Phase II Trial of Weekly Alternating Sequential BIBF 1120 and Afatinib for Advanced Colorectal Cancer*

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Abstract. *Aim: The feasibility of an alternating regimen of BIBF 1120, a potent, oral, triple angiokinase inhibitor, and afatinib (BIBW 2992), a potent ErbB family blocker, was explored in patients with advanced pretreated colorectal cancer (CRC). Patients and Methods: Patients received repeated courses of alternating 7-day treatment periods, first with BIBF 1120 250 mg twice daily and then afatinib 50 mg once daily. The primary endpoint was the objective response rate; the incidence/severity of adverse events (AEs) and pharmacokinetics (PK) were determined. Results: Forty-six patients (≥4 prior lines, most anti-VEGF and/or -EGFR pretreated) received BIBF 1120 and afatinib. No objective responses were observed; the best response was stable disease in 20 patients (43.5%). Seven patients (15.2%) remained progression-free for ≥16 weeks. Median progression-free survival was 1.9 months; median overall survival was 5.5 months. The most frequent drug-related AEs were diarrhoea (80.4%), asthenia (47.8%), nausea (43.5%) and rash (41.3%). PK assessments did not show obvious alterations for either drug. Conclusion: Weekly alternating administration of BIBF 1120 and afatinib is feasible; however, its efficacy was limited in this highly palliative patient population.*

*This work was previously presented at the ASCO 2008 meeting (abstract).

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Monoclonal antibodies targeting vascular endothelial growth factor (VEGF) or the epidermal growth factor receptor (EGFR) represent well-established treatment options for colorectal cancer. The VEGF antibody bevacizumab enhances the efficacy of oxaliplatin-based and irinotecan-based chemotherapy (1-3), presumably by normalization of the tumour vasculature (4-7). EGFR antibodies, such as cetuximab and panitumumab, may act on the tumour cells directly, inhibiting cellular growth, differentiation and proliferation, and inducing antibody-dependent cell-mediated cytotoxicity (8, 9). Monoclonal antibodies against the EGFR have demonstrated activity as monotherapy in pretreated patients (10, 11). Cetuximab in combination with chemotherapy also significantly prolongs progression-free survival (PFS) in the first-line treatment of patients with metastatic colorectal cancer compared with chemotherapy alone (12).

Optimal sequencing of treatment has not been fully established (13-17), even though combining antibodies with standard chemotherapy regimens in early lines of treatment can provide substantial benefits to many patients (18-24). As most patients diagnosed with advanced colorectal cancer (CRC) eventually succumb to their disease, high-intensity therapy at the early disease stage may increase the proportion of patients with a better long-term prognosis and increase the options for secondary surgery with curative intent (14). The recent availability of active and well-tolerated targeted agents has spurned the hopes that intensifying therapy may be achievable. Multitargeted therapies may be useful, particularly in early lines, when treatment aims for long-term benefit or even cure (25). Dual targeting of both the tumour vasculature and the tumour cells appears to be an attractive concept to improve the outcome of up-front therapy. Furthermore, ligands and receptors of the respective targeted pathways may be expressed both by the tumour cells themselves and via cross-talk, as well as overlap of intracellular downstream signalling pathways (26-

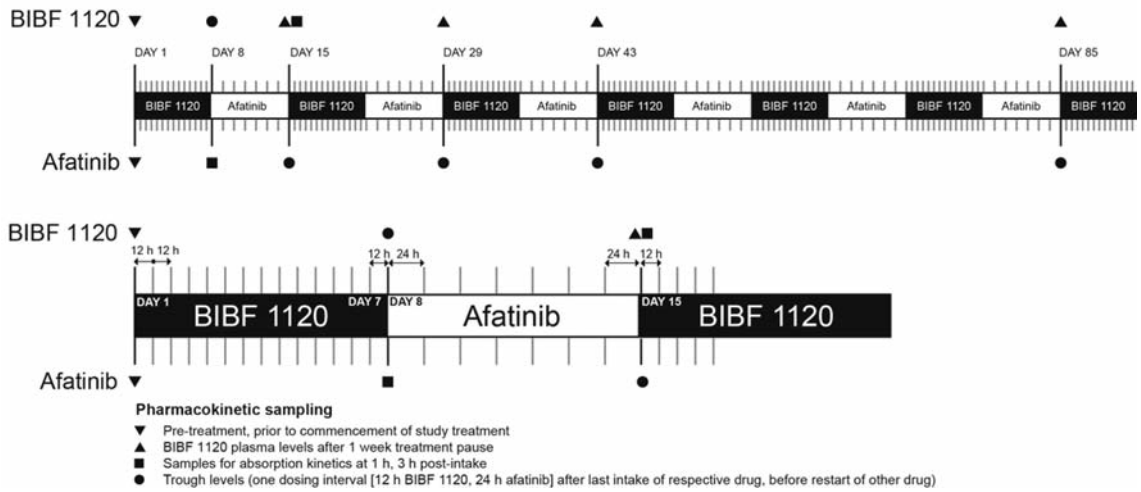

Figure 1. *Treatment schedule and pharmacokinetic sampling.*

34). Simultaneous targeting of tumour cell receptors may also offer the potential for synthetic lethality of therapeutic agents that have little activity as monotherapy (35-38), and cross-talk of pathways may involve mechanisms that can be used to overcome resistance (39).

BIBF 1120 is a novel, potent, triple angiokinase inhibitor targeting VEGFR 1–3, platelet-derived growth factor receptor (PDGFR)-α and -β, and fibroblast growth factor receptor (FGFR) 1–3 tyrosine kinases (40) – three key classes of receptors that are involved in tumour angiogenesis. Preclinical studies show that BIBF 1120 inhibits tumour growth in all animal models investigated to date (40). Phase I dose-escalation studies have investigated oral BIBF 1120 monotherapy in patients with solid tumours (41-43). The trials also provided encouraging dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) data, suggestive of good antivascular efficacy of BIBF 1120 in patients with liver metastases of CRC, as well as one partial response among the 16 patients with CRC who had been treated with twice-daily dosing (41, 44). The maximum tolerated dose (MTD) for BIBF 1120 has been established to be 250 mg twice daily. Phase II trials have confirmed that BIBF 1120 is well tolerated, and provided encouraging evidence of its efficacy in non-small cell lung cancer (NSCLC) and ovarian cancer (45, 46). Phase III trials in both indications are currently ongoing.

Afatinib (BIBW 2992) is a potent and irreversible inhibitor of both the EGFR/human epidermal growth factor receptor (HER)1 and HER2 kinases (47, 48). Preclinical studies demonstrate that afatinib has effective antitumour activity in a variety of human xenograft models (47). Phase I studies have shown that afatinib is well tolerated across a range of different dosing schedules (49-53), the MTD initially being defined as 70 mg once daily for non-continuous dosing of afatinib. Several phase II trials yielded promising results in NSCLC patients with *EGFR* mutations (54). Phase III trials in NSCLC and breast cancer are currently ongoing.

The good tolerability of BIBF 1120 and afatinib when given as single agents suggests that combination therapy is feasible. In view of the overlap of side-effects regarding diarrhoea and other abdominal/gastrointestinal symptoms, a weekly alternating schedule (Figure 1) was chosen. Based on the absence of relevant interaction with liver microsomal cytochrome P450 iso-enzymes, pharmacokinetic (PK) drug–drug interactions were considered unlikely to occur when both drugs are combined. Small-molecule EGFR inhibitors may not be easily combined with either FOLFOX or FOLFIRI when given as continuous monotherapy throughout the cycle of cytotoxic chemotherapy. In contrast, combinations with small-molecule angiogenesis inhibitors appear to be feasible, in particular in terms of gastrointestinal side-effects (25). This trial therefore introduces a regimen using the angiogenesis inhibitor during the first week and the EGFR inhibitor in the second week of a 14-day treatment period that, eventually, might be combined with a cycle of cytotoxic chemotherapy in the future. Even though inhibitors of VEGFRs may be more effective when administered in a continuous schedule, it was speculated that intermittent administration of BIBF 1120 in the proposed alternating regimen may still provide benefits with better tolerability in the combination schedule.

Patients and Methods

Study design. This phase II study assessed the efficacy of the combination regimen of BIBF 1120 and afatinib, using alternating monotherapy rather than concomitant dosing. The trial followed a single-arm, open-label design and was conducted at five sites across France between August 2006 and January 2007. Patients were to receive continuous treatment in repeated cycles of alternating monotherapy with BIBF 1120 250 mg twice daily (*i.e.* MTD

Table I. *Key eligibility criteria*.

Key inclusion criteria
Age >18 years
Signed informed consent
Histologically proven metastatic colorectal adenocarcinoma, measurable disease by RECIST criteria, with documented progression or unacceptable toxicity on the last therapy
Pretreatment with anti-VEGF or anti-EGFR antibodies allowed
WHO (ECOG) performance status <2, <1 if age >75 years
Total bilirubin within normal range, ALT and/or AST <1.5 × ULN; in case of liver metastasis, total bilirubin <1.5 × ULN, ALT and/or AST <2.5 × ULN
Serum creatinine <1.5×ULN
INR <2.5 ULN
Absolute neutrophil count ≥1.5×10^9l, Platelets ≥100×10^9/l
Key exclusion criteria
Prior treatment with small-molecule EGFR, HER2 or VEGFR tyrosine kinase inhibitors
Known hypersensitivity to the trial drugs or their excipients
Treatment with any investigational drug within 28 days of trial onset
Treatment with standard chemotherapy or cetuximab within the last 14 days
Treatment with bevacizumab within the last 28 days
Significant cardiovascular diseases

RECIST: Response Evaluation Criteria In Solid Tumors; VEGFR: vascular endothelial growth factor/receptor; EGFR: epidermal growth factor receptor; ECOG: Eastern Cooperative Oncology Group; ALT: alanine transaminase; AST: aspartate aminotransferase; ULN: upper limit of normal; INR: International Normalized Ratio; HER: human epidermal growth factor receptor.

determined in previous monotherapy trials) for 7 days followed by afatinib monotherapy 70 mg once daily (MTD as of study initiation) for 7 days (Figure 1); a half-cycle (comprising one treatment period of each drug) consisted of one 14-day period, and a full 28-day cycle consisted of two such 14-day periods, to be repeated until progressive disease was observed. After study initiation, the recommended dose for afatinib was reduced to 50 mg; therefore, all but two patients received an initial dose of 50 mg once daily (two patients received 70 mg for a limited number of days only). Dose modifications were foreseen in cases of drug-related undue toxicity (Common Terminology Criteria for Adverse Events [CTCAE] grade ≥2 diarrhoea persisting for eight or more consecutive days, grade ≥3 transaminase elevation or grade ≥2 transaminase elevation in conjunction with grade >1 bilirubin, grade ≥2 vomiting and nausea for eight or more consecutive days despite optimal supportive care, grade >3 hypertension despite optimal supportive care/intervention, grade ≥3 skin rash, and all other grade ≥3 AEs. In the case of gastrointestinal events/diarrhoea, the dose of both drugs was to be reduced – in case of skin AEs, that of afatinib only; if liver laboratory parameters increased, that of BIBF 1120 only; in all other events, both drugs were to be reduced. On the first occurrence of an AE, doses were to be reduced from 250 mg to 150 mg twice daily for BIBF 1120, and from 50 to 40 mg once daily for afatinib. If tolerability did not allow continuation of treatment at reduced doses, treatment was to be discontinued. All patients who discontinued treatment were followed until progression or death.

The trial was carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki (1996 Version), in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP) and in accordance with applicable regulatory requirements. Written informed consent was obtained from each patient prior to their participation in the trial.

Study population. Adult patients (Eastern Cooperative Oncology Group [ECOG] performance status (PS) of ≤2 [or ≤1 if age was >75 years]) were included (Table I) if they had metastatic (stage IV) colorectal adenocarcinoma, had already received prior treatment with both an oxaliplatin- and an irinotecan-containing regimen, and had discontinued the preceding line of therapy with measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.0 (55), due to either progressive disease or undue toxicity. Pretreatment with antibodies targeting VEGF or the EGFR was allowed; patients pretreated with a small-molecule tyrosine kinase inhibitor (TKI) targeting either EGFR, HER2 or VEGFRs, were excluded. Patients were to be entered no earlier than 14 days after completion of the prior treatment (bevacizumab: no earlier than 28 days after last administration), and unacceptable toxicities had to have resolved. Adequate renal and hepatic function were prerequisite.

Efficacy assessments. Radiological imaging was to be performed at baseline and after every second cycle (*i.e*. every 8 weeks). Clinical efficacy was assessed in terms of overall best objective response rate (*i.e*. proportion of patients with complete response [CR] or partial response [PR]) and rate of patients without clinical or radiological progression according to RECIST version 1.0 (55) at 16 weeks. Secondary efficacy endpoints were PFS and overall survival (OS).

Safety and tolerability assessments. Tolerability was evaluated by clinical criteria (performance status, electrocardiogram, AEs according to CTCAE version 3.0(56) and laboratory assessments. A pre-planned interim safety analysis was to be performed once the first six patients had completed the first cycle (4 weeks) of treatment. At the time when this interim safety analysis was eventually performed, 24 patients had entered the trial.

Pharmacokinetic sampling and data analysis. For quantification of plasma levels of BIBF 1120 and afatinib, blood samples were collected on day 1, 8 (*i.e.* after the first 7-day period of BIBF 1120 treatment), 15 (*i.e.* after the first 7-day period of afatinib intake; end of the first 14-day dosing period; half of a cycle), 29 (*i.e.* after one cycle; two 14-day dosing periods), 43 (*i.e.* after three 14-day dosing periods) and 85 (*i.e.* after six 14-day dosing periods; Figure 1). Samples were to be obtained just before the intake of the first dose of the respective study drug that was to be administered. In these samples, trough plasma levels were assessed in the morning on days 15, 29, 43 and 85 for afatinib, and on day 8 only for BIBF 1120. Plasma concentrations just before the restart of therapy with the respective drug in the next half-cycle, at the end of a 7-day interval during which the respective drug had been paused, were assessed in the morning on days 15, 29, 43 and 85 for BIBF 1120, coinciding with the time when trough levels for afatinib were determined (Figure 1).

Changes of plasma levels following administration of the respective drug were to be determined at 1 and 3 hours after intake; for this, additional blood samples were to be obtained on day 8 for afatinib at 1 and 3 hours following the very first dose of afatinib, and on day 15 at 1 and 3 hours after intake for BIBF 1120 (Figure 1).

Plasma concentrations of afatinib and BIBF 1120 were analyzed by a fully validated method using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) in the Department of Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. The lower limit of quantification for BIBF 1120 and metabolites was 0.5 ng/ml plasma, using a plasma volume of 100 μl. For afatinib, the lower limit was 0.5 ng/ml plasma, using a plasma volume of 50 μl.

Statistical analyses. The analyses in this trial were exploratory and descriptive. All patients who received at least one dose of BIBF 1120 and afatinib were included in the efficacy and safety analyses. The inclusion of 40 patients was anticipated to result in a reasonably narrow 95% confidence interval (CI) with a width of 18.8%, assuming a minimal underlying response rate of 7.5%.

Results

Patient population. Patient demographics are shown in Table II. Almost all of the 46 patients suffered from end-stage CRC and had received extensive pre-treatment (Table III). More than half of the patients had received at least four preceding lines of chemotherapy, although two patients were included without having been pretreated with an oxaliplatin-containing regimen. Almost all patients had received prior treatment with antibodies targeting the EGFR or the VEGF pathways; only two patients had not. Most patients had also received antibody treatment as part of the regimen immediately preceding inclusion, including five patients who had received bevacizumab within 28 days prior to study inclusion and two patients who had received cetuximab within 14 days prior to inclusion. Many patients had received both EGFR- and VEGF-targeting agents, and some patients had received targeted antibodies in three preceding lines of treatment (Table III). Figure 2 provides a diagrammatic representation of the flow of patients in the study.

Efficacy. No objective responses were observed (Table IV), and eight patients (17.4%) experienced early clinical progression, discontinuing from the study without undergoing any follow-up radiological assessment. Seven patients (15.2%) had remained progression-free 16 weeks after initiating treatment with BIBF 1120 and afatinib. The median PFS was 1.9 months (Figure 3). Median OS was 5.5 months (Figure 4). The median PFS of the seven patients who had not progressed by week 16 was 5.5 months; two of these patients had a longer PFS in this trial compared to the time to progression with the immediately preceding regimen (167 days actual PFS *vs*. 69 days with the preceding line; 113 days actual PFS *vs*. 80 days with the preceding line), four of the patients remained alive for the observation time of the trial (censored after 199-302 days), and three of the patients died after 142-167 days.

Safety and tolerability. Shortly after study initiation, a protocol amendment was implemented that modified the afatinib starting dose of 70 mg once daily to 50 mg once daily; this amendment, which became effective immediately after inclusion of the first patient, was due to updated safety data for afatinib (a second patient also received a few days' treatment at the dose of 70 mg due to an administrative error).

The first pre-planned safety assessment was performed when 24 patients had been included; the results confirmed adequacy of dosing, and the trial continued to full recruitment. The incidence of AEs regardless of relatedness to the study drugs is presented in Table V. Overall, the AEs reflected the known tolerability profiles of the drugs, as well as the nature of the underlying disease with primarily intra-abdominal spread. Gastrointestinal (GI), asthenia and skin events represented the largely predominating AEs. GI and skin AEs, as well as increases in liver laboratory parameters, were also the most frequent AEs considered to be related to the study drugs (Table VI), with the exception of asthenia – probably as most patients suffered from end-stage disease. No other CTCAE of grade 3 or 4 that was reported as likely to be drug-related occurred in more than a single patient (Table VII).

Dose reductions were most frequently prompted by GI AEs (reduction of BIBF 1120, or afatinib, or both) and increases of liver enzymes (BIBF 1120 only; Table VIII). Few patients only discontinued the drug due to AEs (AEs not related to progressive disease), with drug-related AEs being the reason for discontinuation in only two patients (4.3%) – one suffering from worsening of diarrhoea that had been present and requiring therapy at baseline, the other experiencing CTCAE Grade 4 asthenia.

Increases in liver laboratory parameters (liver enzymes, bilirubin; Table VIII) occurred in several patients. Most patients with relevant changes (increases to CTCAE grades 3 or 4) suffered from liver, or, occasionally, liver hilus lymph node metastases of increasing size during the trial, and also had elevated levels of liver parameters at baseline. However, the

Table II. *Baseline demographics and clinical characteristics of study patients.*

Demographic/clinical characteristic	N (%)
Number of patients enrolled	49*
Number of patients entered	46 (100.0)
Gender	
Male	28 (60.9)
Female	18 (39.1)
Age (years)	
<50	7 (15.2)
50-70	30 (65.2)
>70	9 (19.6)
ECOG performance status	
0	14 (30.4)
1	24 (52.2)
2	8 (17.4)
Race	
White	43 (93.5)
Black	1 (2.2)
Asian	2 (4.3)
Histological type	
Adenocarcinoma	46 (100.0)
Region of primary site	
Left colon	
Descending colon	6 (13.0)
Sigmoid colon	11 (23.9)
Rectum	21 (45.7)
Right colon	
Caecum	1 (2.2)
Ascending colon	4 (8.7)
Transverse colon	3 (6.5)
Metastases (any)	46 (100)
Liver	33 (71.7)
Lung	33 (71.7)

*Three patients died in the screening period. ECOG: Eastern Cooperative Oncology Group.

Table III. *Previous therapies of study patients.*

Previous therapy	N (%)
Prior anticancer therapy	
Surgery	43 (93.5)
Radiotherapy	16 (34.8)
Chemotherapy or other systemic therapy	
Adjuvant	21 (45.7)
First-line metastatic	46 (100.0)
Second-line metastatic	45 (97.8)
Third-line metastatic	38 (82.6)
>Third-line metastatic	26 (56.5)
Prior oxaliplatin and irinotecan	44 (95.7)
Prior irinotecan only	2 (4.3)
Prior treatment with antibody	
Cetuximab	41 (89.1)
Bevacizumab	24 (52.2)
Cetuximab or bevacizumab	44 (95.7)
Cetuximab and bevacizumab (separate lines)	19 (41.3)
Matuzumab*	1 (2.2)
Cetuximab 2×	2 (4.3)
In two subsequent lines	1 (2.2)
In lines separated by line without cetuximab	1 (2.2)
Bevacizumab 2×	7 (15.2)
In two subsequent lines	4 (2.2)
In lines separated by line without bevacizumab	3 (2.2)
Targeted antibody part of three lines of therapy*	8 (17.4)
Investigational agent*†	5 (10.9)
Time since last systemic treatment (days, median [range])	30 (13-269)
Time to progression after preceding line (months, median [95% CI])	4.13 (2.89-5.57)

*Including one patient who had been treated with cetuximab, matuzumab and bevacizumab. †One patient, matuzumab; four patients, pemetrexed. CI: Confidence interval.

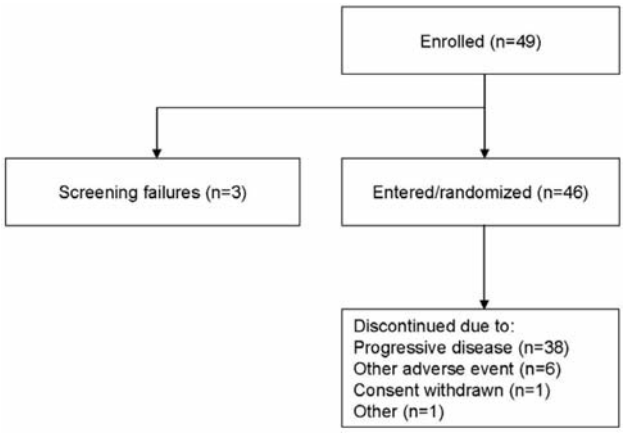

Figure 2. *CONSORT diagram.*

data were suggestive of a clear relationship with hepatic progressive disease only for bilirubin increases. Even though almost all patients with increased liver transaminases, the most common side-effect limiting the dosing of BIBF 1120, also suffered from liver metastases, most of which also progressed until the end of the trial, this increase occurred relatively early (six patients within the first 4 weeks; in the seventh patient after ~8 weeks), and were reversible down to at least grade 1 in five out of seven patients (the other two had progressing liver metastases). One patient had an increase of bilirubin from baseline grade 0 to grade 4 that was not considered to be drug related; the patient had been classified as having no liver metastases at baseline but had a 'liver node' that remained present as a non-target lesion throughout the trial. One patient with multiple large liver metastases was included in the trial 17 days after having received the last dose of bevacizumab; this patient was included despite relevant increased liver parameters at baseline. These were found to be due to thrombosis of the portal vein, with a fatal outcome after 2 weeks of trial participation; the maximum grade of transaminases and bilirubin during trial treatment of this patient was CTCAE grade 3 and 4, respectively.

Table IV. *Overall response of study patients.*

Best response	(n, %)
Objective response	0
SD	20 (43.5)
PD	18 (39.1)
Clinical PD before first reassessment	8 (17.4)
PFS at 16 weeks (n, %)	7 (15.2)
Kaplan–Meier rate PFS at 16 weeks (%, [95% CI])	15.9 (5.1-26.6)
Median PFS (months, 95% CI)	1.9 (1.8-3.2)
Censored patients	2
Median OS (months, 95% CI)	5.5 (4.56-7.61)
Censored patients	21

SD: Stable disease; PD: progressive disease; PFS: progression-free survival; CI: confidence interval; OS: overall survival.

Table V. *Adverse events regardless of relatedness to study drugs (>10% of patients, worst grade).*

Adverse event	Grade 3 n (%)	Grade 4 n (%)	Grade 5 n (%)	Total (any grade) n (%)
Diarrhoea	6 (13.0)	1 (2.2)	–	37
Asthenia	8 (17.4)	1 (2.2)	–	31
Nausea	2 (4.3)	–	–	24
Rash	–	–	–	19
Abdominal pain	3 (6.5)	–	–	18
Anorexia	1 (2.2)	–	–	18
Vomiting	2 (4.3)	1 (2.2)	–	16
Pyrexia	1 (2.2)	–	–	10
Epistaxis	–	–	–	9
Constipation	–	–	–	8
General physical health deterioration	2 (4.3)	1 (2.2)	3 (6.5)	7
Mucosal inflammation	–	–	–	7
Dyspnoea	2 (4.3)	–	1 (2.2)	6
Jaundice	1 (2.2)	2 (4.3)	–	6
ALT increased	4 (8.7)	–	–	5
Ascites	1 (2.2)	–	1 (2.2)	5
AST increased	2 (4.3)	–	–	5
Back pain	1 (2.2)	–	–	5
Dehydration	2 (4.3)	–	–	5
Dry skin	–	–	–	5
Headache	–	–	–	5
Peripheral oedema	–	1 (2.2)	–	5

ALT: Alanine transaminase; AST: aspartate aminotransferase.

None of the 25 deaths that occurred during the study, including 17 patients (40.0%) with additionally reported unrelated AEs, were considered to be related to the study drug, and AEs reported in the context of death in most cases were attributed to progressive disease. One patient with pulmonary metastases died due to pulmonary haemorrhage.

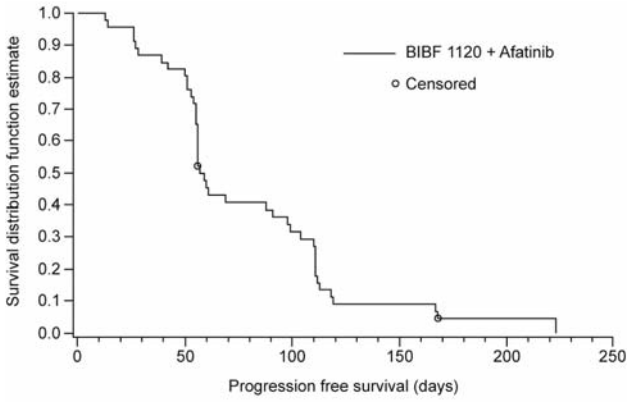

Figure 3. *Kaplan–Meier analysis of progression-free survival (treated set).*

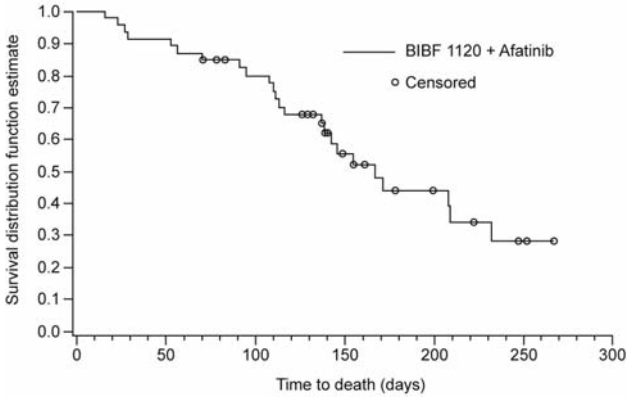

Figure 4. *Kaplan–Meier analysis of overall survival (treated set).*

Pharmacokinetics. On study inclusion, no plasma levels were detectable in the blood samples for either drug prior to first intake of study medication (data not shown; for the PK sampling scheme, refer to Figure 1). In the samples obtained on day 8 prior to the very first intake of afatinib, no plasma levels of afatinib were detectable, with the exception of three patients who had measurable levels in the range otherwise observed shortly after intake of afatinib.

Plasma values of afatinib after drug administration, obtained on day 8, increased during the sampling period to 26.7 ng/ml (gMean [range 0.590 ng/ml to 127 ng/ml]) at 3 hours after intake. Trough values were highly variable at a given time point (gCV 73.6–92.5%), but (overall) remained unchanged during the trial period (Table IX).

Plasma levels of BIBF 1120, obtained on day 15, increased during the sampling period to 20.6 ng/ml (gMean [range 0.841 ng/ml to 127 ng/ml]) at 3 hours after intake. Trough values at 12 hours after the last intake of the first 7-day BIBF 1120 dosing period (day 8) were highly variable (gCV 118%), with a gMean of 21.4 ng/ml (range 3.93 ng/ml to 124 ng/ml). BIBF

Table VI. *Drug-related adverse events of interest*.

Adverse event	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Any grade n (%)
Investigations	1 (2.2)	2 (4.3)	5 (10.9)	1 (2.2)	9 (19.6)
ALT increased	–	1 (2.2)	4 (8.7)	–	5 (10.9)
AST increased	–	3 (6.5)	2 (4.3)	–	5 (10.9)
AP increased	–	–	1 (2.2)	–	1 (2.2)
GGT increased	–	–	–	1 (2.2)	1 (2.2)
Transaminases increased	–	–	1 (2.2)	–	1 (2.2)
Gastrointestinal disorders	10 (21.7)	20 (43.5)	7 (15.2)	1 (2.2)	38 (82.6)
Diarrhoea	14 (30.4)	16 (34.8)	6 (13.0)	1 (2.2)	37 (80.4)
Nausea	13 (28.3)	6 (13.0)	1 (2.2)	–	20 (43.5)
Vomiting	11 (23.9)	4 (8.7)	–	–	15 (32.6)
Abdominal pain	1 (2.2)	1 (2.2)	–	–	2 (4.3)
Skin and subcutaneous tissue disorders	16 (34.8)	11 (23.9)	–	–	27 (58.7)
Rash	10 (21.7)	9 (19.6)	–	–	19 (41.3)
Erythema	4 (8.7)	–	–	–	4 (8.7)
Skin lesion	2 (4.3)	–	–	–	2 (4.3)
Acne	1 (2.2)	1 (2.2)	–	–	2 (4.3)
Dermatitis	1 (2.2)	–	–	–	1 (2.2)
General disorders and administration site conditions	8 (17.4)	10 (21.7)	4 (8.7)	1 (2.2)	23 (50.0)
Asthenia	9 (19.6)	8 (17.4)	4 (8.7)	1 (2.2)	22 (47.8)
General deterioration	–	1 (2.2)	–	–	1 (2.2)
Malaise	–	1 (2.2)	–	–	1 (2.2)

ALT: Alanine transaminase; AST: aspartate aminotransferase; AP: alkaline phosphatase; GGT: gamma-glutamyl transferase. When patients experienced multiple AEs within an system organ class (SOC) category, the worst grade reported for the SOC reflects the worst grade reported amongst those AEs.

1120 plasma concentrations before the restart of BIBF 1120 intake after 7 days of treatment with afatinib were consistently negligible, with values at or below the lower limit of quantification for most patients.

Discussion

This trial demonstrates the feasibility of a sequential combination regimen of small-molecule TKIs, an irreversible EGFR/HER2 inhibitor afatinib and a triple angiokinase inhibitor BIBF 1120. No unexpected drug-related toxicities were observed and the anticipated Gl side-effects were well managed. Only two patients discontinued therapy due to apparent intolerance to the regimen: one patient discontinued for diarrhoea that had already been present at baseline; the other suffered from asthenia in the context of tumour progression. Several patients showed increases in liver enzymes that might not be attributed to progression of CRC manifestations, although most of the patients had extensive, progressing liver metastases. The increase of liver enzymes occurred early, and subsequently resolved to at least maximum CTCAE grade 1 levels after dose interruption and/or reduction. Only some patients had dose reductions of BIBF 1120 to 150 mg twice daily. Reversible liver enzyme elevations have been observed in BIBF 1120 single-agent studies (41, 42), but not for afatinib.

Table VII. *All other drug-related adverse events of grade 3/4*.

Adverse event	Grade 3 n (%)	Grade 4 n (%)	Total Grade 3/4 n (%)
Blood and lymphatic disorders	1 (2.2)	–	1 (2.2)
Anaemia	1 (2.2)	–	1 (2.2)
Metabolism and nutrition disorders	1 (2.2)	1 (2.2)	2 (4.3)
Anorexia	1 (2.2)	–	1 (2.2)
Hypercreatininaemia	–	1 (2.2)*	1 (2.2)*
Musculoskeletal and connective tissue disorders	1 (2.2)	–	1 (2.2)
Arthralgia	1 (2.2)	–	1 (2.2)
Myalgia	1 (2.2)	–	1 (2.2)
Pain in extremity	1 (2.2)	–	1 (2.2)
Renal and urinary disorders	–	1 (2.2)	1 (2.2)
Renal failure	–	1 (2.2)*	1 (2.2)*
Any related adverse event (worst grade event)	14 (30.4)	4 (8.7)	28 (39.1)

*Same patient (renal metastases).

The absorption kinetics obtained for both drugs after 7-day pre-treatment with the respective other combination partner closely resembled those obtained in phase I trials of each drug alone (41, 50). Similarly, trough levels, as well as nadir levels for BIBF 1120, remained unchanged throughout the trial

Table VIII. *Laboratory values, dose reductions and discontinuations of study patients.*

Patients with liver metastases, n (%)	33 (71.7)
Patients with abnormal laboratory values at baseline	
ALT (grade 1), n (%)	7 (15.2)
AST (grade 1/2), n (%)	19/2 (41.3/4.3)
GGT (grade 1/2/3), n, (%)	8/10/14 (17.4/21.7/30.4)
AP (grade 1/2), n (%)	20/ 8 (43.5/17.4)
Total bilirubin (grade 1/2), n (%)	5/ 2(10.9/4.3)
Patients with increase to grade 3/4	
ALT (all grade >2, [grade 3/grade 4])	
Patients without liver metastases	6 (6/0) (13.0% [13.0/0%])
2 (2/0) (4.3% [4.3/0%])	
AST (all grade >2, [grade 3/grade 4])	
Patients without liver metastases	6 (6/0) (13.0% [13.0/0%])
2 (2/0)	(4.3% [4.3/0%])
AP (all grade >2, [grade 3/grade 4])	6 (6/0) (13.0% [13.0/0%])
GGT (all grade >2, [grade 3/grade 4])	15 (14/1) (32.6% [30.4/2.2%])
Total bilirubin (all grade >2, [grade 3/grade 4])	
Patients without liver metastases	8 (4/ 4) (17.4% [8.7/8.7%])
1 (0/1) (2.2% [2.2/0%])	
ALT or AST (all grade >2, [grade 3/grade 4])	
Patients without liver metastases	7 (7/0) (15.2% [15.2/0%])
2 (2/0) (4.3% [4.3/0%])	
Discontinuation of trial therapy, n (%)	
In the absence of PD	6 (13.0)
For AE considered drug-related	2* (4.3)
Dose reduction, n (%)	
BIBF 1120	12 (26.1)
BIBF 1120 only	7 (15.2)
Afatinib	6 (13.0)
Afatinib only	1 (2.2)
BIBF 1120 and afatinib	5 (10.9)
BIBF 1120 or afatinib	13 (28.3)

*One patient for diarrhoea (loperamide for diarrhoea at baseline); one patient for asthenia. ALT: Alanine transaminase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; AP: alkaline phosphatase; PD: progressive disease; AE: adverse event.

Table IX. *Comparison of afatinib trough plasma concentrations for the 50 mg dose afatinib group.*

	Afatinib concentration		
Time point	n	gMean (ng/ml)	gCV (%)
C24,7	34	29.1	80.8
C24,14	30	32.8	75.7
C24,21	27	24.4	92.5
C24,42	14	26.4	73.6

period. These data suggest that PK drug–drug interactions did not occur between these drugs.

This first trial combining BIBF 1120 and afatinib was intended as a first step towards a more intense treatment regimen. In particular, angiogenesis inhibition may need to be maintained continuously with the use of BIBF 1120. Ongoing preclinical studies in colon cancer models suggest that continuous exposure to BIBF 1120 will be needed for optimal activity (57). It should be noted that many patients in this trial presented with advanced disease with palliative treatment intent; almost all patients had already received and failed several lines of prior targeted agents. Antibodies were part of the regimens preceding inclusion into this study, and had been discontinued due to progressive disease.

The lack of clinical efficacy in this study in heavily pre-treated patients may not be conclusive for the sequential treatment approach of combining an EGFR/HER2 inhibitor with an angiogenesis inhibitor. Of note, two patients remained progression-free for a relatively long period of time and had a time to progression that exceeded that observed during the immediately preceding treatment line.

Recent evidence links failure to respond to EGFR antibodies to mutations in the downstream effector pathways (58-61). Whether these may also arise during treatment with EGFR inhibitors and preclude efficacy of retreatment with another EGFR inhibitor has not been investigated. Although afatinib differs from EGFR antibodies by irreversibly targeting the intracellular portion of the receptor, it is conceivable that the same resistance mechanisms may affect treatment with afatinib. Efficacy and tolerability of EGFR antibodies and EGFR TKIs may still differ in sensitive patients, and/or in earlier lines of treatment. Similarly, the activity of VEGF/VEGFR-targeting agents on vasculature (and on tumour cells bearing VEGFR), may be affected by prior treatment with VEGF antibodies, although true resistance to angiogenesis inhibition does not occur, and BIBF 1120 covers broad-spectrum angiokinases.

Since the conception of this trial, phase III data from other trials has matured, suggesting that treatment with EGFR and VEGF antibodies in combination with cytotoxic chemotherapy might not be effective in early lines of treatment (62, 63), and may even adversely affect prognosis in a subpopulation of patients.

Conclusion

This trial demonstrated that a weekly alternating administration of BIBF 1120 and afatinib is feasible, with well-manageable side-effects. The tolerability of the regimen suggests that more intense dosing approaches (*e.g.* combining continuous anti-angiogenic treatment with intermittent afatinib therapy) are feasible. Further phase I and II studies combining afatinib and/or BIBF1120 with chemotherapy in earlier lines of therapy are ongoing.

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Conflict of Interest Statement

Dr Stopfer and Dr Merger are employees of Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. Dr Amellal is an employee of Boehringer Ingelheim, Reims, France. No other author has any conflict of interest.

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