion followed by intravenous infusion of carboplatin over at least 30 min (AUC 5 or 6) on day 1 of 21-day cycle. Combination chemotherapy was repeated every 3 weeks for a maximum of six cycles. After completion of six cycles it was possible to continue pemetrexed monotherapy at the discretion of the investigator until progressive disease (PD). Subsequent cycles of treatment were withheld until the following criteria were satisfied: the neutrophil count $\geq 1500/mm^3$, the platelet count $\geq 100{,}000/mm^3$, hemoglobin >8.0 g/dL, PS ≤ 1, $SpO_2 \geq 90\%$, AST/ALT ≤ 2.5 times upper limit of normal, total bilirubin ≤ 1.5 mg/dL, other nonhematological toxicity $\leq$grade 2, and a decision by the physician. If these criteria were not satisfied within 29 days from the date of dose administration in the cycle, treatment doses were modified as follows: in case of nonhematological toxicity, pemetrexed dose was to be reduced from 500 to 400 mg/m^2 and in case of hematological toxicity, carboplatin dose was to be reduced from AUC 6 to 5 (or 5 to 4). If the toxicity had not resolved within 43 days, the patient was excluded from the study.

While on study, patients received folic acid and vitamin B_{12}. All patients underwent comprehensive baseline assessments including clinical laboratory tests and imaging studies. Patients also received follow-up assessments and monitoring at regular intervals. Toxicity evaluations were based on the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE v3.0).

2.4. Definition of DLT

A DLT was defined as a toxicity occurring in cycle 1 that met one of the following criteria and for which a causal relationship with the study drugs could not be ruled out: grade 4 neutropenia prolonged ≥ 7 days, febrile neutropenia, grade 4 thrombocytopenia, grade 3 thrombocytopenia that required platelet transfusion or was associated with bleeding, or grade 3 nonhematological toxicity (following events were to be DLT if the event does not recover $\leq$grade 2 despite standard/optimal supportive treatment: nausea, vomiting, anorexia, fatigue, constipation, diarrhea, transient increase in AST/ALT, or transient electrolytes abnormally). In case that the patients experienced toxicities which met DLT criteria, treatment doses were modified in subsequent courses.

2.5. Efficacy measures

The efficacy endpoints were tumor response, progression-free survival time and overall survival. Tumor response was evaluated every 6 weeks according to the RECIST guideline [14]. Progression-free survival was defined as the time from enrollment to the date of confirmation of progressive disease (PD) or the date of death from any cause, which is earlier. Overall survival was defined as the time from registration until death from any cause. For patients not known to have died and to have had progression, the patients were censored at the date of the last progression-free assessment.

2.6. Statistical analysis

All patients who received at least one dose of study treatment were included in the safety and efficacy analysis. A maximum of 20 patients were to be enrolled in our study to evaluate the safety of combination therapy with pemetrexed and carboplatin at AUC 5 or AUC 6. At least 14 patients were to be treated at the recommended doses. The probability of adverse events with incidences equal to or greater than 20% not being detected in any of the 14 patients was 4.4%.

The incidence of the adverse events was calculated for each dose group. The distribution of best overall response was summarized in the patients who had target lesions. PFS was estimated using the Kaplan–Meier (K–M) method [15]. This included generating the K–M curve and determining the median with 95% confidence interval.

Table 1
Patient characteristics.

Number of patients	20
Median age, year (range)	64(46–75)
Gender	
Male	13
Female	7
Performance status	
0	4
1	16
Disease stage	
IIIB	3
IV	16
Relapse after surgery	1
Histology	
Adenocarcinoma	17
Squamous cell carcinoma	2
Other	1

3. Results

3.1. Patients

This study was carried out from January 2008 to August 2009 at 2 study centers in Japan. Twenty-one patients were enrolled, and one patient withdrew consent to participate in the study before the treatment. Table 1 shows the demographics and characteristics of the 20 patients. Seven patients were female and 13 were male. The median age was 64 years (range: 46–75). Histologically 17 patients had adenocarcinomas and 2 had squamous cell carcinomas. One patient who had unspecified NSCLC was classified as "other".

3.2. Determination of recommended dose

In cohort 1, a DLT was observed in one of the first 3 patients: grade 4 thrombocytopenia. Following treatment with blood platelet transfusion, the platelet count in this DLT patient recovered rapidly and the thrombocytopenia severity dropped to grade 0. Additional 3 patients were enrolled in cohort 1, but none of these patients developed DLTs. The dose of carboplatin was then escalated to AUC 6 (cohort 2). No DLTs were seen in the first 6 patients of cohort 2, and thus the combination of pemetrexed 500 mg/m^2 plus carboplatin at AUC 6 was determined as the recommended dose. An additional 8 patients were assigned to this dose level. In total, 20 patients were administered the combination of pemetrexed and carboplatin.

3.3. Treatment delivery

The data of treatment delivery was shown in Table 2.

For 6 patients who received carboplatin at AUC 5, the mean relative dose intensities were 90.3% for pemetrexed and 86.1% for carboplatin. Patients received a median of 8.0 cycles of treatment (range, 2–11) including maintenance pemetrexed monotherapy. A total 43 cycles of treatment was delivered overall. Study protocol requirements stipulated dose reductions in 3 cycles (7% of total cycles) and dose delays in 10 cycles (23% of total cycles).

In 14 patients receiving carboplatin at AUC 6, the mean relative dose intensities were 84.6% for pemetrexed and 82.1% for carboplatin. Patients received a median of 5.5 cycles of treatment (range, 1–10). A total 82 cycles of treatment was delivered overall. Study protocol requirements stipulated dose reductions in 12 cycles (15% of total cycles) and dose delays in 29 cycles (35% of total cycles).

As shown in Table 2, hematologic toxicities were a major cause of both dose reductions and dose delays.

Eleven patients (4 patients in cohort 1 [$n = 6$] and 7 patients in cohort 2 [$n = 14$]) completed 6 cycles of the combination therapy. Three of these patients were discontinued due to disease progression (1 patient in cohort 1) and adverse events (2 patients in cohort 2) before maintenance therapy began. The other eight patients (3 patients in cohort 1 and 5 patients in cohort 2) continued pemetrexed monotherapy in a maintenance setting. Six out of 8 patients were discontinued due to disease progression, and 2 out of 8 patients were discontinued due to adverse events (blood creatinine increased and bronchitis, respectively) during maintenance therapy. In maintenance therapy, only one cycle delay due to adverse event was observed, however no dose reductions were observed; the median number of cycles was 4.0 cycles (range, 2–5).

3.4. Safety

The major adverse events during the entire treatment period are shown in Tables 3 and 4. The hematological adverse events reaching ≥ grade 3 were neutropenia (75%), anemia (50%), thrombocytopenia (45%) and leukopenia (15%). Of these events, grade 4 thrombocytopenia was observed in four patients, and no grade 4 leukopenia was observed. Nonhematological toxicities ≥ grade

Table 2
Summary of treatment delivery.

Cohort	Cohort 1 ($N = 6$) PEM 500 mg/m^2 + CBDCA AUC 5		Cohort 2 ($N = 14$) PEM 500 mg/m^2 + CBDCA AUC 6	
Total cycles treated	43		82	
Median cycles (range)	8.0 (2–11)		5.5 (1–10)	
	PEM (mg/m^2)	CBDCA	PEM (mg/m^2)	CBDCA
Planned dose per week	166.7	AUC 1.667	166.7	AUC 2.000
Actual dose per week	150.5	AUC 1.435	141.1	AUC 1.642
Relative dose intensity (%)	90.3	86.1	84.6	82.1
Dose reduction because of AE				
Cycles (%)	3 (7%)		12 (15%)	
Reason (AE, cases)	Neutropenia (1) Platelet decreased (1)		Neutropenia (5) Platelet decreased (4) Hemoglobin decreased (2) ALT increased (1)	
Dose delay because of AE				
Cycles (%)	10 (23%)		29 (35%)	
Reason (AE, cases)	Neutropenia (8) Platelet decreased (3) Bronchitis (1)		Neutropenia (20) Platelet decreased (8) Hemoglobin decreased (6) ALT increased (1)	

PEM: pemetrexed; CBDCA: carboplatin.
Note: Include both results of combination therapy and pemetrexed monotherapy.

Table 3
Summary of adverse events by cohort.

Adverse events	Cohort 1		N=6		Cohort 2		N=14	
	Toxicity grade				Toxicity grade			
	G1	G2	G3	G4	G1	G2	G3	G4
Hematological								
Thrombocytopenia	2	1	2	1	5	3	3	3
Anemia	0	3	3	0	2	4	6	1
Leucopenia	1	4	1	0	5	5	2	0
Neutropenia	0	1	4	1	0	1	9	1
Nonhematological								
Anorexia	2	3	1	0	6	4	1	0
Nausea	3	2	0	0	10	2	1	0
Vomiting	3	1	0	0	0	3	1	0
Fatigue	2	4	0	0	6	2	0	0
AST increased	2	1	0	0	6	1	0	0
ALT increased	0	2	0	0	6	1	1	0
Constipation	3	0	0	0	5	1	0	0
Diarrhoea	2	0	0	0	3	0	0	0
Rash	2	2	0	0	1	2	0	0
Alopecia	2	0	–	–	2	0	–	–
LDH increased	2	1	0	0	1	0	0	0
GGT increased	1	0	0	0	3	0	0	0
ALP increased	1	0	0	0	1	0	0	0
Nasopharyngitis	2	0	0	0	4	0	0	0
Fever	3	0	0	0	0	0	0	0

Events were graded according to CTCAE v3.0.
WBC: white blood cell count; RBC: red blood cell count; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; LDH: lactate dehydrogenase; ALP: alkaline phosphatase.

3 were anorexia (10%), nausea, vomiting and increased ALT (5% each).

There were no treatment related deaths. The adverse events observed in our study were predictable from safety profiles of pemetrexed and carboplatin, with all events well managed. Most of the patients recovered from such adverse events by dose adjustment or discontinuing the study treatment.

Table 4
Summary of adverse events.

Adverse events	N=20			
	Toxicity grade			
	G1	G2	G3	G4
Hematological				
Neutropenia	0	2	13	2
Anemia	2	7	9	1
Thrombocytopenia	7	4	5	4
Leukopenia	6	9	3	0
Nonhematological				
Anorexia	8	7	2	0
Nausea	13	4	1	0
Vomiting	3	4	1	0
Fatigue	8	6	0	0
AST increased	8	2	0	0
ALT increased	6	3	1	0
Constipation	8	1	0	0
Diarrhoea	5	0	0	0
Rash	3	4	0	0
Alopecia	4	0	–	–
LDH increased	3	1	0	0
GGT increased	4	0	0	0
ALP increased	2	0	0	0
Nasopharyngitis	6	0	0	0
Fever	3	0	0	0

Events were graded according to CTCAE v3.0.
WBC: white blood cell count; RBC: red blood cell count; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; LDH: lactate dehydrogenase; ALP: alkaline phosphatase.

Table 5
Overall response.

	Cohort 1 (N=6) n (%)	Cohort 2 (N=14) n (%)	Total (N=20) n (%)
Overall response rate	4 (66.7)	8 (57.1)	12 (60.0)
Complete response	0 (0.0)	0 (0.0)	0 (0.0)
Partial response	4 (66.7)	8 (57.1)	12 (60.0)
Stable disease	0 (0.0)	4 (28.6)	4 (20.0)
Progressive disease	1 (16.7)	1 (7.1)	2 (10.0)
Not evaluable	1[a] (16.7)	1 (7.1)	2 (10.0)

[a] One patient had no target lesion.

3.5. Efficacy

There were 12 partial responses (4 patients in cohort 1 and 8 patients in cohort 2) and no complete responses, yielding an overall response rate of 60.0% (95% CI: 36.1–80.9%, Table 5). All 20 treated patients were assessable for progression-free survival and overall survival. With a median follow-up time of 13 months (range of 2.4–16.7 months), 13 patients were still alive. Median progression-free survival for all patients was 7.6 months (95% CI: 4.8–8.0 months; Fig. 1), whereas median overall survival was not reached.

4. Discussion

This was the first dose-escalation study which examined the recommended dose of pemetrexed and carboplatin combination therapy as a first-line therapy for treatment of advanced NSCLC. Six patients were treated with pemetrexed 500 mg/m^2 and carboplatin at AUC 5 (cohort 1), and 14 patients were treated with pemetrexed 500 mg/m^2 and carboplatin at AUC 6 (cohort 2).

DLT was observed in 1 patient in cohort 1. This was grade 4 thrombocytopenia, however the low platelet baseline of this patient (13.6×10^4/mm^3, grade 1) might have made this patient more susceptible to this DLT. This indicates the need for suitable precautions in clinical practice prior to starting treatment, especially for patients who have a low platelet count. No DLT was

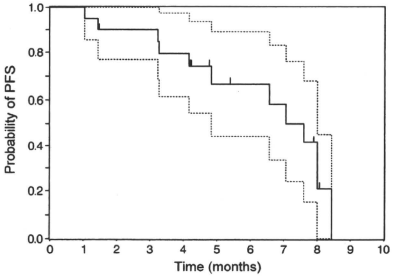

Fig. 1. Progression-free survival time. Solid line shows Kaplan–Meier curve and dashed line shows 95% CI, and events were observed in 12 patients out of 20 patients.

observed in the first 6 patients of cohort 2 (pemetrexed 500 mg/m^2 and carboplatin AUC 6). Other grade 3/4 hematological toxicities were also observed, but these were manageable with dose delays and reductions, or supportive care. Dose delays and reductions were made in both cohorts, however mean weekly dose intensities of pemetrexed and carboplatin exceeded 80% of the planned dose intensities in both cohorts. This demonstrates that pemetrexed and carboplatin can be combined in this population of NSCLC patients without compromising the dose of either agent.

For first-line and maintenance therapy, phase III studies have demonstrated pemetrexed to be efficacious. This has resulted in FDA approvals for pemetrexed as a first-line therapy for NSCLC in 2008 and as a maintenance therapy for NSCLC in 2009. Therefore pemetrexed-based platinum therapy followed by pemetrexed maintenance therapy may be an alternative treatment option for patients with nonsquamous NSCLC, although survival benefit of this regimen was not shown then further clinical study is ongoing [11].

In the present study, 8 patients (cohort 1: 3 patients, cohort 2: 5 patients) started pemetrexed monotherapy in a maintenance setting after 6 cycles of pemetrexed and carboplatin. The median number of cycles was 4.0 cycles of maintenance therapy without dose reduction. Only two of the 8 patients discontinued treatment due to adverse events, and there were no unexpected adverse events or cumulative toxicity in the maintenance phase. This showed that pemetrexed maintenance therapy was tolerable following pemetrexed-based combination therapy. In a recent pemetrexed maintenance phase III study, which demonstrated superior OS, the induction therapy consisted of 4 cycles of a non-pemetrexed agent containing a platinum doublet. In our study, 85% (17/20) of patients were able to complete the first 4 cycles of the pemetrexed and carboplatin combination therapy, and 60% (12/20) were able to continue this combination to at least the fifth cycle. This means that if the combination therapy was limited to 4 cycles, more patients would be able to continue pemetrexed monotherapy as maintenance therapy.

The response rate of 60.0% (95% CI: 36.1–80.9%) and the median PFS of 7.6 months (95% CI: 4.8–8.0 months) are certainly encouraging even though it was a small sample size. Two previous phase II studies of combination therapy with pemetrexed and carboplatin showed lower response rates of 24.0% and 31.6% [8,9]. One possible reason for this difference is the histological type of NSCLC. Recent studies have shown superior efficacy for pemetrexed in nonsquamous NSCLC and inferior efficacy in squamous NSCLC. The phase II studies described above included 12.0% and 30.8% of squamous NSCLC patients, respectively. However, all but two of the patients in our study were nonsquamous NSCLC patients, and thus the higher response rate was not surprising.

Another possible reason is ethnic difference. There are no confirmed data showing that ethnic difference is related to different patient responses to pemetrexed. However, the results of subgroup analyses in the phase III study of pemetrexed and cisplatin as a first-line therapy for NSCLC have shown higher response rates in East Asian (Korea and Taiwan only) populations, 42.6% [16] compared with 29.5% (Data on file) in non-East Asian populations. Response rates for East Asian patients were also comparatively high in a Japan phase II study of second or third line pemetrexed monotherapy [17]. In that study, Ohe et al. reported an 18.5% response rate in the pemetrexed 500 mg/m^2 group. On the other hand, Hanna et al. reported only a 9.1% response rate in the pemetrexed arm from a randomized phase III study of pemetrexed versus docetaxel that included a comparatively small number of Asian patients [4]. Although there are limitations when comparing the results from different studies, the efficacy results in our study compare favorably with those reported in the above first-line studies. The reason for this apparent difference between non-East Asian and East Asian populations remains unknown, however pemetrexed does seem to have better efficacy in East Asian patients with nonsquamous NSCLC.

There is the possibility that further pemetrexed studies might be restricted to patients with nonsquamous NSCLC patients because of the pemetrexed label indications. However, in present study, one responder was observed among the squamous NSCLC patients, and in other studies there were also some responders in the squamous population. Pemetrexed is now in clinical development for head and neck cancer treatment, the major histological tumor type being squamous cell carcinoma. Preclinical data suggest that tumoral expression of thymidylate synthase (TS), which is usually lower in nonsquamous compared with squamous NSCLC, may be responsible for the differential activity of pemetrexed. Results such as these, especially with the number of therapeutic choices steadily increasing, have shown that the development of predictive biomarkers like tumoral TS expression is more important than ever.

In the present study, the combination of pemetrexed 500 mg/m^2 and carboplatin AUC 5 or 6 was confirmed to be feasible and effective for chemotherapy-naive Japanese patients with advanced NSCLC. According to the protocol definition, we concluded that carboplatin at AUC 6 is recommended for use in combination with pemetrexed 500 mg/m^2. And pemetrexed maintenance monotherapy continued after this combination was safe and tolerable. A larger-scale study is needed to confirm these findings overall for advanced NSCLC patients. In addition, large-scale phase III studies of a pemetrexed, carboplatin plus molecular-target drugs combination are currently underway in both Japan and abroad.

Conflicts of interest

I. Okamoto, K. Takeda and K. Nakagawa disclose consultant or advisory relationship with Eli Lilly Japan K.K. R. Sekiguchi, K. Tominaga, S. Enatsu and Y. Nambu have been full-time employees of Eli Lilly Japan K.K. H. Daga, M. Miyazaki, K. Yonesaka, H. Kiyota, J. Tsurutani, S. Ueda, Y. Ichikawa and M. Takeda have no conflict of interest to disclose.

Acknowledgment

The study was sponsored and supported by Eli Lilly Japan K.K.

References

[1] Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899–909.

[2] Clinical practice guidelines for the treatment of unresectable non-small-cell lung cancer. Adopted on May 16, 1997 by the American Society of Clinical Oncology. J Clin Oncol 1997;15:2996–3018.
[3] Socinski MA, Crowell R, Hensing TE, Langer CJ, Lilenbaum R, Sandler AB, et al. Treatment of non-small cell lung cancer, stage IV: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 2007;132(3 Suppl): 277S–89S.
[4] Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22(9):1589–97.
[5] Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26(21):3543–51.
[6] Scagliotti GV, Hanna N, Fossella FV, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. Oncologist 2009;14(3):253–63.
[7] Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 2009;374(9699):1432–40.
[8] Scagliotti GV, Kortsik C, Dark GG, Price A, Manegold C, Rosell R, et al. Pemetrexed combined with oxaliplatin or carboplatin as first-line treatment in advanced non-small cell lung cancer: a multicenter, randomized, phase II trial. Clin Cancer Res 2005;11(2 Pt 1):690–6.
[9] Zinner RG, Fossella FV, Gladish GW, Glisson BS, Blumenschein Jr GR, Papadimitrakopoulou VA, et al. Phase II study of pemetrexed in combination with carboplatin in the first-line treatment of advanced non-small cell lung cancer. Cancer 2005;104(11):2449–56.
[10] Grønberg BH, Bremnes RM, Fløtten O, Amundsen T, Brunsvig PF, Hjelde HH, et al. Phase III study by the Norwegian lung cancer study group: pemetrexed plus carboplatin compared with gemcitabine plus carboplatin as first-line chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 2009;27(19):3217–24.
[11] Patel JD, Hensing TA, Rademaker A, Hart EM, Blum MG, Milton DT, et al. Phase II study of pemetrexed and carboplatin plus bevacizumab with maintenance pemetrexed and bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer. J Clin Oncol 2009;27(20):3284–9.
[12] Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 1989;7(11):1748–56.
[13] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1):31–41.
[14] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92(3):205–16.
[15] Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:475–80.
[16] Orlando M, Lee JS, Yang C, Simms L, Park K. Efficacy of pemetrexed-cisplatin (PC) in East Asian patients (pts): subgroup analysis of a phase III study comparing PC versus gemcitabine-cisplatin (GC) in first-line treatment of advanced non-small cell lung cancer (NSCLC). Poster presented at: annual meeting of the American Society of Clinical Oncology 29 May to 2 June 2009, Florida.
[17] Ohe Y, Ichinose Y, Nakagawa K, Tamura T, Kubota K, Yamamoto N, et al. Efficacy and safety of two doses of pemetrexed supplemented with folic acid and vitamin B_{12} in previously treated patients with non-small cell lung cancer. Clin Cancer Res 2008;14(13):4206–12.

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

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

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

Abstract

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

Introduction

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

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

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

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

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

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

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

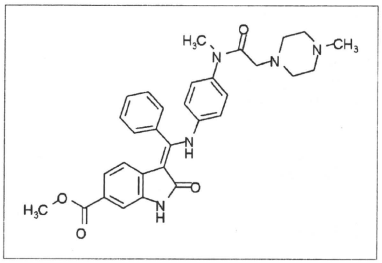

Figure 1. Structure of BIBF 1120.

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

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

Patients and Methods

Patient eligibility

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

Study design

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

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

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

Safety and efficacy assessments

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

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

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.

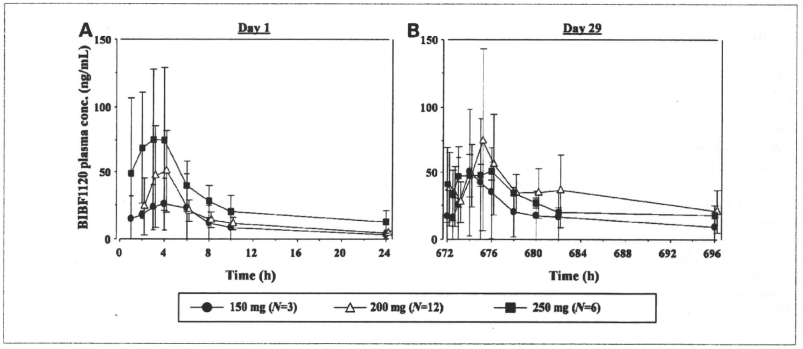

Figure 2. Mean (± SD) plasma concentration–time profiles of BIBF 1120 after single (A; day 1) and multiple (B; day 29) administration of 150, 200, and 250 mg BIBF 1120 twice daily.

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

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.

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

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.

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

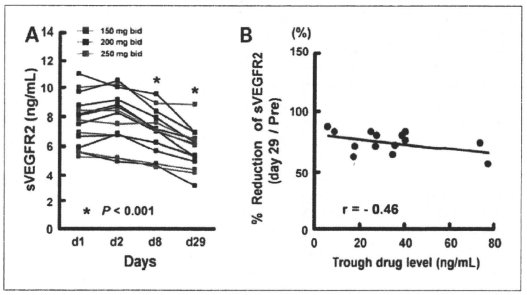

Figure 3. sVEGFR2 levels in plasma after BIBF 1120 treatment. A, plasma sVEGFR2 levels decreased during the 4-week treatment period. B, the decrease in sVEGFR2 at cycle 1, day 29 showed a modest inverse correlation with trough plasma drug levels of BIBF 1120 ($r = -0.46$).

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

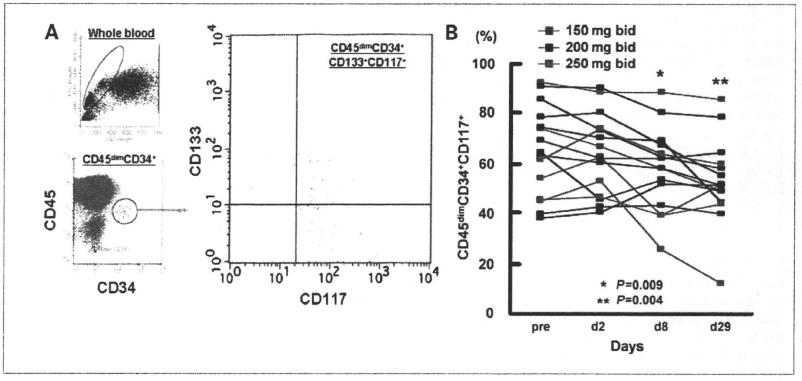

Figure 4. Levels of circulating CD117-BMD progenitor cells after BIBF 1120 treatment. A, representative flow cytometric analysis for determining the number of CD117-positive–BMD progenitor cells defined as $CD45^{dim}CD34^{+}CD117^{+}$. B, circulating levels of $CD45^{dim}CD34^{+}CD117^{+}$ cells decreased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

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

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,

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

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

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

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

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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

References

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

Published OnlineFirst August 5, 2010; DOI:10.1158/1535-7163.MCT-10-0379

of indolinones as triple angiokinase inhibitors and the discovery of a highly specific 6-methoxycarbonyl-substituted indolinone (BIBF 1120). J Med Chem 2009;52:4466–80.
11. Hilberg F, Roth GJ, Krssak M, et al. BIBF1120: triple angiokinase inhibitor with sustained receptor blockade and good anti-tumor efficacy. Cancer Res 2008;68:4774–82.
12. Mross K, Stefanic M, Gmehling D, et al. Phase I study of the angiogenesis inhibitor BIBF 1120 in patients with advanced solid tumors. Clin Cancer Res 2010;16:311–9.
13. du Bois A, Huober J, Stopfer P, et al. A phase I open-label dose-escalation study of oral BIBF 1120 combined with standard paclitaxel and carboplatin in patients with advanced gynecological malignancies. Ann Oncol 2010;21:370–5.
14. Ledermann JA, Rustin GJ, Hackshaw A, et al. A randomized phase II placebo-controlled trial using maintenance therapy to evaluate the vascular targeting agent BIBF 1120 following treatment of relapsed ovarian cancer (OC). J Clin Oncol 2009;27:15s (abstr 5501).
15. von Pawel J, Kaiser R, Eschbach C, et al. Efficacy, safety and pharmacokinetic (PK) results of a phase II study with the triple angiokinase inhibitor BIBF 1120 in patients suffering from advanced non small cell lung cancer (NSCLC). J Thorac Oncol 2008;3:S61.
16. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
17. Faivre S, Djelloul S, Raymond E. New paradigms in anticancer therapy: targeting multiple signaling pathways with kinase inhibitors. Semin Oncol 2006;33:407–20.
18. Beaudry P, Force J, Naumov GN, et al. Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. Clin Cancer Res 2005;11:3514–22.
19. Beerepoot LV, Mehra N, Vermaat JS, Zonnenberg BA, Gebbink MF, Voest EE. Increased levels of viable circulating endothelial cells are an indicator of progressive disease in cancer patients. Ann Oncol 2004;15:139–45.
20. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood 2001;97:3658–61.
21. Stamper IJ, Byrne HM, Owen MR, Maini PK. Modelling the role of angiogenesis and vasculogenesis in solid tumour growth. Bull Math Biol 2007;69:2737–72.
22. Summer R, Kotton DN, Sun X, Fitzsimmons K, Fine A. Translational physiology: origin and phenotype of lung side population cells. Am J Physiol Lung Cell Mol Physiol 2004;287:L477–83.
23. Blair A, Sutherland HJ. Primitive acute myeloid leukemia cells with long-term proliferative ability *in vitro* and *in vivo* lack surface expression of c-kit (CD117). Exp Hematol 2000;28:660–71.
24. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 2002;109:337–46.