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PHASE II STUDIES

Trastuzumab and bevacizumab combined with docetaxel, oxaliplatin and capecitabine as first-line treatment of advanced HER2-positive gastric cancer: a multicenter phase II study

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Summary *Objective* To investigate the efficacy of bevacizumab and trastuzumab combined with docetaxel, oxaliplatin, and capecitabine (B-DOCT) as first-line treatment of advanced human epidermal growth factor receptor 2 (HER2)-positive gastric cancer (GC). *Methods* In this multicentre, single-arm, phase II study, tumor HER2 status was determined centrally prior to treatment. Patients with advanced HER2-positive adenocarcinoma of the stomach or gastroesophageal junction (immunohistochemistry 3+ or immunohistochemistry 2+/silver in-situ hybridization positive) were treated with six cycles of bevacizumab 7.5 mg/kg (day 1), docetaxel 50 mg/m^2 (day 1), oxaliplatin 100 mg/m^2 (day 1), capecitabine 850 mg/m^2 b.i.d. (days 1–14), and trastuzumab 6 mg/kg (day 1) every three weeks, followed by maintenance with bevacizumab, capecitabine, and trastuzumab until disease progression. The primary objective was to demonstrate an improvement of progression-free survival (PFS) to >7.6 months (observed in the ToGA trial) determined according to the lower

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limit of the 95 % confidence interval (CI). Secondary endpoints were safety, objective response rate (ORR), and overall survival (OS). *Results* Twenty-five patients with HER2-positive tumors were treated with B-DOCT between March 2011 and September 2014. At a median follow-up of 17 months, median PFS was 10.8 months (95%CI: 9.0–NA), OS was 17.9 months (95%CI: 12.4–NA). One-year PFS and OS were 52 % and 79 %, respectively. The ORR was 74 % (95%CI: 52–90 %). Two patients became resectable during treatment with B-DOCT and achieved a pathological complete response. The most common treatment-related grade ≥ 3 adverse events were: neutropenia (16 %), diarrhoea (16 %), and hypertension (16 %). *Conclusions* B-DOCT is a safe and active combination in HER2-positive GC, supporting further investigations of DOC with HER2/vascular endothelial growth factor (VEGF) inhibition in HER2-positive GC.

Keywords Gastric cancer · Trastuzumab · Bevacizumab · HER2 · Adenocarcinoma · Stomach

Introduction

Gastric cancer (GC) has a poor prognosis, with a median survival of approximately one year for patients with advanced disease [1]. Chemotherapy is the main treatment option available, and different fluoropyrimidine and platinum-based combinations have shown to improve overall survival (OS) [1]. Two phase III studies have shown that triplet combinations with docetaxel added to cisplatin and 5-fluorouracil (DCF) are associated with better OS than doublet combinations [2, 3]. However, the use of DCF regimens has been limited due to high rates of severe (grade ≥ 3) toxicity, with typically 60–80 % of the patients experiencing grade ≥ 3 neutropenia [2, 3]. There are different reports on the safety and efficacy of docetaxel, oxaliplatin, and capecitabine (DOC), showing that DOC has a favourable safety profile compared to DCF, and that it can be administered fully on an outpatient basis [4–6]. In a phase 1a/1b study which we reported recently, DOC had promising efficacy with a median progression-free survival (PFS) of 6.5 months and OS of 11.0 months [6], which is in the same range as shown for DCF [2, 3].

A key driver of tumor progression that is overexpressed in 7–34 % of GC tumors is the human epidermal growth factor receptor 2 (HER2) [7, 8]. Trastuzumab is a humanized IgG1 monoclonal antibody directed at HER2, which has shown to substantially improve outcome of patients with HER2-positive GC. In patients with immunohistochemistry (IHC) 3+, or IHC 2+/fluorescence in-situ hybridization (FISH) positive disease, trastuzumab improved PFS from 5.5 months to 7.6 months, and OS from 11.8 to 16.0 months, when added to doublet chemotherapy [9, 10]. Trastuzumab and docetaxel have demonstrated synergistic antitumor activity in preclinical investigations in HER2-overexpressing breast cancer (BC) cell lines, and this combination has become a standard regimen in the first-line treatment of metastatic BC [11]. In GC, there is limited experience with the combination of trastuzumab plus docetaxel-based chemotherapy (Table S1 of Online Resource material). Also for the combination of trastuzumab and oxaliplatin there are indications for a synergistic antitumor effect [12]. Thus far, one Korean study has investigated the combination of capecitabine and oxaliplatin plus trastuzumab, for which a notable PFS was demonstrated, of 9.8 months [13].

A crucial aspect of tumor development is angiogenesis, of which vascular endothelial growth factor A (VEGF-A) is one of the main regulators. In GC, as well as in other cancers, tumor expression of VEGF-A has been shown to correlate with tumor progression and patient outcome [14–16]. A positive association between HER2 overexpression and VEGF expression was demonstrated in HER2-positive BC, which correlated with an aggressive phenotype and poor patient outcome [16]. Preclinical investigations in HER2-positive GC xenografts show that combined blockade of HER2 and VEGF result in greater growth-inhibition than either agent alone [17, 18]. In addition, it was shown in a trastuzumab-resistance xenograft model that anti-VEGF treatment could overcome trastuzumab resistance, suggesting that combined HER2 and VEGF inhibition might lead to improved antitumor effect in HER2-positive tumors [19].

Bevacizumab is a humanized IgG1 anti-VEGF-A monoclonal antibody, which has shown to confer a significant improvement of PFS in GC, from 5.3 to 6.7 months when added to doublet chemotherapy ($p = 0.004$), but no significant improvement of OS (from 10.1 to 12.1 months, $p = 0.100$) [20]. A pre-planned analysis showed that Asian patients had less benefit from treatment with bevacizumab (HR 0.92 for PFS, 95%CI: 0.74–1.14, $n = 188$), while Europeans had a more pronounced, significant benefit (HR 0.71 for PFS, 95%CI: 0.54–0.93, $n = 125$). Furthermore, a subsequent biomarker evaluation showed that non-Asian patients with high plasma VEGF-A levels had a significant OS benefit when treated with bevacizumab [21]. A recent study in HER2-positive BC showed that in positron emission tomography (PET)-predicted non-responders on treatment with neoadjuvant docetaxel and trastuzumab, addition of bevacizumab increased the pathological complete response (pCR) rate from 24 % to 44 %, again demonstrating the potential usefulness of bevacizumab in HER2-positive tumors to improve antitumor effect [22].

The reported preclinical and clinical findings support investigations of combined HER2 and VEGF inhibition in combination with docetaxel-based triplet chemotherapy in advanced GC. We performed a multicenter phase II study to investigate the feasibility and efficacy of bevacizumab combined with DOC and trastuzumab (B-DOCT).

Patients and methods

Patients

B-DOCT was a multicenter, single-arm, phase II study undertaken between March 2011 and September 2014 at ten hospitals in The Netherlands (NCT01359397). Eligible patients fulfilled the following major inclusion criteria: age 18 years or older, histologically confirmed, metastatic and/or irresectable (primary or recurrent) adenocarcinoma of the stomach or gastro-esophageal junction, measurable or evaluable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 guidelines [23], World Health Organization (WHO) performance score ≤ 2, able and willing to undergo procedures for assessment of tumor HER2 status, baseline left ventricular ejection fraction (LVEF) ≥50 %, and adequate bone marrow, hepatic, and renal function. Major exclusion criteria were: increased risk of gastrointestinal perforation in response to treatment due to deep ulceration of the tumor as assessed by endoscopy, previous radiotherapy on the abdominal cavity, active gastrointestinal bleeding, arterial thrombosis or cerebrovascular accident within 6 months prior to enrollment, therapeutic use of oral anticoagulants or low molecular weight heparins or non-steroidal anti-inflammatory drugs, uncontrolled hypertension (systolic blood pressure > 180 mmHg or diastolic blood pressure > 100 mmHg) or history of heart disease (e.g. heart failure, angina pectoris), other malignancy within the past 5 years (excluding basal cell carcinoma of the skin, or cervical carcinoma in situ), history of brain metastases, and prior chemotherapy or investigational treatment for advanced disease (neoadjuvant chemotherapy was permitted if completed >6 months before enrollment). Surgery and radiotherapy (other than on the abdominal cavity) were allowed up to 4 weeks prior to enrollment.

Patients were screened prior to treatment for tumor HER2 status, and only patients with HER2-positive disease received treatment with the B-DOCT regimen. Patients with HER2-negative tumors were treated with B-DOC (B-DOCT without trastuzumab), the results of which will be reported elsewhere. The study was approved by the medical ethics committees of all participating institutions and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent prior to study procedures.

Central testing of tumor HER2 status

Tumor HER2 status was determined centrally by a pathology laboratory with extensive experience in HER2 testing of GC. HER2 status was determined on existing tissue or newly obtained tissue, of the primary tumor or (if not available) a metastatic lesion, using IHC and dual silver in-situ hybridization (SISH). HER2 IHC was performed using the PATHWAY HER2/neu 4B5 antibody on a Benchmark XT platform (Ventana Medical Systems, Inc., Tucson, Arizona, USA) and immunoreactivity was scored as IHC 0, 1+, 2+, or 3+, according to previously published methods [7, 24]. SISH was performed on all samples, irrespective of IHC score, on the Benchmark XT platform. HER2 status was defined according to regulatory guidelines as HER2-negative in all cases with IHC 0/1+, or IHC 2+ with a HER2:chromosome 17 ratio < 2 by SISH, and as HER2-positive in all cases with IHC 3+, or ICH 2+ with a HER2:chromosome 17 ratio of ≥2 by SISH. In biopsy specimens at least one cluster of 5 positive cells was required for HER2-positivity, in resection specimen at least 10 % of neoplastic cells. The total numbers of HER2 and chromosome 17 signals were counted in at least 20 tumor cell nuclei in two different areas.

Treatment plan

Patients received outpatient treatment in three-weekly cycles with bevacizumab 7.5 mg/kg on day 1, docetaxel 50 mg/m^2 on day 1, oxaliplatin 100 mg/m^2 on day 1, capecitabine 850 mg/m^2 b.i.d. on days 1–14, and trastuzumab 6 mg/kg on day 1 (with a loading dose of 8 mg/kg in the first cycle), for a planned total of six cycles. The chemotherapy regimen has been described in detail in the report of the phase Ia/1b study [6]. After six cycles of B-DOCT and in absence of disease progression, patients received maintenance treatment with bevacizumab (7.5 mg/kg), capecitabine (at a dose of 1000 mg/m^2 b.i.d.), and trastuzumab (6 mg/kg) until disease progression or unacceptable toxicity. Dose modifications for docetaxel, oxaliplatin, and capecitabine were allowed as per protocol, but no dose reductions of bevacizumab or trastuzumab were allowed. Discontinuation of individual drugs was allowed if required, according to the investigator's judgement and in the best interest of the patient. The prophylactic use of growth factors was not permitted.

Procedures

Baseline assessments (physical examination, hematology, chemistry, and urinalysis) were performed within 14 days of treatment initiation. Tumor measurements (using computed tomography or magnetic resonance imaging), an electrocardiogram, and determination of the LVEF were allowed up to 28 days prior to start of treatment. During treatment, physical examination, hematology, chemistry, and urinalysis were repeated prior to each cycle. The LVEF was measured every 12 weeks. Tumor assessments were performed every two cycles during cycles 1–6, followed by every three cycles. Response was evaluated according to RECIST 1.1 [23]. After completion of study treatment, survival status was monitored.

Endpoints and statistical analysis

The primary endpoint of the study was PFS. Secondary endpoints were safety, OS, objective response rate (ORR), and disease control rate.

Our hypothesis was that treatment with B-DOCT would improve PFS compared to the standard treatment for HER2-positive GC, i.e. chemotherapy plus trastuzumab (without bevacizumab). Efficacy of chemotherapy plus trastuzumab has been studied in one pivotal study, the ToGA study [9, 10]. In the ToGA study, PFS of patients with HER2-positive disease (IHC3+ or IHC2+/FISH+, i.e. the same criteria as in this study), was 7.6 months [9, 10]. Our hypothesis was that B-DOCT would improve PFS by 2.5 months and, thus, a PFS of 10.1 months was anticipated. We assessed the obtained 95 % confidence interval (CI) to determine whether it was higher than and excluded 10.1 months, as this would indicate a relevant improvement of PFS compared to the ToGA study.

This study was part of a larger project in which the patients who screened HER2-negative were treated with B-DOC (B-DOCT without trastuzumab). The project was powered on this population of patients with HER2-negative disease (the efficacy results of which will be reported elsewhere; *submitted manuscript*). No formal power or sample size calculation was performed for the study described here (although a posteriori calculation of power showed that the power to detect the anticipated 2.5 month difference with 25 included patients was 27 %).

All efficacy and safety analyses were performed on the population of patients who received at least one dose of all study medications. Time-to-event endpoints were estimated using the Kaplan-Meier method. PFS was defined as the time between study registration and first documented disease progression per RECIST, or death, whichever came first. Patients who withdrew or could not continue treatment due to adverse events before documented progression were monitored for disease progression or, in case they were lost to follow-up, censored at their off-study date. In the rare case that a patient became operable during treatment and received surgery, the patient was censored at the time of surgery so that PFS would not become overestimated (an underestimation is more likely to occur [25]).

OS was defined as the time from study registration to death from any cause. Response was evaluated according to RECIST 1.1 [23]. The ORR (the proportion of patients who achieved complete or partial response) and disease control rate (the proportion of patients who achieved complete response, partial response, or stable disease) were determined in the subset of patients with measurable disease. Confidence intervals on the ORR and disease control rate were calculated using the exact method. Duration of response was estimated in the subset of patients who reached a partial or complete response, using the Kaplan-Meier method.

We calculated parameters related to drug exposure for each drug, including: the number of cycles administered, the duration of treatment, the cumulative dose administered, the proportion of patients with a dose reduction, and the relative dose intensity (RDI). RDI was defined as the cumulative dose given in the cycle, divided by the length of the cycle, further divided by the reference dose intensity (i.e. the planned dose intensity). The RDI was calculated for each drug per cycle, during the time that patients were on treatment. If a patient had discontinued one of the drugs but remained on treatment with the remaining drugs, the RDI for that drug was considered 0 % in the subsequent cycles that the patient remained on treatment. The overall RDI during treatment exposure was calculated by dividing the sum of all cycles' RDI by the number of cycles on treatment with any of the drugs. In the case of docetaxel or oxaliplatin, the cumulative RDI was divided by the number of cycles treated with the respective drug, unless treatment had been discontinued before the sixth cycle due to an AE or patient refusal, then the cumulative RDI was divided by 6. In a separate analysis the population median RDI was calculated per cycle for each study drug and plotted against the cycles. In calculations of the population median RDI per cycle, patients who stopped treatment for progressive disease or death from any cause were excluded from the analysis of subsequent cycles. All statistical analyses were performed using R v3.1.0.

Results

Patients

A total of 91 patients were screened for tumor HER2 status between March 2011 and September 2014 in ten centres in The Netherlands. Twenty-eight patients were screened HER2-positive (31 %), of which 25 patients were eligible for treatment with B-DOCT. Three patients were ineligible, one patient had a LVEF of <50 %, one patient had a deeply ulcerating tumor not allowing safe treatment with bevacizumab, and one patient required radiotherapy on the primary tumor. The median age was 62 years (range: 38–73), and 21/25 patients (84 %) were male. Central pathology review showed that most patients had IHC 3+/SISH positive disease (76 %). Four patients had IHC 2+/SISH positive disease (16 %), and 2 patients had IHC 3+ disease with unknown amplification status (8 %). Table 1 summarizes the complete baseline characteristics of the patients enrolled.

Efficacy

Median follow-up was 17.1 months (95%CI: 14.2–NA). At clinical data cut-off, 15 patients (60 %) had progressed. Ten patients were censored, two patients because they received

gastrectomy with curative intent, one patient due to an adverse event deemed unrelated to treatment (myocardial infarction), and seven patients who were still on treatment (28 %). At the time of analysis, 13/25 patients (52 %) had deceased and 12 patients (48 %) were alive. A total of 7 lines of additional therapy after B-DOCT had been initiated, in 6 patients (24 %, Table 1).

Table 1 Patient and disease characteristics

Characteristic	Overall Population $N = 25$
Age, years	
median (range)	62 (39–73)
Sex	
Female	4 (16 %)
Male	21 (84 %)
WHO performance score	
0	11 (44 %)
1	12 (48 %)
2	2 (8 %)
Race	
Caucasian	25 (100 %)
Other	0 (0 %)
Site of primary cancer	
Gastric	8 (32 %)
Gastro-esophageal junction	17 (68 %)
Lauren classification	
Diffuse	0 (0 %)
Intestinal	22 (88 %)
Mixed	3(12 %)
Extent of disease	
Locally advanced	3 (12 %)
Metastatic	22 (88 %)
Previous treatment	
Surgery	0 (0 %)
Radiotherapy	0 (0 %)
Neoadjuvant chemotherapy	0 (0 %)
Measurable disease	
Yes	24 (96 %)
No (evaluable only)	1 (4 %)
HER2 status	
SISH positive/IHC 2+	4 (16 %)
SISH positive/IHC 3+	19 (76 %)
SISH unknown/IHC 3+	2 (8 %)
Second-line treatment [a]	
Phase I	3 (12 %)
Irinotecan	2 (8 %)
Taxane + trastuzumab	1 (4 %)
Capecitabine + bevacizumab + trastuzumab	1 (4 %)

WHO World Health Organization, *NOS* not otherwise specified;

HER2 human epidermal growth factor receptor 2, *SISH* silver in situ hybridization, *IHC* immunohistochemistry

[a] six patients received a total of seven lines of treatment after B-DOCT

Median PFS was 10.8 months (95%CI: 9.0–NA), and OS was 17.9 (95%CI: 12.4–NA, Fig. 1). One-year PFS and OS were 47 % and 79 %, respectively. A total of 23/25 patients (92 %) were evaluable for response. One patient had non-measurable disease, and one other patient went off-study before the first tumor evaluation due to an adverse event in cycle 1 (posterior reversible encephalopathy syndrome, described below). The ORR was 74 % (95%CI: 52–90 %), with one patient (4 %) having a complete response on radiological evaluation, 16 patients achieving partial response (70 %), 5 patients (22 %) with stable disease, and one patient (4 %) with progressive disease on first evaluation. The disease control rate was 95 % (95%CI: 77–100 %). The median duration of response was 11.9 months (95%CI: 7.6–NA).

Two patients became resectable during the course of treatment. The first patient was a 68 year old male with a distal, intestinal type, HER2-positive tumor (IHC 3+), with several small pathological locoregional lymph nodes. A laparoscopic procedure showed a large tumor (T_4) fixed to the pancreas, which was therefore deemed irresectable ($T_4N_1M_0$). There were no visible peritoneal metastases and peritoneal lavage cytology was negative, and no distant metastases were present. The patient received four cycles of treatment and, although the tumor was deemed non-measurable per RECIST at baseline, there appeared to be a substantial reduction in tumor size at the end of cycle 4. It was decided to perform partial gastrectomy, and a total of 24 lymph nodes were removed. Pathological examination revealed that all removed tissues were free off neoplastic cells (pCR). The patient is currently alive and disease-free, two years after treatment.

The second patient was a 69 year old male with an intestinal type HER2-positive tumor (IHC 3+) of the corpus, with several locoregional lymph nodes and a 12 mm PET-positive para-aortic lymph node ($T_3N_1M_1$). A laparoscopic procedure showed no peritoneal metastases and peritoneal lavage cytology was negative. A fine needle puncture of the PET-positive para-aortic lymph node did not yield representative tissue. Thus, the suspected metastasis could not be pathologically confirmed. The patient received six cycles of treatment with B-DOCT. After four cycles a partial response was achieved, with an ongoing response observed after six cycles. It was then decided to perform surgery for gastrectomy, with removal of the omentum and lymph nodes. Also in this patient pathological examination of all removed tissues showed that a pCR was achieved. This patient received adjuvant treatment with capecitabine, bevacizumab, and trastuzumab for one year, and is currently alive and disease-free two and a half years after treatment.

Safety

The reported hematological and non-hematological treatment-related adverse events with a frequency of ≥5 %, or which

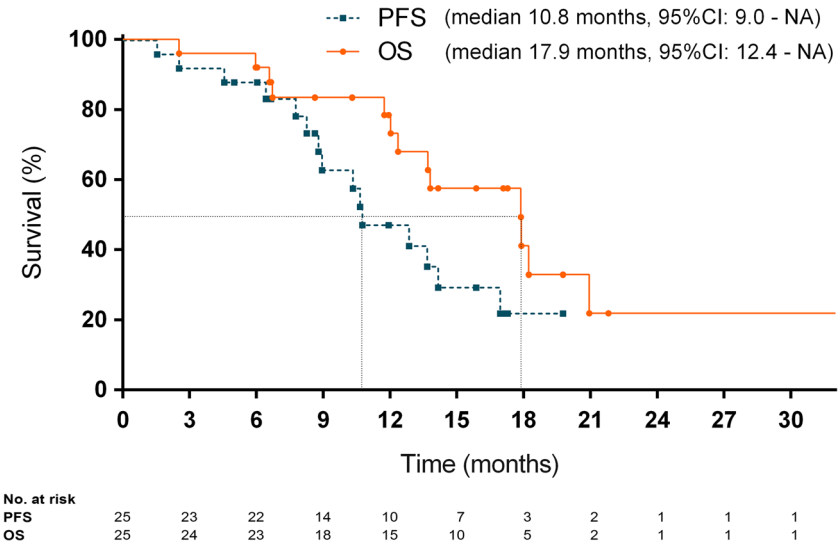

Fig. 1 Progression-free survival and overall survival of patients treated with bevacizumab plus docetaxel, oxaliplatin, capecitabine and trastuzumab (B-DOCT). The figure shows the progression-free survival and overall survival of the overall population of patients treated with bevacizumab plus docetaxel, oxaliplatin, capecitabine and trastuzumab (B-DOCT).
NA = not available (not reached);
OS = overall survival;
PFS = progression-free survival

occurred as grade ≥ 4, are listed in Table 2. The most common hematological adverse events were neutropenia and leukocytopenia, which occurred as grade 3 in 16 % and 16 % of the patients, respectively. Febrile neutropenia occurred in a single patient (4 %). The most common non-hematological treatment-related adverse events occurring as grade ≥ 3 were diarrhea (16 %) and hypertension (16 %). Only one patient had a grade 4 treatment-related adverse event (4 %), a sigmoid perforation thought to be probably related to treatment with bevacizumab (no other risk factors were present).

Three patients (12 %) had to stop treatment due to an adverse event. One patient due to the sigmoid perforation which occurred in cycle 3, one patient due to a posterior reversible encephalopathy syndrome in cycle 1 (deemed probably related to treatment), and one patient who had a myocardial infarction in cycle 2 (deemed unrelated to treatment). Twenty-one treatment-related serious adverse events were reported among 15 patients (60 %). The adverse events most commonly associated with serious adverse events were diarrhea (16 %) and nausea/vomiting (16 %). There were no treatment-related deaths.

The mean LVEF at baseline was 62 % (± 7.5 %, range: 51–79 %). A decrease in LVEF of ≥10 % to an absolute value of <50 % occurred in 4 patients (16 %). A decrease to <45 % occurred in one patient (4 %). Trastuzumab was discontinued due to a decrease of LVEF in three patients, in one patient after 9 cycles with a decrease from 76 % to 48 %, in one patient after 9 cycles (from 71 % to 45 %), and in one patient after 23 cycles (from 56 % to 43 %). Decreases in LVEF were reversible in all patients and none of the patients developed clinical heart failure.

Drug exposure

Of the total number of cycles in which patients were treated, bevacizumab was administered in 314/329 cycles (95 %), docetaxel in 129/132 (98 %) of cycles, oxaliplatin in 129/132 (98 %) of cycles, capecitabine in 287/329 (87 %) of cycles, and trastuzumab in 320/329 (97 %) of cycles. The RDI over the course of the cycles is shown in Fig. 2, and complete data on drug exposure are summarized in Table 3. Treatment could be administered on an outpatient basis in 323/329 administered cycles (98 %), in 2 % of the administrations patients had to stay overnight for logistical reasons.

Discussion

In this study we investigated the feasibility and efficacy of B-DOCT as first-line treatment of advanced HER2-positive GC. B-DOCT was well-tolerated and associated with a notable PFS, of 10.8 months (95%CI: 9.0–NA), which was longer than patients treated with trastuzumab plus doublet chemotherapy in the ToGA study, who had a median PFS of 7.6 months (an estimated improvement of 3.1 months, 41 %). The results from this study also compare favourably with the other studies that have investigated trastuzumab plus chemotherapy as first-line treatment of GC, in which PFS ranged between 5.1 and 9.8 months (Table S1). We found an ORR of 74 % (95%CI: 52–90 %), which is higher than that reported in previous studies (range 32–68 %). Overall survival, of 17.9 months in this study, is in the higher range compared to previous studies (range: 12.9–21.0 months). Of note, two patients who became resectable during treatment with B-DOCT achieved a pCR, and are currently alive and disease-free more than two years after surgery. The safety profile of B-DOCT was similar to that shown for DOC, confirming the favourable safety profile of DOC compared to DCF regimens [2–6]. The incidence of grade ≥ 4 events was low (4 %), and safety findings regarding bevacizumab and trastuzumab are in line with phase III studies [26, 27].

Table 2 Treatment-related adverse events occurring as grade ≥ 3 in the overall population

MedDRA preferred term [a]	Grade 1–2		Grade 3		Grade 4		Any grade	
	No.	%	No.	%	No.	%	No.	%
Hematological toxicity								
Febrile neutropenia			1	4 %			1	4 %
Anemia	1	4 %	2	8 %			3	12 %
Neutropenia	5	20 %	4	16 %			9	36 %
Leukocytopenia	8	32 %	4	16 %			12	48 %
Non-hematological toxicity								
Diarrhoea	13	52 %	4	16 %			17	68 %
Fatigue	15	60 %	2	8 %			17	68 %
Nasea/vomiting	14	56 %	2	8 %			16	64 %
Hand-foot syndrome	10	40 %	2	8 %			12	48 %
Stomatitis	7	28 %	1	4 %			8	32 %
Abdominal pain	6	24 %	1	4 %			7	24 %
Esophageal pain			1	4 %			1	4 %
Decreased appetite	4	16 %	2	8 %			6	24 %
Dysphagia			1	4 %			1	4 %
Cholecystitis			1	4 %			1	4 %
Malaise	5	20 %	1	4 %			6	24 %
Hypertension	1	4 %	4	16 %			5	20 %
Pyrexia	2	8 %	1	4 %			3	12 %
Weight decreased	2	8 %	1	4 %			3	12 %
Dry skin	1	4 %	1	4 %			2	8 %
Hiccups	1	4 %	1	4 %			2	8 %
Mucosal inflammation	1	4 %	1	4 %			2	8 %
Pulmonary embolism			2	8 %			2	8 %
Posterior reversible encephalopathy syndrome			1	4 %			1	4 %
Sigmoid perforation [b]					1	4 %	1	4 %
Hyperglycemia			1	4 %			1	4 %
Hypokalemia			1	4 %			1	4 %
Hyponatremia			1	4 %			1	4 %
Syncope			1	4 %			1	4 %

[a] *MedDRA* Medical Dictionary for Regulatory Affairs (http://www.meddra.org/)

[b] Requiring colectomy

The incidence of treatment-emergent hypertension was relatively high (16 %), which could be explained by the long average treatment duration, and is in line with the frequency described for women treated with docetaxel, trastuzumab, and bevacizumab [26]. Cases of hypertension could be managed using standard antihypertensive treatments. One patient experienced a posterior reversible encephalopathy syndrome, a rare side effect that has been described in a number of patients treated with (fluoropyrimidine-based) chemotherapy, but that has also been associated with bevacizumab and trastuzumab treatment [28–30].

Bevacizumab has shown to improve outcome in different cancers and is approved for a number of tumor types [31–35]. Phase III studies in GC, however, have shown only a limited improvement of outcome [20, 31]. In the AVAGAST study, bevacizumab conferred a significant improvement of PFS from 5.3 to 6.7 months when added to chemotherapy (p = 0.004), while improvement of OS was non-significant (from 10.1 to 12.1 months, p = 0.100) [20]. It was shown, however, in a pre-planned subgroup analysis that patients of non-Asian ethnicity had greater benefit from bevacizumab than Asians [20]. Differences in tumor biology and general prognosis of Asian and non-Asian GC patients might underlie the differences observed in treatment response to anti-VEGF-A [14, 15, 20, 36]. Asian GC patients have been found to have significantly lower VEGF-A concentrations than non-Asians, and biomarker studies in GC (as well as in BC) have consistently shown that patients with high plasma concentrations of

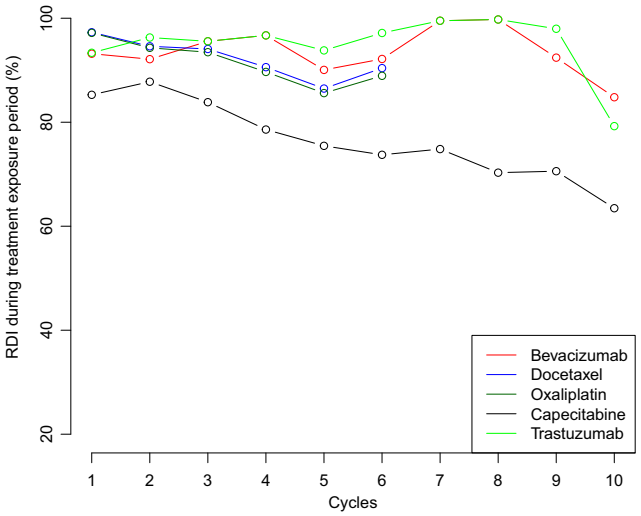

Fig. 2 Relative dose intensity achieved for bevacizumab plus docetaxel, oxaliplatin, capecitabine, and trastuzumab. The figure shows the relative dose intensity (RDI) achieved during treatment, per cycle, for each of the administered drugs. RDI was defined as the cumulative dose given in the cycle, divided by the length of the cycle, divided by the reference dose intensity. If a patient had discontinued a drug but remained on treatment with the remaining drugs, the RDI for the drug in that cycle was considered 0 %. Open circles depict the median RDI among the patients on treatment in the respective cycle. RDI = relative dose intensity

VEGF-A (and/or low neuropilin levels) have more benefit than patients with lower concentrations [14, 15, 21, 37]. This suggests that selection of GC patients based on plasma concentrations of angiogenic factors could be a feasible strategy to enrich patient populations that derive the most benefit from treatment with bevacizumab. Further investigations of bevacizumab in GC should therefore proceed in patient populations enriched based on angiogenic biomarkers.

The validity of targeting the VEGF/VEGFR (VEGF receptor) axis in GC has been confirmed recently in studies with other anti-angiogenic drugs. Ramucirumab, a fully humanized monoclonal antibody directed against VEGFR-2, has shown to prolong OS of GC patients treated in second-line [38, 39]. Also apatinib, an oral small-molecule VEGFR-2 tyrosine kinase inhibitor, has shown to prolong OS in second-line [40]. However, in a recently presented randomized placebo-controlled phase II study of ramucirumab as first-line treatment of advanced GC in combination with FOLFOX, there was no improvement on the primary endpoint PFS, or OS [41]. Since activity of the VEGF-VEGFR-2 signalling pathway increases with disease progression in GC, it could be that benefit from antiangiogenic therapy is greater for patients treated in second-line than in first-line [42, 43].

Preclinical and clinical findings suggest that bevacizumab could be effective in a proportion of tumors to overcome trastuzumab-resistance [17–19, 22]. A recent clinical study in BC showed that bevacizumab increased the proportion of patients who achieved a pCR, from 24 % to 44 %, in patients with HER2-positive tumors who were predicted by PET to be non-responders to neoadjuvant treatment with docetaxel plus trastuzumab [22]. These findings indicate that combining anti-HER2 and anti-VEGF might be effective to overcome trastuzumab-resistance, at least in subsets of patients. Combination of trastuzumab with pertuzumab could be effective to further improve efficacy of trastuzumab-based regimens [19]. There are preclinical indications that addition of bevacizumab to trastuzumab and pertuzumab could further improve antitumor response in HER2-positive tumors [19].

We acknowledge the limitations of this single-arm phase II study, including its small sample size and the fact that outcome was compared with a historical cohort. It does, however, demonstrate that the combination of bevacizumab and trastuzumab with DOC as a chemotherapy backbone is feasible, is well-tolerable, and has promising activity in HER2-positive GC. Further investigations of DOC plus trastuzumab in GC are warranted.

Table 3 Drug exposure and dose adjustments

Bevacizumab	
Number of cycles administered (IQR)	12 (8–18)
Treatment duration, weeks (IQR)	38 (26–58)
Cumulative dose, mg/kg (IQR)	83 (57–121)
Relative dose intensity during treatment (IQR)	99 % (79–100 %)
Docetaxel	
Number of cycles administered (IQR)	6 (6–6)
Treatment duration, weeks (IQR)	18 (18–19)
Cumulative dose, mg/m^2 (IQR)	294 (247–303)
Patients with dose reduction, no. (%)	6 (24 %)
Relative dose intensity adjusted for first six cycles (IQR)	95 % (88–101 %)
Oxaliplatin	
Number of cycles administered (IQR)	6 (6–6)
Treatment duration, weeks (IQR)	18 (18–19)
Cumulative dose, mg/m^2 (IQR)	585 (469–602)
Patients with dose reduction, no. (%)	6 (24 %)
Relative dose intensity adjusted for first six cycles (IQR)	96 % (88–99 %)
Capecitabine	
Number of cycles administered (IQR)	12 (4–16)
Treatment duration, weeks (IQR)	38 (12–54)
Cumulative dose, grams (IQR)	266 (87–374)
Patients with dose reduction, no. (%)	14 (56 %)
Relative dose intensity during treatment (IQR)	82 % (67–89 %)
Trastuzumab	
Number of cycles administered (IQR)	12 (8–18)
Treatment duration, weeks (IQR)	38 (26–59)
Cumulative dose, mg/kg (IQR)	68 (50–110)
Relative dose intensity during treatment (IQR)	97 % (93–100 %)

DOC docetaxel, oxaliplatin, plus capecitabine, *IQR* interquartile range

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Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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