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ORIGINAL ARTICLE

Phase 1b trial of nintedanib in combination with bevacizumab in patients with advanced solid tumors

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Abstract

Purpose Vascular endothelial growth factor (VEGF) inhibitors have produced demonstrable but limited benefit for various cancers. One mechanism of resistance includes revascularization, secondary to upregulation of alternative pro-angiogenic platelet-derived growth factor receptor and fibroblast growth factor receptor pathways. Nintedanib is an oral, triple kinase inhibitor that blocks these pathways and may improve anti-tumor activity by overcoming resistance to anti-VEGF therapies. The primary objective of this first in-human study was to evaluate the safety and tolerability of nintedanib in combination with bevacizumab.

Methods Patients were treated with escalating doses of nintedanib (150 mg or 200 mg oral twice daily) and bevacizumab (15 mg/kg once intravenously every 3 weeks) until disease progression or unacceptable toxicity using standard 3 + 3 phase 1 design. Plasma levels of angiogenic biomarkers were correlated with clinical outcomes.

Results Eighteen patients with advanced tumors [lung ($n = 9$), colon ($n = 8$), and cervical ($n = 1$)] previously treated with at least two lines of chemotherapy including bevacizumab ($n = 9$, 50%) were enrolled. The highest dose of nintedanib was 200 mg twice a day with no observed dose-limiting toxicities (DLT). Common adverse events (AE) were fatigue (grade 1–3) and diarrhea (grade 1–2). Durable clinical response was observed in 55% patients pretreated with bevacizumab (1 complete and 4 stable response). Better disease control was correlated with higher than median baseline values for VEFGR2 and E-selectin, and lower levels for SDF-1α.

Conclusion Nintedanib was well-tolerated with bevacizumab with no DLT. Significant clinical activity was observed, including in bevacizumab-pretreated patients, suggesting nintedanib can overcome bevacizumab resistance.

Keywords Nintedanib · Bevacizumab · Metastasis · Solid tumors · Vascular endothelial growth factors

Introduction

Angiogenesis is a complex biologic process that plays a crucial role in tumor growth, progression, and metastasis. The vascular endothelial growth factor (VEGF) family (including ligands, VEGF-A to VEGF-D and receptors, VEGFR-1 to VEGFR-3) is considered to be one of the most important pathways involved in the regulation of tumor angiogenesis [1]. In addition, emerging evidence suggests that upregulation and activation of alternative pro-angiogenic pathways, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), placental growth factor (PlGF), transforming growth factors (TGF-β), and angiopoietins, may be involved in the acquisition of resistance to anti-VEGF agents [2–5]. Therefore, their inhibition could be associated with improved anti-tumor activity. The simultaneous abrogation of these pathways may result in effective growth inhibition of both endothelial and perivascular cells, which may be more effective than inhibition of endothelial cell growth alone via the VEGF pathway.

Nintedanib (BIBF1120) is an oral, triple angiokinase, adenosine triphosphate (ATP) competitive inhibitor that targets VEGFRs (VEGFR-1,2,3), PDGFRs (PDGFR-α/β), and FGFR (FGFR-1,2,3) pathways. Preclinical models have demonstrated that nintedanib may have a direct anti-tumor effect on malignant cells that overexpress platelet-derived growth factor receptor (PDGFR) and/or fibroblast growth

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factor receptor (FGFR) (e.g., H1703 NSCLC cells). In mouse xenograft models, nintedanib, as a single agent and in combination with standard chemotherapies, suppressed tumor growth of a broad range of various human tumor types, including renal cell, colorectal, ovarian, non-small cell lung, and prostate [6, 7].

Earlier studies with nintedanib demonstrated favorable pharmacokinetic and excretion profiles with metabolic characteristics independent of cytochrome P450-catalyzed metabolic pathways [8]. Available pharmacokinetic data indicate that the systemic exposure needed for its biological activity can be achieved with starting doses of 100 mg once daily [8]. Phase I dose escalation studies revealed that nintedanib is generally well-tolerated with mild to moderate adverse effects, such as gastrointestinal symptoms (nausea, diarrhea, and vomiting) and reversible elevations of liver enzymes. Initial signs of clinical activity, including an encouraging rate of patients with tumor stabilization, have been observed in patients with various solid tumours [9].

LUME-Lung 1 is a phase 3 trial that reported progression-free survival (PFS) and overall survival (OS) benefit in combination with docetaxel in NSCLC [10]. LUME-Colon 1 showed only a marginal increase in PFS over placebo in refractory metastatic colorectal cancer (mCRC) patients, with a toxicity profile similar to other anti-angiogenic agents and no benefit in OS [11]. Nintedanib showed similar efficacy to sorafenib in hepatocellular carcinoma with a favorable and manageable adverse events (AE) profile [12]. In comparison to bevacizumab, nintedanib showed a similar level of safety and efficacy, along with a comparable exposure and dose intensity of mFOLFOX6 [13].

Bevacizumab, a humanized monoclonal antibody against VEGF-A, is the first anti-angiogenic agent approved for mCRC and is associated with modest PFS and minimal OS benefit [14–16]. It has shown clinical benefit in combination with chemotherapy in several advanced tumors. However, the clinical benefit (duration of response) is limited, most likely secondary to the fact that tumors develop salvage or alternative pathways of angiogenesis. Therefore, by targeting the most active salvage pathways of tumor angiogenesis with the addition of nintedanib to bevacizumab, a potential benefit may be expected via delayed tumor growth.

Based on the phase I dose escalation trials with nintedanib monotherapy, the maximum tolerated dose was defined to be 250 mg for twice daily dosing [17]. The maximum tolerated dose for combination therapy of nintedanib in combination with pemetrexed, docetaxel, paclitaxel/carboplatin and FOLFOX is 200 mg bid. The predominant adverse events were nausea, diarrhea, vomiting, abdominal pain and fatigue of mostly low to moderate severity. Dose-limiting toxicities were mainly confined to reversible hepatic enzyme elevations which increased dose-dependently [10, 18, 19]. Most cases occurring at doses of 250 mg and above, and a very low incidence at doses below 200 mg and were reversible after discontinuation of nintedanib treatment. Therefore, the starting dose in this dose escalation study was determined to be 150 mg oral twice a day.

In this phase 1 study, we sought to determine whether nintedanib could be safely combined with bevacizumab in patients with advanced solid tumors with an approved indication for bevacizumab. Furthermore, plasma levels of angiogenic markers were correlated with preliminary antitumor activity.

Patients and methods

Study design and endpoints

This is an investigator-initiated (IIS), phase I, open-label, single center, dose escalation trial of the combination of nintedanib plus bevacizumab in patients with advanced solid tumors. The criteria for dose escalation was based on a standard 3 + 3 design, for dose escalation of nintedanib up to 200 mg twice daily in combination with fixed dose bevacizumab 15 mg/kg. The primary endpoint was to determine the maximum tolerated dose (MTD) and the dose-limiting toxicity (DLT) of nintedanib combined with bevacizumab. Secondary endpoints included: (1) evaluation of anti-angiogenic biomarkers during treatment, and (2) determination of clinical efficacy as measured by PFS and response rate.

The protocol was approved by the University of Alabama at Birmingham (UAB) Institutional Review Board and followed the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All patients signed informed consent forms, which fully disclosed the investigational nature of the trial, prior to enrollment. The study was supported by Boehringer Ingelheim and UAB Cancer Center core grant (CA 13148). This study was registered at ClinicalTrials.gov (NCT02835833).

Patients

Adult patients (≥ 18 years of age) with a histologically confirmed diagnosis of an advanced tumor for which bevacizumab has an indication (renal cell carcinoma, colorectal adenocarcinoma, non-squamous non-small cell lung cancer, platinum-refractory ovarian carcinoma, and cervical carcinoma) were eligible. Eligible patients had: (1) progressed on at least one line of standard systemic therapy; (2) Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; (3) adequate organ function as defined by adequate organ function as defined by normal serum bilirubin, AST/ALT ≤ 2.5× upper limit of normal (ULN), serum creatinine < 1.5× ULN, absolute neutrophil count (ANC) > 1500, platelets > 100 k and hemoglobin

> 9.0 without transfusion support in the past 28 days, urinalysis ≤ 1 + protein and (4) no contra-indications to anti-angiogenic therapy.

Patients were ineligible if they had: (1) previously experienced serious toxicities while on bevacizumab therapy; and (2) hypersensitivity to nintedanib. Patients with a history of prior brain metastasis were eligible provided the lesions were fully treated, asymptomatic, and stable as evidenced by repeat MRI brain imaging within 2 weeks prior to starting study treatment. Patients were also excluded if they: (1) required therapeutic anti-coagulation; (2) had a history of clinically significant hemorrhagic or thromboembolic event(s) in the past 6 months and/or surgery within the past 4 weeks, prior to start of study treatment; (3) had significant cerebrovascular and cardiovascular events within the past 6 months; (4) had proteinuria of ≥ grade 2; and (5) had pulmonary hemorrhage or hemoptysis within 6 months of starting study treatment.

Treatment

The starting dose of nintedanib was 150 mg, administered orally, twice every day with potential to escalate to a dose of 200 mg twice daily or deescalate to 100 mg twice daily. Bevacizumab was administered intravenously on day 1 of every 21-day cycle, at the dose of 15 mg/kg. Nintedanib administration was started on day 2 of each cycle (Table 1). Premedication with a 5-HT3 antagonist was used if needed. Intrapatient dose escalation was not permitted. Treatment duration was until disease progression, unacceptable toxicity, or patient refusal, whichever occurred first. Dose escalations were not allowed in patients requiring dose reductions due to toxicity. No dose adjustments for bevacizumab were allowed. If a patient experienced toxicities > grade 3, then the drug was held for subsequent cycles until toxicities returned to ≤ grade 1. For treatment-related grade 2/3 toxicity, the treatment was interrupted until resolution of toxicity to grade ≤ 1. If nintedanib was held, then bevacizumab was also placed on hold during treatment-interrupted period.

Table 1 Dose escalation table of nintedanib given orally in combination with a fixed dose of bevacizumab given IV (21-day cycle)

Dose level	Nintedanib (mg)	Bevacizumab (mg/kg)
1 ($n=3$)	150	15
2 ($n=3$)	200	15
2a ($n=12$)	200	15

Total number of patients, 18

2a expansion cohort

Study assessments

Safety

Once patients went off study, follow-up assessments with clinical examination and imaging were conducted every 2 months until 2 years after enrollment, in accordance with standard of care for treatment of patients with advanced solid tumors. Safety was evaluated at baseline, at regular intervals during treatment, and for 28 days after completing study therapy. Safety assessments including physical examination, hematologic parameters, serum chemistry, and urine analysis were performed every 3 weeks. Toxicities were characterized by type, frequency, seriousness, and relationship to study drug, and were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03. DLTs were assessed during the first cycle. The following AEs were considered DLT if they were attributable to study drug: (1) any grade 3 or 4 non-hematologic toxicity, as defined by CTCAE 4.03, even if believed to be unrelated to the study medications (except transient electrolyte abnormality, alopecia, untreated vomiting or diarrhea, and isolated elevation of gamma glutamyl transpeptidase); and (2) any grade 4 hematologic toxicity lasting ≥ 7 days or longer or associated with bleeding or requiring transfusions.

Serum biomarker correlative analysis

Angiogenic biomarkers were assessed in patient plasma ($n=18$) at the following time points: baseline, at second and fourth cycle, and the end of treatment. Biomarkers analyzed include pro-angiogenic factors VEGF, interleukin-8 (IL-8), ICAM-1, angiopoietin-1 and 2, KDR/VEGFR2, E-selectin, transforming growth factor-β (TGF-β), FGF-2, placental growth factor (PIGF), human stromal cell-derived factor-1α (SDF-1α), endocan, PDGF-AA, endoglin, and anti-angiogenic factors such as thrombospondin-1. Blood samples were collected in two EDTA tubes (2–4 ml each) and were sent to a UAB research biomarker core facility. The samples were immediately spun down and the separated plasma was stored in two aliquots at − 80 °C. All samples were analyzed at study completion. Enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN) was used to analyze the levels of PDGF-AA, thrombospondin-1, SDF-1α, and endoglin. The remaining biomarkers were analyzed using electrochemiluminescence (Meso Scale Discovery, Gaithersburg, MD).

Anti-tumor activity

Assessment of tumor response using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1)

guidelines was performed at baseline, beginning of cycle three (6 weeks), and then every two cycles of therapy until progressive disease.

Statistics

An algorithm-based 3 + 3 dose escalation design was used to find the maximum tolerated dose (MDT) of combination therapy and to characterize the AEs and DLTs. Descriptive statistics (means, medians, standard deviations, and ranges for continuous data and percentages for categorical data) were used to summarize patient characteristics, treatment administration, safety, and efficacy. Response rates, along with corresponding 95% confidence intervals, were calculated, based on the exact binomial distribution. PFS was defined as the time from the start of study to the first documentation of tumor progression or death and censored on the last day of study. OS was defined as the time from the start of study to death and censored on the last day of study. PFS and OS were evaluated with Kaplan–Meier method. Biomarker differences between responders and non-responders were assessed by the Wilcoxon test. Based on median at baseline, biomarkers were categorized as low and high levels. The association between PFS/OS and biomarker levels was evaluated using Cox proportional hazards model and the association between response status and biomarker levels was evaluated using Fisher's exact test. All analysis was performed using SAS 9.4 (Cary, NC) and p values smaller than 0.1 were considered significant.

Results

From June 2016 to June 2017, a total of 18 patients with advanced tumors [lung ($n = 9$), colon ($n = 8$), and cervical ($n = 1$)] pretreated with at least two lines of chemotherapy were treated with nintedanib combined with bevacizumab at 15 mg/kg in 2 dose escalation groups (dose levels 1 and 2): 150 mg × 2 ($n = 3$) and 200 mg × 2 ($n = 3$), respectively. Twelve patients were treated in dose expansion cohort (200 mg × 2). Nine patients (50%) were pretreated with bevacizumab. Baseline patient characteristics are reported in Table 2. Median patient age was 59 years (range 30–76). The majority of patients were male (67%) and white (72%). The most common tumor types were non-small cell lung ($n = 9$, 50%) and colorectal carcinoma ($n = 8$, 44%). Two patients (11%) had brain metastases. At baseline, patients had either ECOG 0 ($n = 6$) or 1 ($n = 12$). The majority of patients had received two or more lines of systemic anti-cancer therapy prior to the enrollment to this study. The median number of prior therapies was 3 (range 2–5). Median duration of treatment with this combination across all dose levels was 9.5 months (range 4–19). All 18 patients who received treatment on protocol completed at least one cycle.

Table 2 Patient demographics and baseline disease characteristics

Variables	$N = 18$	%
Age in years (median and range)	58.5 (30–76)	
Male	12	66.7
Race		
Black	5	27.8
White	13	72.2
Smoking history		
Current	1	5.7
Former	14	77.8
Never	3	16.7
Diagnosis		
Lung	9	50.0
Colorectal	8	44.4
Cervical	1	5.6
Histology		
Adenocarcinoma of lung	9	50.0
Adenocarcinoma of colorectum	8	44.4
Adenocarcinoma of the cervix	1	5.6
Performance status (ECOG)		
ECOG 0	6	33.3
ECOG 1	12	66.7
Weight loss	10	55.6
LDH abnormal	1	5.6
Brain metastasis	2	11.1
Pretreated with bevacizumab	9	50.0

During the dose escalation portion of the study, no DLTs were observed at nintedanib doses of 150 mg and 200 mg, twice a day. Therefore, the dose level of 200 mg twice a day was used in the expansion cohort.

Toxicity, as described in Table 3, was assessed in all treated patients ($n = 18$). Overall, 16 patients (89%) had no toxicity greater than grade 1/2 by the end of first cycle. Grade 3/4 toxicity was observed in 5 patients (28%) at the completion of the study. The most common non-hematologic grade 1/2 toxicities were fatigue ($n = 15$, 83%) and diarrhea ($n = 11$, 61%) at the completion of the study. Other adverse effects include nausea, proteinuria, elevated transaminases, hypertension, epistaxis and hypertension (Table 3). The grade 3 fatigue ($n = 3$, 17%), occurred beyond cycle 1 of the study. The grade 3 elevation of transaminases was seen in one patient that was resolved with brief treatment interruption. No grade 2–4 hematologic or grade 4 non-hematological toxicity was seen in this study. Eight patients (44%) developed grade 1 anemia during treatment course and required no dose interruption or adjustments. During the first cycle of treatment, one patient developed grade 3 proteinuria and another patient had grade 3 nausea. Treatment was

Table 3 Adverse events

AE	End of cycle # 1 Grade 3/4	*n* (%) All grades, *n* (%)	End of all cycles Grade ¾, *n* (%)	*n* (%) All grades, *n* (%)
Hematological[a]	0	2 (11)	0	8 (44)
Nausea	1 (5)	9 (50)	1 (5)	10 (55)
Diarrhea	0	6 (33)	1 (5)	11 (61)
Fatigue	0	12 (67)	3 (17)	15 (83)
Hypertension	0	2 (11)	1 (5)	4 (22)
Proteinuria	1 (5)	5 (1)	1 (5)	3 (17)
Epistaxis	0	4 (22)	0	5 (28)
Elevated transaminase	0	0	1 (6)	1 (6)

Grade 3 and 4 adverse events were possibly related to the therapy in all 18 evaluable patients

[a]Anemia, thrombocytopenia and neutropenia

briefly interrupted to control proteinuria and nausea. Eventually, both patients continued the study until progression with dose modification (dose reduced to 100 mg oral twice a day) of nintedanib. Grade 3 hypertension was observed in one patient that required brief interruption of treatment and management with anti-hypertensives. No patients required treatment with granulocyte colony-stimulating factors for management of neutropenia. Overall, no deaths due to toxicity were reported. At the time of last follow-up, no patients remained on study. Majority of the patients ($n=16$, 89%) discontinued treatment as a result of disease progression and two patients (11%) voluntarily discontinued the study due to grade 2 diarrhea (after 10 cycles) and grade 2 fatigue (after 8 cycles). Dose reduction was required in three patients (one in 150 mg and two in 200 mg dose level), due to grade 2 diarrhea and grade 3 emesis and elevation of transaminases, respectively.

Anti-tumor activity

All the 18 patients enrolled in this study were evaluated by RECIST response and included in the efficacy analyses. Figure 1 depicts patient duration of response. The overall response rate (ORR) was 11% [one complete response (CR) and one partial response (PR) (Table 4)]. The complete responder who had lung cancer was treated in the first cohort (150 mg twice a day) and previously received two lines of chemotherapy (one with bevacizumab and nivolumab). This patient achieved CR by the third cycle and was maintained on study for 10 cycles before discontinuing the study due to grade 2 diarrhea. Another patient with colon cancer who had received prior surgery, chemotherapy, and radiation achieved PR with 200 mg twice a day dose group by the fifth cycle. This patient was not pretreated with bevacizumab and was maintained on the study for seven cycles before discontinuing the study due to grade 2 fatigue.

Stable disease (SD) was achieved by 11 patients (61%). Five of these cancer patients ($n=1$ cervical, $n=2$ colorectal, and $n=2$ lung) had SD for ≥ 5 months. Disease control rate (DCR) (combined 1 CR, 1 PR, and 11 SD) was achieved in 13 patients (72%). No responses were observed in five patients (28%). Patients who did not respond ($n=5$, 28%) included two patients with colorectal (pretreated with bevacizumab) and three with lung adenocarcinoma of which two were pretreated with bevacizumab. After a median follow-up of 9.5 months, the median PFS was 4 months (95% CI 2–5) and median OS was 14 months (95% CI 8-NR). The PFS rate at 6, 12, and 18 months was 22%, 11%, and 11%, respectively, and the OS rate at 6, 12, and 18 months was 83%, 70%, and 28%, respectively. Durable clinical response (DCR) was observed in pre-bevacizumab treated patients (1 CR, 4 SD). The median OS for colon ($n=8$) was not achieved, with 57% alive at 18 months; median OS for lung ($n=9$) was 13 months, with 13% alive at 18 months. Figure 1 depicts the duration of response for individual patients. Fifty percent of patients ($n=3$ lung, $n=5$ colon, and $n=1$ cervical) were pretreated with bevacizumab. One lung cancer patient achieved CR (33%), three colon cancer patients (60%), and the cervical cancer patient achieved SD. Overall, the disease control rate was 55.5% in patients previously treated with bevacizumab.

Exploratory biomarker analysis

The results of exploratory association analysis are shown in Table 5. In the analysis of 15 angiogenic markers, higher (relative to median) concentrations of VEGFR2 ($p=0.0669$) and E-selectin ($p=0.0173$) and lower levels (relative to median) of SDF-1α ($p=0.017$) were associated with better PFS. Also, lower PIGF levels correlated with better DCR ($p=0.0294$). Percentage of change in plasma levels from baseline was assessed. Increased levels of ICAM ($p=0.0837$) and PIGF (0.005) and decreased levels of IL-8

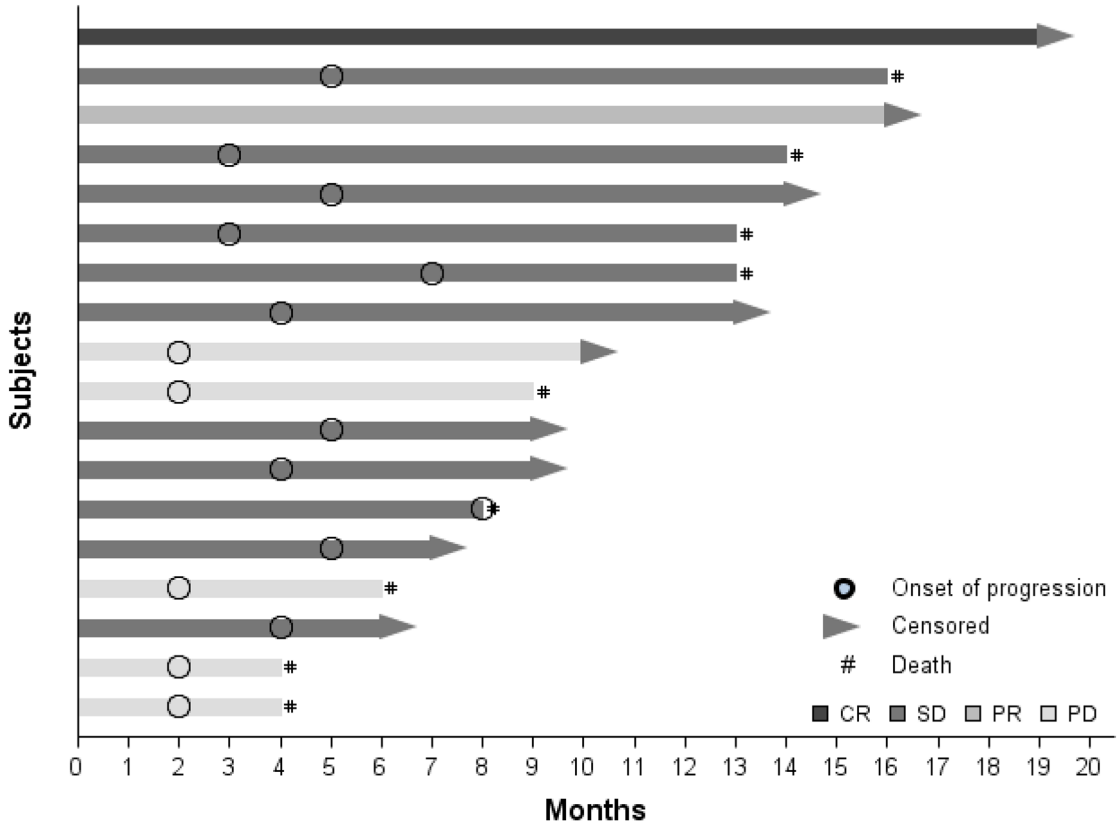

Fig. 1 Duration of response. *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Table 4 Response evaluation

Response	Frequency (%)
CR	1 (5.6%)
PR	1 (5.6%)
SD	11 (61.1%)
PD	5 (27.8%)
ORR (PR or CR)	2 (11.1%)
DCR (PR, CR or SD)	13 (72.2%)

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *ORR* overall response rate, *DCR* disease control rate

($p = 0.0565$) were associated with better DCR (data not shown here).

Discussion

Tumor angiogenesis results from the interplay of overlapping signaling pathways, thus the inhibition of one pathway could possibly lead to compensatory mechanisms of the others that could promote resistance. Recent strategies have focused on developing new multi-targeted drugs with the ability to simultaneously block several angiogenic signaling pathways, while maintaining an acceptable safety and tolerability profile [20]. Nintedanib is a next-generation tyrosine kinase inhibitor that targets three receptor pathways (VEGF, FGFR, and PDGFR). This unique targeting profile has the potential to effectively control tumor growth and dissemination, while also avoiding intrinsic and/or acquired resistance to VEGF inhibition alone. Upregulation of PDGFR and FGFR signaling has been validated as one of the common tumor escape mechanisms to a sustained VEGF/VEGFR blockade [21, 22].

Table 5 Association of potential angiogeneic biomarkers with clinical response parameters

Biomarkers (low versus high)	OS		PFS		DCR	
	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value	Risk difference (95% CI)	*p* value
IL-8	1.57 (0.39, 6.35)	0.5256	0.79 (0.29, 2.11)	0.6314	−0.11 (−0.52, 0.30)	1.0000
ICAM-1	0.22 (0.04, 1.11)	0.0661	0.42 (0.15, 1.19)	0.1025	0.33 (−0.05, 0.72)	0.2941
Angiopoietin-2	0.62 (0.17, 2.35)	0.4843	0.62 (0.23, 1.67)	0.3441	0.11 (−0.30, 0.52)	1.0000
KDR/VEGFR2	1.00 (0.22, 4.37)	0.9992	2.71 (0.93, 7.84)	0.0669	−0.11 (−0.52, 0.30)	1.0000
E-selectin	1.29 (0.26, 6.37)	0.7588	4.67 (1.31, 16.63)	0.0173	−0.11 (−0.52, 0.30)	1.0000
Angiopoietin-1	1.20 (0.32, 4.50)	0.7908	0.99 (0.37, 2.64)	0.9756	−0.11 (−0.52, 0.30)	1.0000
TGF-β	0.82 (0.22, 3.14)	0.7737	0.63 (0.23, 1.72)	0.3667	−0.11 (−0.52, 0.30)	1.0000
bFGF	0.31 (0.06, 1.54)	0.1516	0.60 (0.21, 1.70)	0.3385	0.11 (−0.30, 0.52)	1.0000
PIGF	0.37 (0.09, 1.51)	0.1671	0.77 (0.29, 2.06)	0.6017	0.56 (0.23, 0.88)	0.0294
VEGF	0.70 (0.19, 2.61)	0.5901	0.90 (0.34, 2.41)	0.8319	0.11 (−0.30, 0.52)	1.0000
SDF-1α	0.40 (0.08, 2.03)	0.2677	0.21 (0.06, 0.76)	0.0173	0.33 (−0.05, 0.72)	0.2941
Endocan	0.55 (0.13, 2.42)	0.4289	0.61 (0.21, 1.78)	0.3643	−0.11 (−0.52, 0.30)	1.0000
PDGF-AA	1.56 (0.38, 6.36)	0.5384	1.77 (0.63, 4.98)	0.2769	0.05 (−0.36, 0.46)	1.0000
Endoglin	0.40 (0.10, 1.63)	0.203	0.80 (0.30, 2.13)	0.6473	0.33 (−0.05, 0.72)	0.2941
Thrombospondin	0.62 (0.16, 2.33)	0.4788	0.79 (0.29, 2.11)	0.6314	0.11 (−0.30, 0.52)	1.0000

This is the first in-human study that evaluates the combination of nintedanib and bevacizumab in heavily pretreated, solid tumor patients. It is based on the rationale that the combination can improve anti-tumor activity by complementing VEGF inhibition and simultaneously overcoming resistance to VEGF inhibition. This study demonstrates that the combination of nintedanib and bevacizumab was safe and well-tolerated with grade 1/2 fatigue and diarrhea, which emerged as the most common adverse effects. As there were no DLTs, the last dose level tested in the escalation phase (nintedanib 200 mg twice a day and bevacizumab 15 mg/kg intravenously every 3 weeks) was chosen as the expansion phase dose. Only 3 patients (17%) experienced grade 3 treatment-related fatigue, which resolved after temporary hold of treatment. Three patients (17%) had transient grade 1 transaminase elevations during the course of treatment requiring no dose interruptions. The DLT in an earlier study with dosing of 250 mg twice daily was reversible for liver enzyme elevations [9]. Therefore, 200 mg twice daily administrations in our study allowed an increase in total daily exposure without additional toxicity. The most common non-hematologic toxicities (observed in ≥ 20% of patients) were fatigue, nausea, and diarrhea. The majority of non-hematologic AEs were reversible. There was no incidence of hematologic toxicity > grade 1 in this study. Eight patients developed grade 1 anemia while on study and none required blood transfusion. One patient developed sepsis, but it was not attributed to study medications. One patient developed proteinuria, grade 3, after first cycle; the onset of proteinuria was possibly related to bevacizumab.

This study was not powered to assess efficacy; however, the combination of nintedanib with bevacizumab suggests anti-tumor activity in terms of disease control at 72% for the whole cohort and 55% for patients previously treated with bevacizumab. 2 of the 18 patients (11%) achieved confirmed responses (one CR in 300 mg/day dosing cohort and one PR in 400 mg/day dosing cohort). Of the two responders, one received bevacizumab prior to the study (CR patient) and the other was bevacizumab naïve (PR patient). The responses were durable for at least 5 months in 8 patients (44%). These results appear promising with favorable side effect profiles in these pretreated patients. Therefore, it signals that the anti-VEGF activity in bevacizumab-naïve and bevacizumab-pretreated patients. Patients, who did not respond or progressed on this study, subsequently received other treatments as tolerated.

Plasma levels of angiogenic biomarkers were correlated with clinical outcomes. Tissue biopsy at the time of study entry or up on progression was not required per protocol. We therefore chose blood-based biomarker analysis where serial samples were collected at pre-specified intervals as it could assess the changes in molecular phenotype and also possible inter- and intra-tumoral heterogeneity. A previous work demonstrated prognostic significance of variations in plasma angiogenic biomarkers relative to median values [17, 23, 24]. Better DCR was correlated with lower baseline PIGF levels, as well as increased level from baseline. Longer PFS was associated with higher than median baseline values for VEFGR2 and E-selectin and lower values for SDF-1α.

Our study has several notable strengths. It represents the first time that a study with the combination of anti-VEGF and a triple angiokinase inhibitor has shown to be safe, tolerable, and associated with clinical activity. Also, we identified several plasma biomarkers that could potentially

prognosticate responders to this dual combination. However, our study was limited by enrolling relatively few patients from a single academic center; therefore, our results require validation in larger multi-center studies.

In conclusion, nintedanib 200 mg twice a day combined with bevacizumab shows a favorable safety profile with preliminary clinical efficacy. Nintedanib has the potential to overcome acquired resistance to anti-angiogenic drugs. Thus, this combination is a viable treatment option which warrants further investigation in larger studies.

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Compliance with ethical standards

Conflict of interest Dr. Francisco Robert: He is a member of speaker bureau for Boehringer Ingelheim. He is not involved with any of the drugs in this clinical study. No affiliations with or involvement in any organization or entity with any financial interest. All the authors declare that they have no potential conflicts of interest.

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