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An open-label, multicentre biomarker-oriented AIO phase II trial of sunitinib for patients with chemo-refractory advanced gastric cancer

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ABSTRACT

Background: Sunitinib monotherapy in pretreated patients with advanced gastric cancer (AGC) was investigated. Preplanned analyses of tumour biomarkers on treatment outcome were performed.

Patients and methods: Patients received sunitinib 50 mg/day for 4 weeks with 2 weeks rest until disease progression or unacceptable toxicity. The primary end-point was objective

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Sunitinib
Tyrosine kinase inhibitor
Objective response
Biomarkers

response rate (ORR). Secondary end-points included progression-free survival (PFS), overall survival (OS) and safety.

Results: Fifty-two patients were enrolled and treated (safety population, SP). In the intention to treat population ($n = 51$); the ORR was 3.9%, median PFS was 1.28 months [95% CI, 1.18–1.90], median OS was 5.81 months [95% CI, 3.48–12.32], the estimated one-year survival rate was 23.7% [95%CI: 12.8–36.5]. In subgroup analyses, tumour VEGF-C expression compared with no expression was associated with significantly shorter median PFS (1.23 versus 2.86 months, logrank $p = 0.0119$) but there was no difference in tumour control rate ($p = 0.142$). In the SP, serious adverse events occurred in 26 patients, leading to 13 deaths, all sunitinib unrelated. Thirty-eight patients died from progressive disease, nine died <60 days after treatment start.

Conclusion: Sunitinib monotherapy was associated with limited tumour response and good/moderate tolerability in this setting.

1. Introduction

Worldwide gastric cancer (GC) is the fourth most common malignancy and annually the second most common cause of cancer death. In Europe the incidence of GC in 2008 was approximately 150,000 cases.[1,2] Although GC mortality has markedly declined, the 5-year survival rate for patients with locally advanced disease is <20%, and approximately 30% for those undergoing surgery. Many chemotherapeutic agents demonstrate activity in advanced GC (AGC) with combination therapies reported to prolong survival and improve quality of life over single-agent therapy.[3] Currently cisplatin/5-fluorouracil (5-FU)-based chemotherapeutic regimens are the mainstay of treatment for metastatic disease.[3] In recent phase III trials, oxaliplatin, docetaxel, capecitabine and irinotecan demonstrated activity, increasing first-line treatment options,[4–7] with irinotecan also improving patient quality of life[8] for AGC patients. Median survival currently ranges from 8 to 11 months.[4,7] However, the efficacy data reported from a number of phase II and III studies are disappointing for AGC patients receiving second-line treatment with median survival ranging from 5 to 6 months.[9,10] The identification of less toxic and more effective treatment strategies is needed to improve the survival of AGC patients, particularly those whose disease has progressed during or after chemotherapy.

Tumour angiogenesis, growth and metastasis can be inhibited by blocking receptor tyrosine kinases (RTKs) overexpressed in GC, including vascular endothelial growth factor receptors (VEGFRs), epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptors (PDGFRs).[11–13] VEGF, EGFR and PDGF-A tumour expression is reportedly associated with progression and poor survival in GC patients.[14–16] Therapies that specifically target RTK signalling through a single receptor pathway have been investigated in phase II studies in AGC including gefitinib[17] and erlotinib[18] as single-agents, and bevacizumab[19] and cetuximab,[20,21] both in combination with chemotherapy. However, many tumours co-express several RTKs[11] and drugs targeting multiple RTKs involved in angiogenesis growth and metastasis may provide additional benefits relative to single receptor targeted inhibition.

Sunitinib malate (SUTENT®; Pfizer Inc., New York, NY) is an oral, multi-targeted tyrosine kinase inhibitor of VEGFR1, -2 and -3, PDGFR-α and -β, and several other related RTKs.[22–24] In a murine xenograft model of GC, sunitinib exhibited anti-angiogenic and antitumour activity at 40 mg/day (Pfizer Inc., Data on file). Single agent sunitinib (50 mg/day) for 4 weeks followed by 2 weeks off treatment, demonstrated superior efficacy to standard treatment with acceptable toxicity in patients with gastrointestinal stromal tumours (refractory or intolerant to imatinib), and in patients with advanced renal cell carcinoma.[25,26]

In this trial we investigated single agent sunitinib for objective response, safety and survival in patients with previously treated AGC. Pre-planned exploratory analyses of the influence of selected molecular biomarkers on trial end-points were also performed.

2. Patients and methods

2.1. Patient population

Inclusion criteria were: male or female patients aged ⩾18 years old with histologically proven metastatic adenocarcinoma of stomach or 0esophagogastric junction or lower oesophagus (Barrett carcinoma); measurable metastatic disease according to Response Evaluation Criteria in Solid Tumours (RECIST-criteria, version 1.0); failure of at least one prior irinotecan- or cisplatin-based palliative chemotherapy (progression of disease or unacceptable toxicity). Further inclusion criteria included: written informed consent; recovery from toxicity of previous chemotherapy or surgical procedures; life expectancy >12 weeks; Karnofsky Performance Status (KPS) ⩾70, adequate organ function, no other severe chronic or acute medical or psychiatric disorders; at least 3 weeks from last chemotherapy and at least 4 weeks from major surgery. Exclusion criteria were: tumour types other than adenocarcinoma; known brain or leptomeningeal metastases; intake of non-permitted concomitant drugs as defined per protocol; any prior or concomitant radiotherapy of target lesions; chronic gastrointestinal, thromboembolic or cardial disorders; hypertension; active uncontrolled infec-

tion or any other severe acute or chronic medical condition; pregnancy or lactation; hypersensitivity to treatment components; drug and alcohol abuse.

2.2. Study design and treatment

This prospective, open-label, multicentre, uncontrolled phase II trial was conducted in 13 clinical trial centres in Germany after approval of the leading and local ethics committees and the competent authority. The trial was performed according to ICH-GCP and the Declaration of Helsinki. The study was registered in the public NCT Clinical Trials Registry (ClinicalTrials.gov) under NCT00411151 before start of recruitment on Dec 11, 2006.

Patients received oral sunitinib (50 mg) once daily in 6-week cycles (4 weeks active treatment, then two weeks rest). Sunitinib was administered until tumour progression, unacceptable toxicity or discontinuation for any other reason. Study visits were performed on days 1, 15, 29 and 36 of every treatment cycle, and data including, vital signs, KPS, laboratory analyses, concomitant diseases and medication, adverse events (AEs) and tumour assessments (day 36 of cycle 1 and all following even cycles) were collected.

2.3. Safety and efficacy assessments

Pretreatment investigations included: medical history, physical examination, toxicity, electrocardiogram, KPS assessment, vital signs, laboratory analyses and a pregnancy test. Baseline tumour assessments comprised computer tomography (CT) of the abdomen and chest.

Toxicity was graded according to National Cancer Institute Common Terminology Criteria (NCI CTC), version 3.0. Depending on the severity of toxicity, sunitinib was interrupted or the dose reduced to 37.5 or 25 mg/day based on protocol recommendations. Dose re-escalation to the previous dose level was permitted at the start of the next cycle if toxicities did not exceed grade 1 at day 36 of the previous cycle. Sunitinib therapy was discontinued following: dose interruption >14 days within the active treatment cycle or >4 weeks between consecutive active treatment cycles; severe side-effects. Safety evaluation included the recording of AEs between the first day and up to day 28 after the last dose of sunitinib. Progressive disease (PD) was not reported as an AE unless the malignancy was fatal during the safety reporting period.

Tumour response was measured by CT-scans and assessed locally and graded by RECIST criteria, version 1.0. Partial response (PR) was confirmed by a repeat assessment within 4–6 weeks.

End of treatment visits were performed according to the protocol. Patients were followed-up for one year in three-monthly intervals where survival and progression status and further anticancer treatment were documented.

2.4. Protein expression

Immunohistochemical (IHC) staining for tumour VEGF-C, VEGFR3, PDGFRα and HER2 was performed as described previously.[27] Staining was evaluated by intensity (0–3) and the extent of the area stained (0–4). These classifications were added and divided into the categories negative and positive (weak–moderate–strong). Evaluation of staining was performed by two independent, blinded investigators.

2.5. Serum analysis

Serum samples were collected and stored at −80 °C. Samples were tested for VEGF-A, VEGF-C, VEGF-D and sVEGFR2 concentrations by quantitative ELISA (R&D, Minneapolis). Serum biomarker concentrations were calculated with the aid of a standard curve. For comparison biomarker levels were also measured in the serum from controls ($n = 7$) comprising healthy volunteers recruited from the hospital.

2.6. Gene mutation analysis

For each tumour two 5 μM sections of formalin-fixed paraffin-embedded (FFPE) tissue were deparaffinised and DNA were extracted with the high pure PCR Template Preparation Kit (ROCHE Diagnostic Spa, Indianapolis, USA), according to the manufacturer's instructions. *KRAS* (exon 12/13), *BRAF* (V600) and *PIK3CA* (exon 9 and 20) gene mutations were screened for by high resolution melting analysis using LightCycler 480 High Resolution Melting Master (ROCHE Diagnostic Spa) according to the manufactures instructions. Tumour DNA was amplified using gene specific PCR primers (sequences available on request to the author) by real-time PCR on a light cycler 480 instrument (ROCHE Diagnostic Spa). Data were analysed by the LightCycler 480 Gene Scanning Software Module for base deletion and variant identification (ROCHE Diagnostic Spa).

2.7. Statistical considerations

The primary end-point was the objective response rate (ORR) defined as the percentage of patients with a confirmed reduction in tumour size compared to baseline. ORR was summarised in terms of percentage, with a 95% Clopper-Pearson confidence interval (CI). The primary analysis population was the intention-to-treat (ITT) population comprising all patients enrolled in the study who received at least one dose of study treatment. The evaluable patient population (EPP) was defined as patients who had received at least one cycle of treatment and for which at least one tumour assessment has been performed after baseline. Sensitivity ORR was also analysed in the per protocol population (PPP), which consisted of all patients in the EPP without major protocol violations.

Secondary end-points were progression-free survival (PFS), overall survival (OS) and one-year survival. PFS was defined as the time from first dose of trial medication until first documentation of objective tumour progression or death due to any cause. OS was defined as the time from first dose of trial medication to death due to any cause. PFS and OS curves were generated using the Kaplan–Meier method. Median event time and 2-sided 95% confidence interval for the median was provided for PFS and OS. One-year survival was estimated by the OS distribution function for patients surviving for at least one year after first dose of trial medication.

Safety analysis was performed in the safety population (SP) comprising all patients who received at least one dose of study medication.

Sample size was determined under the following considerations: The historical ORR for the population under investigation was assumed up to 5%. A trial of 50 patients has 89.6% power for testing that the ORR for sunitinib is higher than 5% if the assumed response rate is 20% (overall 2-sided significance level of 0.05). If at least 8 responders out of 50 patients (16%) were observed then the lower bound of the 95% confidence interval would be 5% or higher. Enrolled patients dropping out before receiving any dose of trial medication were replaced to achieve 50 patients treated with sunitinib.

3. Results

3.1. Patients

Fifty-two patients were enrolled and treated with sunitinib and comprised the SP. The ITT population contained 51 patients as tumour diagnosis could not be confirmed in one patient. Fourteen patients were excluded (because no tumour assessment under study treatment had been documented), giving rise to the EPP ($n = 38$). Non-evaluable patients terminated study treatment before tumour staging in cycle 1 could be assessed, this was due to death, clinical/symptomatic deterioration, toxicity related to study medication or other reasons. Overall, 37 patients were excluded to give a PPP ($n = 15$) due to the following major protocol violations: violation of any inclusion or exclusion criterion (23 patients, 45%), any missing tumour assessment (25 patients, 49%), continuation of treatment in spite of progression (5 patients, 10%), interruption of sunitinib intake of more than 4 weeks (two patients, 4%) and reduced start dose (one patient 2%). Patient disposition is described in Fig. 1. Patient and disease characteristics at baseline in the ITT population are summarised in Table 1. Most patients had adenocarcinoma of the stomach or gastroesophageal junction. Almost all patients were previously treated with irinotecan- or cisplatin-based palliative chemotherapy. Thirty-eight patients (74%) received sunitinib as second-line therapy and 10 patients (20%) received sunitinib as third-line therapy. Three other patients had received more than three lines of previous therapy.

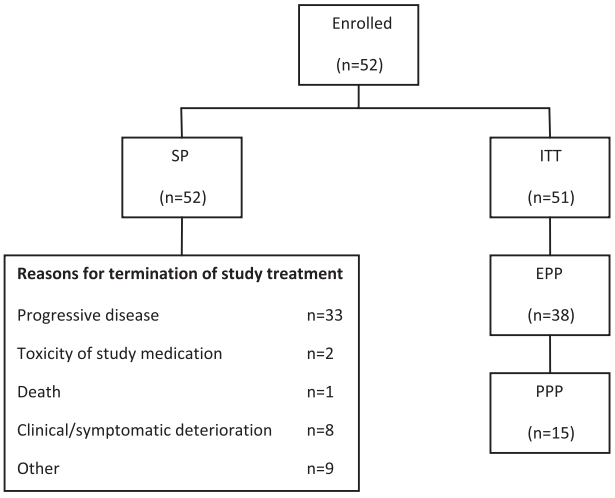

Fig. 1 – Patient disposition. EPP, evaluable patient population; PPP, per protocol population; SP, safety population.

3.2. Treatment characteristics

The median duration of sunitinib treatment in the ITT population was 28 days (range, 1–157) and the median number of cycles was 1 (range, 1–6). Thirty patients (59%) terminated their sunitinib therapy in cycle 1, 15 (29%) in cycle 2, one (2%) in cycle 3, one (2%) in cycle 4, two (4%) in cycle 5 and two (4%) in cycle 6. The median dose of study medication over the total study period was 1400 mg (range 50–7850 mg). Fifty-one patients had an initial dose of 50 mg sunitinib, one patient started with 25 mg sunitinib. Five (10%) patients had a dose reduction to 37.5 mg sunitinib. Three patients interrupted their treatment with sunitinib in cycle 1 for 1–8 days. Sunitinib was terminated in two patients during cycle 1 due to toxicities.

3.3. Treatment activity

Best overall response in the study populations is shown in Table 2. No CRs were reported. In the ITT population, 4% of patients displayed PRs, 16% had stable and 55% PD. The ORR was 3.9%. Best overall response was comparable in the EPP and PPP (Table 2).

PFS in the ITT population is shown in Fig. 2A. Median PFS was 1.28 months [95%CI, 1.18–1.90], and was the same in the EPP. Median PFS in the PPP was 1.18 months [95% CI, 1.15–1.28]. The OS for the ITT population is shown in Fig. 2B with a median OS of 5.81 months [95% CI, 3.48–12.32] and a one-year survival probability of 23.7% [95% CI, 12.8%–36.5%]. Median OS was higher in the EPP and PPP at 8.41 months, [95% CI, 5.09–9.36] and 9.36 months, [95% CI, 5.81–12.32] respectively.

3.4. Safety and tolerability

The majority of AEs (79%) were grade 1–2, most common were fatigue, nausea, leukopenia and thrombocytopenia (Table 3). Approximately 55% of AEs observed were suspected to be related to sunitinib treatment. Treatment-related grade 3 AEs were reported in 14 patients including anaemia ($n = 2$), neutropenia ($n = 4$), leukopenia ($n = 5$), thrombocytopenia ($n = 2$), vomiting ($n = 2$), fatigue ($n = 3$), diarrhoea ($n = 1$), palmar-plantar erythrodysaesthesia syndrome ($n = 1$), increased ALT ($n = 1$) and increased blood bilirubin ($n = 1$). Treatment-related grade 4 AEs were reported in five patients including neutropenia ($n = 3$), increased blood amylase ($n = 1$) and increased lipase ($n = 1$).

Forty serious AEs (SAEs) occurred in 26 (50%) patients. Five SAEs were suspected to be related to sunitinib including anaemia ($n = 1$), vomiting ($n = 2$), pneumonia ($n = 1$), thrombocytopenia ($n = 1$). Deaths due to SAE were reported in 13 (25%) patients, none were considered sunitinib-related. There were no suspected unexpected serious adverse reactions recorded. For the majority of AEs (80%) no specific action for sunitinib therapy was taken. Only two patients discontinued sunitinib treatment due to toxicity (increased bilirubin and thrombocy-

Table 1 – Patient and disease characteristics at baseline in the ITT population ($n = 51$).

Characteristic	Number	%
Age (years)		
Median	59	
Range	28–81	
Gender		
Male	39	76
Female	12	24
Race		
European	40	78
Caucasian	11	11
KPS		
100%	17	33
90%	15	29
80%	18	35
70%	2	4
Localisation of adenocarcinoma		
Stomach	36	71
Gastrooesophageal junction	11	22
Lower oesophagus	5	10
Grading		
2	18	35
3	26	51
4	2	4
Previous therapies		
Irinotecan	20	39
Cisplatin	28	55
Missing	3	6
Localisation of target lesions		
Primary tumour	3	6
Lung	8	16
Liver	28	55
Lymph node truncus coeliacus	10	20
Lymph node mediastinum	11	22
Lymph node spleen/pancreas	3	6
Lymph node paraaortal caudal	9	18
Peritoneum	2	4
Soft tissue	5	10
Other	13	25
Total size of target lesions (mm)		
Median	61	
Range	10–236	
Missing	3	
Localisation of non-target lesions		
Primary tumour	1	2
Bone	1	2
Lung	4	8
Liver	15	29
Lymph node truncus coeliacus	4	8
Lymph node mediastinum	3	6
Lymph node para-aortal caudal	3	6
Peritoneum	3	6
Other	9	18

KPS, Karnofsky Performance Status.

topenia, respectively). There were no relevant changes in vital signs during the study.

At the end of follow-up 38 (73%) patients had died due to tumour progression; nine (17%) of these within 60 days from the start of treatment.

3.5. *Tumour biomarker analyses*

Protein expression was performed by IHC in the EPP for VEGF-C, VEGFR3 and PDGFRα in 28 evaluable patient tumours and for HER2 in 23 evaluable patient tumours (Fig. 3). Associations

Table 2 – Best overall response in patient treatment populations.

	ITT (n = 51)	%	EPP (n = 38)	%	PPP (n = 15)	%
	n		n		n	
Best overall response						
CR	0	0	0	0	0	0
PR	2	4	2	5	1	7
SD	8	16	8	21	2	13
PD	28	55	28	74	12	80
Missing	13	25	0	0	0	0
ORR (CR + PR)	2	3.9	2	5.3	1	6.7
[95% CI] (%)	[0.48–13.46]		[0.64–17.75]		[0.17–31.95]	

CR, complete response; EPP, evaluable patient population, ITT, intent to treat; PPP, per protocol population; PR, partial response; PD, progressive disease, SD, stable disease.

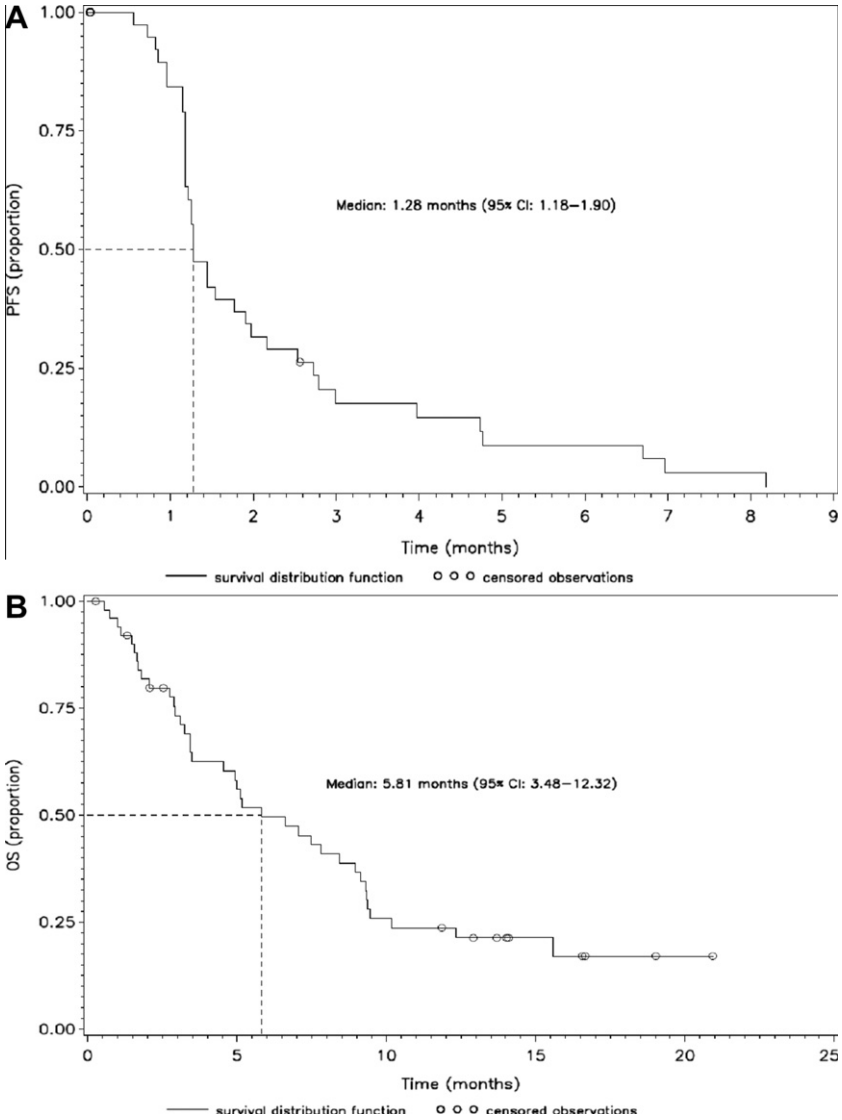

Fig. 2 – Progression-free (A) and overall survival (B) in the ITT population (n = 51).

between tumour biomarker expression and clinical outcome are presented (Table 4). In this subgroup (n = 28) median PFS (1.44 months [1.08–1.80]) and median OS (7.79 [4.60–10.97]) were comparable with the ITT population. Of note, tumour VEGF-C expression was associated with an increase in PD and significantly shorter PFS (Table 4, Fig. 4) compared with

Table 3 – Frequency of AEs regardless of relationship in ⩾10% of patients in the SP (n = 52).

AE	n	%
Non-haematologic		
Fatigue	19	37
Nausea	16	31
Mucosal inflammation	9	17
Anorexia	9	17
Vomiting	8	15
Diarrhoea	8	15
Dysgeusia	6	12
Headache	5	10
Paraesthesia	5	10
Cough	5	10
Abdominal pain	5	10
Palmar-plantar erythrodysaesthesia syndrome	5	10
Blood bilirubin increased	5	10
Haematological		
Leukopenia	11	21
Thrombocytopenia	11	21
Neutropenia	8	15
Anaemia	7	13

Abbreviations: AE, adverse events; SP, safety population.

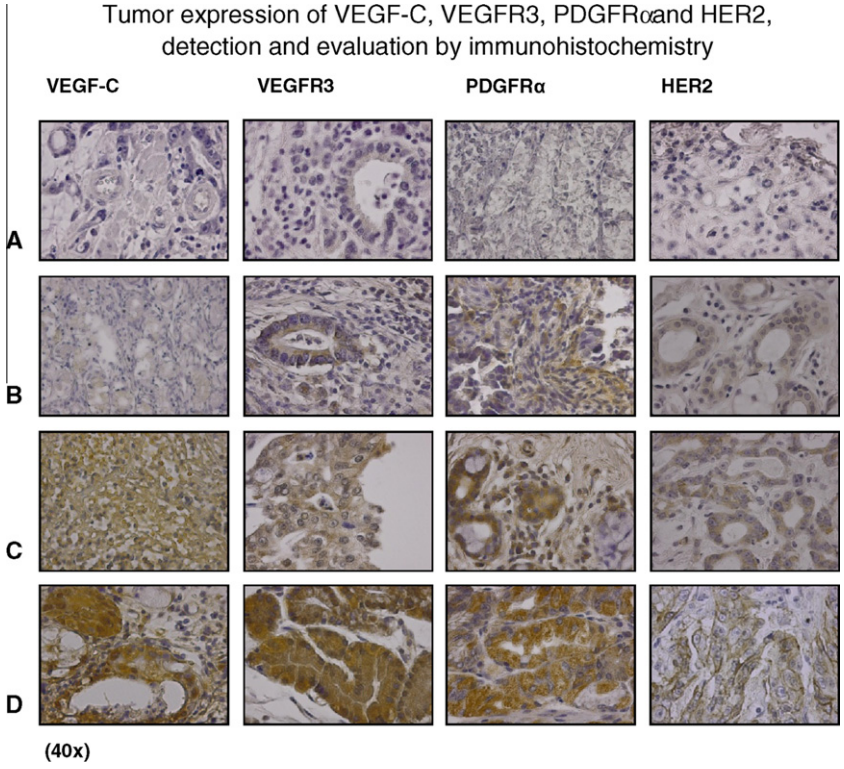

Fig. 3 – Tumour expression of VEGF-C, VEGFR3, PDGFRα and HER2. (A) No staining; (B) weak staining; (C) moderate staining; (D) strong staining.

patients whose tumours did not express VEGF-C (median PFS 1.23 months versus 2.86 months, logrank $p = 0.0119$).

Twenty-nine patients were evaluable for gene mutation analyses; no *KRAS* or *BRAF* mutations were detected. *PIK3CA* mutations were detected in 2/29 (7%) patients, one exhibited PD and the other died 54 days after start of treatment.

3.6. *Serum biomarker analysis*

Serum analysis was performed repeatedly in nine patients with SD or PR during follow up of at least 2 cycles. There were no significant differences found in serum biomarker concentrations in patients following one cycle of treatment

Table 4 – Association between tumour biomarker expression with clinical outcome.

Biomarker	Total, N	IHC	Tumour control			PFS		
			PD, *n*	SD/PR, *n*	*p*-Value	0–50 d, *n*	>50 d, *n*	*p*-Value
VEGFR3	28	Negative	2	2		1	3	
		Positive	19	5	0.253	15	9	0.285
VEGF-C	28	Negative	4	4		1	7	
		Positive	17	3	0.142	15	5	0.004
PDGFRα	28	Negative	0	1		0	1	
		Positive	21	6	0.25	16	11	0.429
HER2	23	Negative	12	2		9	5	
		Positive[a]	4	5	0.066	2	7	0.089

[a] Moderate and strong positive staining.

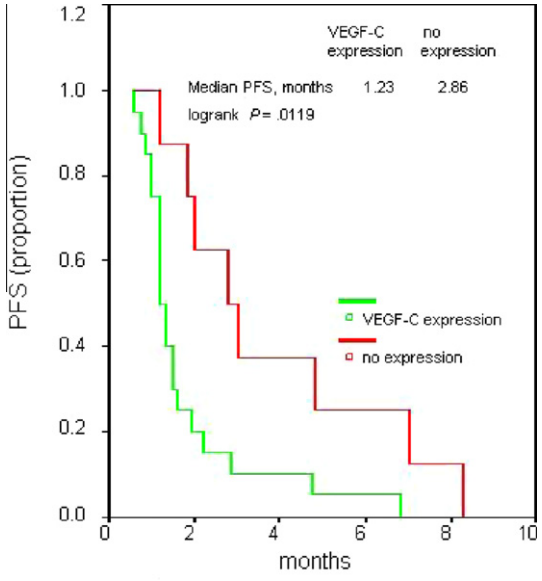

Fig. 4 – Progression-free survival in patients by tumour VEGF-C expression (*n* = 20) and no expression (*n* = 8).

compared with levels at baseline and those from normal controls, or in patients displaying a tumour response compared with non-responders. Some differences of note were a decrease in sVEGFR2 concentrations in patients after one cycle of treatment compared with baseline levels, and an increase in VEGF-A concentration in patients displaying a tumour response compared with non-responders (data not shown).

4. Discussion

In this study, sunitinib monotherapy in heavily pretreated patients with AGC led to RECIST defined PRs in 4% of patients and SD was recorded in 16% of patients. In support similar ORR were recorded in the EPP (5.3%) and PPP (6.7%) compared with the ITT population, therefore classifying patients without any tumour assessment as non-responders had no impact on ORR. These data are comparable to a recent phase II study of sunitinib monotherapy in 78 Korean patients with AGC, the ORR rate was 2.6% and 32% of patients had SD of >6 weeks, including four patients with SD lasting >24 weeks giving a clinical benefit of 7.7%.[28]

Observations with targeted agents in other tumour types, for example imatinib in GIST[29] and sunitinib in RCC[30], suggest that one-dimensional RECIST measurements can miss important information about changes in tumour density and metabolic response.[45] This raises the question as to whether ORR is the most suitable primary end-point for assessing sunitinib in AGC. Of note in the present study the tumour control rate was 19.6% in the ITT in this setting.

In the present study and in the Korean study PFS and OS times are comparable, and furthermore these values are similar to those reported in the second-line setting for single-agent chemotherapy, including docetaxel,[31,32] paclitaxel,[33,34] irinotecan[35] and mitomycin C[36] and for various combinations of chemotherapy. Whilst the current and the Korean trial provide evidence that sunitinib affects the late clinical course of AGC, they do not support further investigation of sunitinib monotherapy in this setting.

The type and frequency of reported AEs were generally consistent with those previously reported for single-agent sunitinib.[25,26,38,39]

There are an increasing number of reports on the ability of tumour biomarkers to identify those patients who will derive benefit from treatment with targeted-agents.[40,41,37] The biomarkers in the present analyses were chosen based on their role in RTK signalling or in facilitating tumour proliferation and metastasis. Mutations to *KRAS*[42], *BRAF*[42] and *PIK3CA*[43] genes and deregulated expression of VEGF/VEGFR[14,16,44], PDGF[15] proteins have been reported in GC.[45] The number of patients in the present biomarker analyses was small and the data should therefore be considered as hypothesis forming. No associations were found between tumour biomarker expression and patient or disease characteristics at baseline. However, there were some noteworthy associations with clinical outcome which would require confirmation in larger studies. We found that patients with tumours expressing VEGF-C were more likely to have PD and experienced reduced PFS times than patients whose tumours were negative for expression. In support, VEGF-C expression is essential during lymphogenesis which is associated with poor prognosis in GC patients.[46,47] Furthermore expression of the VEGFC/VEGFR3 axis is reported to be important in stimulating tumour progression and influencing patient clinical outcome in this setting.[48,49]

We also investigated the association between serum biomarker concentrations with patient prognosis, and no signif-

icant associations were found. However, due to the small sample size examined these data should be interpreted with caution. A number of studies have reported significant associations between serum VEGF-C concentrations with clinical outcome in GC patients,[28,50,51] although to date the relationship between serum concentration and tumour expression is unclear. In summary, currently the prognostic value of VEGF ligands and VEGFRs in GC remains contradictory and requires further investigation in the second-line setting.[11,14,16,28,44,50,51]

In conclusion, the preliminary activity and manageable toxicity observed in this study suggests that single-agent sunitinib has defined clinical value as second-line treatment for AGC and its role in combination with chemotherapy warrants further investigation. Following, additional positive *in vitro* data[52] we are currently conducting a randomised phase II study of second-line FOLFIRI +/− sunitinib (NCT01020630, clinical trials.gov), which will provide more insight into the use of multiple-RTK inhibitors such as sunitinib in the treatment of AGC.

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Conflict of interest statement

M. Moehler has received research funding from company Pfizer. M. Moehler and T.Trarbach have reported honoraria or a consultancy role with Pfizer. All other authors have declared no conflict of interest.

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