Original Article

Bevacizumab Combined With Docetaxel, Oxaliplatin, and Capecitabine, Followed by Maintenance With Capecitabine and Bevacizumab, as First-Line Treatment of Patients With Advanced HER2-Negative Gastric Cancer: A Multicenter Phase 2 Study

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BACKGROUND: The current study was a multicenter, single-arm, phase 2 study performed to investigate the feasibility and efficacy of bevacizumab combined with docetaxel, oxaliplatin, and capecitabine (B-DOC) in patients with advanced human epidermal growth factor receptor 2 (HER2)-negative, previously untreated, gastric or gastroesophageal adenocarcinoma. **METHODS:** Tumor HER2 status was determined centrally. Patients received 6 cycles of bevacizumab at a dose of 7.5 mg/kg, docetaxel at a dose of 50 mg/m^2, and oxaliplatin at a dose of 100 mg/m^2 (all on day 1) combined with capecitabine at a dose of 850 mg/m^2 twice daily (days 1-14) every 3 weeks followed by maintenance with capecitabine and bevacizumab in patients with disease control. The primary objective was to demonstrate a progression-free survival (PFS) of >6.5 months, according to the 95% confidence interval (95% CI). Secondary endpoints included safety, objective response rate, overall survival (OS), analyses of circulating tumor cells (CTCs), and pharmacogenetic analyses. **RESULTS:** Sixty eligible patients were enrolled. The median PFS was 8.3 months (95% CI, 7.2-10.9 months). The objective response rate was 70% (95% CI, 55%-83%) and the disease control rate was 96% (95% CI, 85%-99%). The median OS was 12.0 months (95% CI, 10.2-16.1 months). According to CTC-AE v4.0, the most common treatment-related grade ≥3 adverse events were neutropenia (20%), leukocytopenia (18%), diarrhea (15%), and nausea/vomiting (15%). The presence of CTCs at baseline was strongly predictive of PFS (hazard ratio [HR], 3.8; P =.007) and OS (HR, 3.4; P =.014). The methylenetetrahydrofolate reductase (*MTHFR*) 677C>T genotype was strongly associated with PFS (HR, 4.7 for TT vs CC or CT; P =.0007) and OS (HR, 5.9; P =.0001). **CONCLUSIONS:** The B-DOC regimen plus maintenance was feasible and active. CTCs were found to be prognostic in patients treated with B-DOC. Docetaxel-based triplet chemotherapy as a backbone for targeted therapies is feasible and deserves further study. ***Cancer*** **2016;122:1434-43**. © *2016 American Cancer Society.*

KEYWORDS: bevacizumab, capecitabine, clinical trial, docetaxel, gastric cancer, phase 2.

INTRODUCTION

Gastric cancer (GC) has a poor prognosis, with a cancer-related mortality rate of 75%.[1] For patients with advanced disease, palliative chemotherapy is the main treatment option.[2] The majority of regimens are based on fluoropyrimidines

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and platinum, and 5-fluorouracil (5-FU)-based combination chemotherapy has been shown to provide superior overall survival (OS) and quality of life compared with single-agent 5-FU.[2]

An important disadvantage of 5-FU-based and cisplatin-based regimens is that prolonged hospitalization is required for administration. Recent studies have focused on the oral fluoropyrimidine capecitabine and newer platinum compounds such as oxaliplatin.[3] The Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer 2 (REAL-2) study demonstrated that 5-FU and cisplatin can be substituted by capecitabine and oxaliplatin, which are at least noninferior and can be administered on an outpatient basis.[3]

In recent years, attention has been focused on taxane-containing triplet combinations.[2,4] The addition of docetaxel to cisplatin and 5-FU (DCF) improved time to disease progression from 3.7 months to 5.6 months ($P<.001$) and improved quality of life.[4,5] However, an important limitation of DCF was the high incidence of severe treatment-related adverse events (AEs), with grade $\geq$3 neutropenia reported to occur in 82% of the patients.[4] Therefore, reduced-dose (modified) DCF (mDCF) regimens have subsequently been investigated.[6,7] We recently reported the results of a phase IA/IB study of docetaxel at a dose of 50 mg/m^2, oxaliplatin at a dose of 100 mg/m^2, and capecitabine at a dose of 850 mg/m^2 twice daily (DOC) every 3 weeks.[7] At the established recommended phase 2 dose, DOC was found to have a favorable safety profile, with an incidence of severe neutropenia of 24%, at therapeutic drug levels.[7] DOC conferred a median progression-free survival (PFS) of 6.5 months and an OS of 11.0 months, which is in keeping with other studies investigating DOC regimens.[7] In addition, DOC could be fully administered on an outpatient basis.

Bevacizumab is a humanized immunoglobulin G1 monoclonal antibody directed at the vascular endothelial growth factor A (VEGF-A), which has been shown to improve outcome in multiple solid tumors. In patients with GC, bevacizumab improved PFS from 5.3 to 6.7 months (hazard ratio [HR], 0.80; $P=.004$), but there was no significant improvement in OS noted (from 10.1 to 12.1 months; HR, 0.87 [$P=.100$]). Preplanned subgroup analyses have demonstrated that non-Asian individuals benefit more from bevacizumab than Asian individuals.[8,9] In patients treated in Europe, bevacizumab was found to improve PFS by 2.5 months, from 4.4 months to 6.9 months (HR, 0.71; 95% confidence interval [95% CI], 0.54-0.93), and the objective response rate (ORR) was also improved significantly (odds ratio, 1.79; 95% CI, 1.02-3.15).[8] Maintenance treatment with bevacizumab and capecitabine has been shown to be an effective strategy with which to delay disease progression in patients with colorectal cancer without compromising quality of life.[10] In patients with GC, maintenance treatment could similarly be beneficial, but to our knowledge only limited data are available.[11]

Based on our experience with DOC and the potential to further improve patient outcomes through the addition of bevacizumab and maintenance treatment, we initiated a multicenter, open-label, phase 2 study to investigate the efficacy of bevacizumab combined with docetaxel, oxaliplatin, and capecitabine (B-DOC) plus maintenance in patients with human epidermal growth factor receptor 2 (HER2)-negative disease. Patients received 6 cycles of B-DOC followed by maintenance with capecitabine and bevacizumab. The study was supported by pharmacogenetic analyses and measurements of circulating tumor cells (CTCs).

MATERIALS AND METHODS

Patients and Study Design

This prospective, multicenter, single-arm phase 2 study was performed at 10 centers in the Netherlands (ClinicalTrials.gov identifier NCT01359397). Eligible patients were aged $\geq$18 years; had histologically confirmed unresectable and/or metastatic (primary or recurrent) adenocarcinoma of the stomach or gastroesophageal junction; measurable or evaluable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1; hereafter referred to as RECIST 1.1)[12]; a World Health Organization (WHO) performance status of 0 to 2; and adequate bone marrow, hepatic, and renal function (including proteinuria $\leq$2+). Patients were centrally tested for tumor HER2 status before treatment (see online Supporting Information), and patients with HER2-negative disease (ie, those with immunohistochemistry [IHC] 0 or 1+ disease, regardless of silver in-situ hybridization [SISH] result; or IHC 2+, SISH-negative disease) were eligible. As per study protocol, patients with HER2-positive disease could be included in a substudy to receive B-DOC plus trastuzumab, the results of which are reported separately.[13] Major exclusion criteria were previous radiotherapy to the abdomen, active gastrointestinal bleeding, arterial thrombosis or cerebrovascular accident within 6 months before enrollment, therapeutic use of anticoagulants or nonsteroidal antiinflammatory drugs, and previous treatment for advanced disease (neoadjuvant

chemotherapy was permitted if administered >6 months before enrollment).

The first 5 patients were treated at the primary study center to monitor safety and recognize potential unexpected toxicities. An additional exclusion criterion was added by protocol amendment to exclude patients at an increased risk of gastrointestinal perforation in response to treatment due to deep ulceration of the tumor as assessed by endoscopy, when one of the first patients experienced gastrointestinal perforation as a result of tumor response.

The study was approved by the medical ethics committees of the participating institutions and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent.

Treatment Plan and Assessments

Patients received outpatient treatment with bevacizumab at a dose of 7.5 mg/kg (day 1), docetaxel at a dose of 50 mg/m^2 (day 1), oxaliplatin at a dose of 100 mg/m^2 (day 1), and capecitabine at a dose of 850 mg/m^2 twice daily (days 1-14) every 3 weeks for 6 cycles. Subsequently, patients with stable disease or response continued to receive maintenance treatment, with bevacizumab at a dose of 7.5 mg/kg (day 1) and capecitabine at a dose of 1000 mg/m^2 twice daily (days 1-14) every 3 weeks until disease progression or unacceptable toxicity occurred. The chemotherapy treatment plan was described in detail previously.[7] Dose modifications for docetaxel, oxaliplatin, and capecitabine were allowed as per protocol, and no dose reductions of bevacizumab were performed. If individual study drugs had to be discontinued, patients could continue on the remaining drugs if it was in the best interest of the patient. Prophylactic leukocyte growth factor support was not allowed.

Screening assessments were performed within 14 days of treatment initiation. Tumor assessment and electrocardiogram were allowed ≤28 days before first drug administration. During treatment, physical examination, hematology, biochemistry, and urinalysis were repeated before each cycle. Tumor assessments (using computed tomography or magnetic resonance imaging) were performed before treatment and at the end of cycles 2, 4, and 6 followed by every 3 cycles.

Circulating Tumor Cells

Blood for the detection of CTCs was collected before treatment for patients enrolled at the Netherlands Cancer Institute. A fully validated fluorescence-activated cell sorting assay was used to enumerate CTCs.[14] The lower limit of quantification of the assay is 2 CTCs per 8 mL of blood.[13] Therefore, samples with <2 CTCs per 8 mL of blood were considered CTC negative, and samples with ≥2 CTCs per 8 mL of blood were considered as CTC positive. In view of stochastic sampling error at low numbers of CTCs, three 8-mL blood samples were drawn and the average number of CTCs per 8 mL of blood was calculated to classify patients as CTC positive or negative.

Pharmacogenetics

Associations with PFS, OS, and toxicity were investigated for polymorphisms in genes encoding pharmacokinetic and dynamic enzymes related to fluoropyrimidine and platinum pharmacology (see online Supporting Information).

Endpoints and Statistical Analysis

The primary endpoint was PFS, and the secondary endpoints were safety, ORR, OS, disease control rate (DCR), and translational research. PFS, OS, and safety analyses were performed on the overall population of eligible patients who had received at least 1 dose of all study drugs.

A historical cohort of patients treated with DOC between 2007 and 2010 in a phase IA/IB study performed in 2 Dutch centers was found to have a PFS of 6.5 months.[7] Therefore, a median PFS of ≤6.5 months was considered insufficient to warrant further investigation of B-DOC. A median PFS of 9.0 months was desired. A total of 60 evaluable patients would have 67% power to reject the null hypothesis of 6.5 months against 9.0 months at a 2-sided α of .05. The 95% CI was calculated to determine whether it excluded 6.5 months.

Time-to-event endpoints were estimated using the Kaplan-Meier method. PFS was defined as the time from study registration to first documented disease progression as per RECIST 1.1 or death from any cause, whichever came first. Patients who withdrew from or could not continue treatment due to AEs before documented disease progression occurred were monitored for disease progression or, in case they were lost to follow-up, censored at their date of last contact. OS was defined as the time from study registration to death from any cause. Response was evaluated according to RECIST 1.1.[12] The ORR and DCR were determined in the subset of patients with measurable disease who had at least 1 on-treatment tumor assessment. The 95% CIs for the ORR and DCR were calculated using the exact method. The duration of response was estimated in the subset of patients who achieved a partial or complete response using the Kaplan-Meier method.

Multiple parameters related to drug exposure were calculated for each drug, including the number of cycles administered, the duration of treatment, the cumulative dose administered, the percentage of patients with a dose reduction, and the relative dose intensity (RDI) (methods discussed in online Supporting Information).

For the CTC analysis, groups were compared with regard to efficacy endpoints using log-rank tests and the effects reported as HRs. For the pharmacogenetic analysis, genotypes were compared using log-rank tests for efficacy endpoints and the results reported as HRs. For associations with toxicity, groups were compared using chi-square tests. Multivariable analyses to investigate associations between CTCs and pharmacogenetic variants with survival endpoints were performed using Cox regression, with adjustment for age, sex, extent of disease, and WHO performance status. An exploratory (multivariable) analysis to compare the outcomes of patients treated with B-DOC in the current study with patients treated previously with DOC was performed, adjusting for age, sex, extent of disease, and WHO performance status. The threshold for statistical significance was set at $P < .05$. No correction for multiple comparisons was applied. Statistical analyses were performed using SPSS statistical software (version 17.0; IBM Corporation, Armonk, NY) and R statistical software (version 3.1.1; R Foundation, Vienna, Austria).

RESULTS

Patients

A total of 91 patients were screened for tumor HER2 status between March 2011 and September 2014, 63 of whom (69%) were found to have HER2-negative disease. Sixty patients were eligible and treated with B-DOC (Table 1). Two patients were ineligible due to an early decline in WHO performance status and 1 patient was ineligible due to elevated liver function tests. The median age of the patients was 58 years (range, 27-75 years), and 43 of 60 patients (72%) were male. Approximately one-half of the patients had a tumor localized in the stomach (48%), whereas the remaining patients had a tumor at the gastroesophageal junction (52%).

TABLE 1. Patient Characteristics

Characteristic	Overall Population N = 60
Median age (range), y	58 (27–75)
Sex	
Female	17 (28%)
Male	43 (72%)
WHO performance status	
0	33 (55%)
1	24 (40%)
2	3 (5%)
Race	
White	57 (95%)
Other	3 (5%)
Site of primary cancer	
Gastric	29 (48%)
Gastroesophageal junction	31 (52%)
Lauren classification	
Intestinal	35 (58%)
Diffuse	16 (27%)
Mixed	7 (12%)
NOS	2 (3%)
Extent of disease	
Locally advanced	7 (12%)
Metastatic	53 (88%)
Previous treatment	
Surgery	10 (17%)
Neoadjuvant chemotherapy	7 (12%)
Measurable disease	
Yes	52 (87%)
No (evaluable only)	8 (13%)
Second-line treatments[a]	
Irinotecan-based	7 (12%)
Paclitaxel (plus ramucirumab)	3 (5%)
Radiotherapy	2 (3%)
DOC/DOF (plus bevacizumab)	3 (5%)
CAP/CAPOX plus bevacizumab	2 (3%)
Chemoradiotherapy	1 (2%)
Experimental NOS	1 (2%)
Anthracycline-based triplet	1 (2%)
5-FU	1 (2%)
CRT postoperatively	1 (2%)

Abbreviations: 5-FU, 5-fluorouracil; CAP, capecitabine; CAPOX, capecitabine plus oxaliplatin; CRT, chemoradiotherapy; DOC, docetaxel, oxaliplatin, and capecitabine; DOF, docetaxel, oxaliplatin, and 5-fluorouracil; NOS, not otherwise specified; WHO, World Health Organization.
[a] Twenty-two lines of treatment after the administration of bevacizumab, docetaxel, oxaliplatin, and capecitabine (B-DOC) were administered to 16 patients.

Efficacy

The median follow-up was 19.0 months. At the clinical data cutoff (April 20, 2015), a total of 43 patients (72%) had reached the primary endpoint and 13 patients remained on treatment. The remaining 4 patients were censored for reasons other than remaining on treatment: 1 patient received a new treatment (chemoradiotherapy) before documented disease progression on B-DOC, 1 patient who had initially unresectable disease had his disease become resectable during treatment and underwent surgery, 1 patient refused further treatment before disease progression, and 1 patient stopped treatment in view of clinical deterioration (disease related) before documented radiological disease progression. At the clinical data cutoff, 39 patients (65%) had died and 21 patients (35%) were alive. Sixteen patients (27%) received a total of 22 lines of therapy after treatment with B-DOC (Table 1).

The median PFS was 8.3 months (95% CI, 7.2-10.9 months) and the median OS was 12.0 months (95% CI,

10.2-16.1 months) (Fig. 1). In total, 47 of 60 patients (78%) were evaluable for response. Ten patients had nonmeasurable disease and 3 other patients did not complete the first 2 cycles of therapy and therefore had no on-treatment tumor assessment. Of the latter 3 patients, 1 patient developed a fatal gastric hemorrhage in the first cycle, 1 died of rapid disease progression in the second cycle, and 1 patient withdrew from treatment after severe nausea/vomiting with dehydration developed in the first cycle. A complete response was achieved in 1 patient who was continuing to receive treatment at the time of the data cutoff 16 months after the initiation of treatment (2%), a partial response was noted in 32 patients (68%), and stable disease was noted in 12 patients (26%); 2 patients (4%) were found to have progressive disease at the time of first tumor assessment. The confirmed ORR was 70% (95% CI, 55%-83%). All responses occurred during the induction phase, with the median number of cycles to response being 4. The DCR was 96% (95% CI, 85%-99%), and the median duration of response was 5.6 months (95% CI, 4.6 months to not reached).

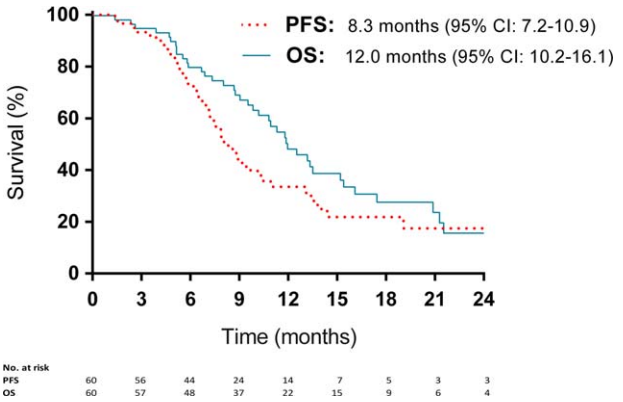

Figure 1. Progression-free survival (PFS) and overall survival (OS) of human epidermal growth factor receptor 2 (HER2)-negative patients treated with bevacizumab, docetaxel, oxaliplatin, and capecitabine. 95% CI indicates 95% confidence interval.

The patients with nonmeasurable disease had a median PFS of 13.8 months (95% CI, 7.6 months to not reached) versus 7.9 months (95% CI, 7.0-10.2 months) for patients with measureable disease ($P = .054$). The median OS was 20.9 months (95% CI, 9.6 months to not reached) versus 11.8 months (95% CI, 9.9-15.4 months), respectively ($P = .110$).

TABLE 2. Treatment-Related Adverse Events Reported as Grade ≥3 (Common Terminology Criteria for Adverse Events (CTC-AE) v4.0) in the Overall Population

MedDRA-Preferred Term	Grade 1 to 2[a]		Grade 3		Grade 4		Grade 5		Any Grade	
	No.	%	No.	%	No.	%	No.	%	No.	%
Hematological toxicity										
Anemia	4	7%	1	2%					5	7%
Leukocytopenia	23	38%	8	13%	3	5%			34	57%
Neutropenia	22	37%	5	8%	7	12%			34	57%
Febrile neutropenia			1	2%	2	3%			3	5%
Nonhematological toxicity										
Diarrhea	35	58%	9	15%					44	73%
Nausea and/or vomiting	30	50%	9	15%					39	65%
Stomatitis	23	38%	1	2%					24	40%
Dehydration	0	0%	5	8%					5	8%
Mucosal inflammation	3	5%	2	3%					5	8%
Esophageal perforation					1	2%			1	2%
Gastric hemorrhage							1	2%	1	2%
Fatigue	32	53%	8	13%					40	67%
Dyspnea	2	3%	2	3%					4	7%
Hypertension	4	7%	3	5%					7	12%
Pulmonary embolism	0	0%	3	5%	1	2%			4	7%
Peripheral sensory neuropathy	30	50%	8	13%					38	63%
Hand-foot syndrome	21	35%	5	8%					26	43%
Hypokalemia	1	2%	1	2%	1	2%			3	5%
Pneumosepsis					1	2%	1	2%	1	2%
Nail toxicity[b]	17	28%	2	3%					19	32%
Anorexia	7	12%	3	5%					10	17%
Increased lacrimation	6	10%	1	2%					7	12%
Dry skin	7	12%	1	2%					8	13%

Abbreviation: MedDRA, Medical Dictionary for Regulatory Affairs.
[a] Worst-grade toxicity per patient was calculated; table reports the numbers of patients.
[b] Includes nail discoloration, nail disorder, nail infection, nail toxicity, and onychomadesis.

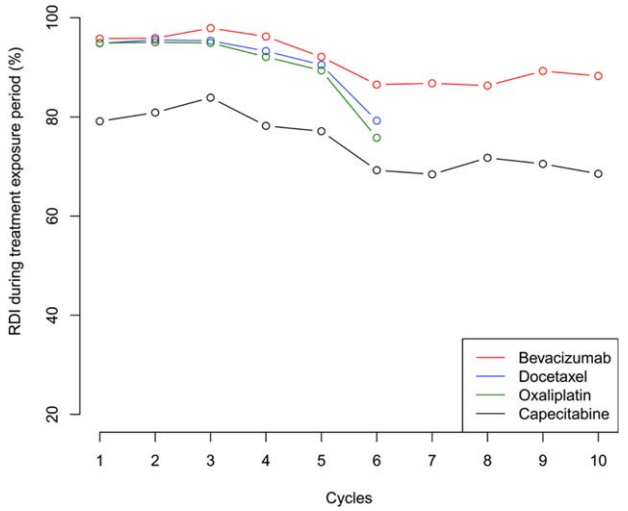

Figure 2. Relative dose intensity (RDI) achieved for the combination of bevacizumab, docetaxel, oxaliplatin, and capecitabine (B-DOC) during treatment, per cycle, for each of the administered drugs. RDI was defined as the cumulative dose given in the cycle divided by the length of the cycle divided by 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 to be 0%. Open circles depict the median RDI among the patients receiving treatment in the respective cycle.

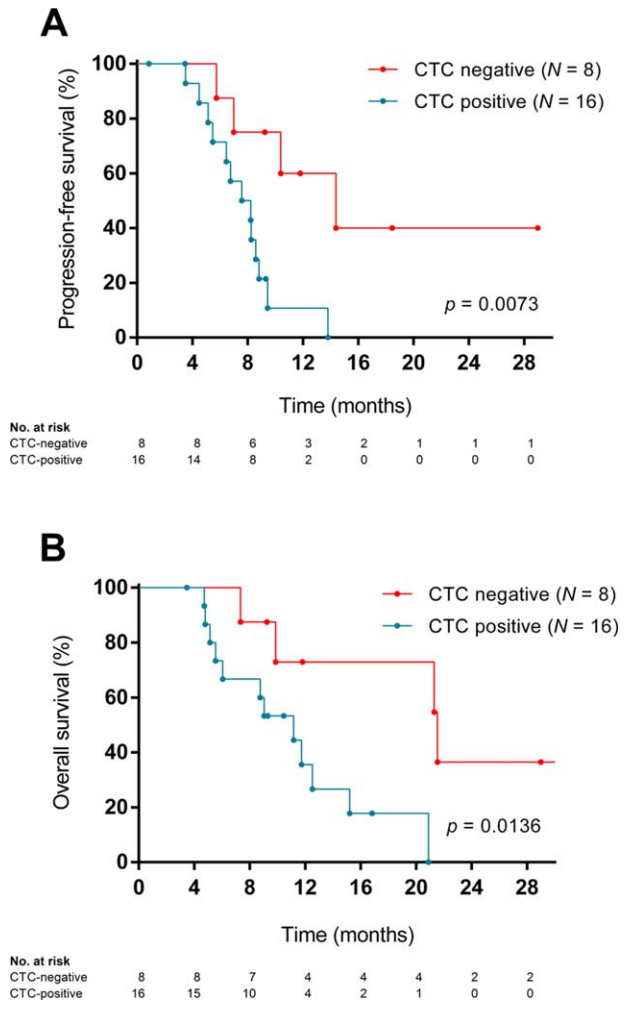

Figure 3. Outcome of patients according to the presence of circulating tumor cells (CTCs) at baseline. The figure shows the (A) progression-free survival and (B) overall survival of patients according to the presence of CTCs before treatment. Patients with ≤2 CTCs per 8 mL of peripheral blood were classified as CTC negative, whereas patients with >2 CTCs per 8 mL of peripheral blood were classified as CTC positive. Multivariable analysis revealed indications toward associations between CTC-positive status and progression-free survival (hazard ratio, 2.5; 95% confidence interval, 0.76-8.27 [*P* = .133]) and overall survival (hazard ratio, 2.7; 95% confidence interval, 0.82-8.98 [*P* = .101]).

Safety

The reported treatment-related AEs that occurred as grade ≥3 are listed in Table 2. All patients experienced at least 1 treatment-related AE, 78% experienced a grade ≥3 treatment-related AE, and 18% of patients experienced a grade 4 AE. The most common grade ≥3 hematological toxicities were neutropenia (20%) and leukocytopenia (18%). Febrile neutropenia occurred at grade 3 in 1 patient (2%), and at grade 4 in 2 patients (3%). The most prominent nonhematological grade ≥3 toxicities were diarrhea (15%), nausea/vomiting (15%), fatigue (13%), and peripheral sensory neuropathy (13%), all of which were limited to grade 3. A total of 35 treatment-related serious AEs were reported among 26 patients (43%).

Six patients (10%) discontinued treatment due to treatment-related AEs. These cases were associated with neuropathy (grade 2), dehydration (grade 3), esophageal (tumor) perforation (grade 4), myocardial infarction (grade 3), gastric hemorrhage (grade 5), and pneumosepsis (grade 5). All cases of treatment discontinuation due to AEs occurred during the induction phase; no patients discontinued due to treatment-related AEs during maintenance. Of the 2 cases with fatal toxicities (3%), one case of sepsis occurred during cycle 5 (possibly related to chemotherapy) and one case of gastric hemorrhage occurred during cycle 1 (most likely related to bevacizumab).

Drug Exposure

Of the total number of cycles in which patients were treated, bevacizumab was administered in 676 of 708 cycles (95%), docetaxel in 315 of 319 cycles (99%), oxaliplatin in 315 of 319 cycles (99%), and capecitabine in 689 of 708 cycles (97%). Overall, the RDI during treatment for bevacizumab was 97% (interquartile range

[IQR], 92%-99%), the RDI for docetaxel was 92% (IQR, 84%-99%), the RDI for oxaliplatin was 89% (IQR, 83%-97%), and the RDI for capecitabine was 73% (IQR, 59%-85%). The RDI over the course of treatment is shown in Figure 2. Complete data regarding drug exposure are listed in Supporting Information Table S1. In 98% of all administered cycles, treatment could be administered on an outpatient basis.

Circulating Tumor Cells

Twenty-two patients treated with the B-DOC regimen were included in the CTC analysis. Two additional patients who were screened as HER2 positive and were treated with B-DOC plus trastuzumab participated in the CTC investigations. Both patients were included in the analysis (but were not included in any of the other analyses). In total, 16 of 24 patients (67%) were CTC positive at baseline; the median number of CTCs per 8 mL of blood was 36 (range, 2-428 CTCs) (Supporting Information Table S2). Clinical characteristics did not differ between patients with and without detectable CTCs, although CTC-positive patients more often tended to have tumors located at the gastroesophageal junction. Patients who were CTC positive had inferior PFS and OS compared with patients who were CTC negative (Fig. 3). The median PFS for patients who were CTC positive at baseline was 7.9 months compared with 14.4 months for patients who were CTC negative (HR, 3.8; 95% CI, 1.56-10.5 [P =.007]). Similarly, the OS was 10.2 months for CTC-positive patients and 21.5 months for CTC-negative patients (HR, 3.4; 95% CI, 1.45-10.4 [P =.014]). Multivariable analysis confirmed that CTC-positive status was associated with inferior PFS (HR, 6.7; 95% CI, 1.43-31.03 [P =.016]) and a trend toward inferior OS (HR, 4.3; 95% CI, 0.82-22.90 [P =.084]).

Pharmacogenetic Analyses

A total of 56 of 60 patients (93%) participated in the pharmacogenetic analysis. The TT genotype of methylenetetrahydrofolate reductase (*MTHFR*) 677C>T was strongly associated with inferior PFS (vs CC/CT: HR, 4.7; 95% CI, 1.75-12.8 [P =.0007]) and OS (vs CC/CT: HR, 5.9; 95% CI, 2.12-16.5 [P =.0001]) (Fig. 4). Multivariable analysis confirmed that PFS was inferior in patients with the TT genotype (HR, 3.9; 95% CI, 1.13-13.7 [P =.031]), as was OS (HR, 4.8; 95% CI, 1.37-16.8 [P =.014]). None of the other polymorphisms were found to be associated with PFS or OS. The *MTHFR* 677C>T polymorphism was also found to be associated with gastrointestinal toxicity (Supporting Information Table S3). In addition, excision repair cross-complementation group 2 (*ERCC2*) 2251A>C was associated with gastrointestinal and hematological toxicity, and thymidylate synthase (*TYMS*) 3′UTR 6-bp ins/del was associated with overall toxicity (Supporting Information Table S3).

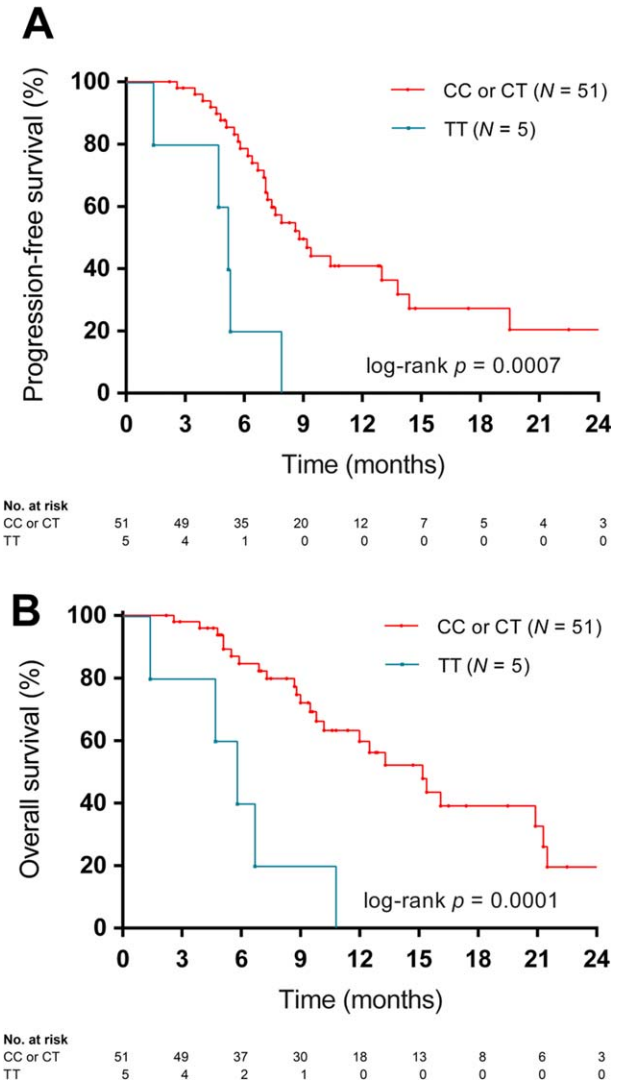

Figure 4. Associations between methylenetetrahydrofolate reductase (*MTHFR*) 677C>T genotypes and outcome. (A) Progression-free survival (PFS) and (B) overall survival (OS) of patients are shown according to *MTHFR* 677C>T genotype. Patients carrying a wild-type (CC) or heterozygous (CT) genotype had a median PFS of 8.8 months versus 5.2 months for patients with a homozygous mutant (TT) genotype. Similarly, the OS was 15.2 months for patients with CC or CT genotypes versus 5.8 months for those with a TT genotype. Multivariable analysis (with adjustment for age, sex, extent of disease, and World Health Organization performance status) confirmed that PFS was inferior in patients with the TT genotype (hazard ratio, 3.9; 95% confidence interval, 1.13-13.7 [P = .031]), as was OS (hazard ratio, 4.8; 95% confidence interval, 1.37-16.8 [P = .014]).

DISCUSSION

In this phase 2 study, treatment with B-DOC plus maintenance in patients with HER2-negative advanced GC was associated with a PFS of 8.3 months (95% CI, 7.2-10.9 months), which was longer than the 6.5 months observed in patients treated with DOC.[7] The median OS was 12.0 months (95% CI, 10.2-16.1 months), which is close to the 11.0 months observed for DOC.[7,8] Thus, although the anticipated PFS of 9.0 months was not achieved, there was a strong trend toward improvement in PFS, and a lesser trend toward improved OS. The safety profile of B-DOC was similar to that of DOC, which compares favorably with the safety profile of DCF regimens.[4,6] Neutropenia occurred as grade $\geq$3 in 20% of the patients, which is a much lower incidence than the 82% reported for DCF or the incidence of 61% reported for mDCF.[4,6] Febrile neutropenia occurred in 5% of the patients, which also is lower than the rate of 29% observed for DCF and lower than the rate of 13% observed for mDCF.[4,6] The most important nonhematological grade $\geq$3 toxicities were diarrhea (15%), nausea/vomiting (15%), fatigue (13%), and peripheral sensory neuropathy (13%). These toxicities were all limited to grade 3, and generally were well managed using standard supportive measures. No new bevacizumab-related safety signals were identified in the current study.

The results of the current study are in keeping with the beneficial effect of bevacizumab on PFS demonstrated in the AVAGAST study (from 5.3 to 6.7 months when added to chemotherapy; $P = .004$). Although the results of the AVAGAST study demonstrated that bevacizumab conferred a significant improvement in PFS, a similar improvement in OS was not significant (from 10.1 to 12.1 months; $P = .100$).[8] We compared the outcome of the patients treated with B-DOC plus maintenance in the current study with patients treated previously with DOC in an exploratory multivariable analysis (Supporting Information Table S4). The results of the analysis indicated that patients treated with B-DOC were at a significantly lower risk of disease progression compared with those treated with DOC (HR, 0.5; 95% CI, 0.32-0.87 [$P = .013$]), but were not at a lower risk of death (HR, 1.0; 95% CI, 0.58-1.64 [$P = .924$]) (Supporting Information Table S4). Although exploratory in nature, these findings suggest that in an unselected population of patients with GC treated with first-line therapy, bevacizumab provides a benefit with regard to PFS but not OS. Whether this improvement in PFS translates into a meaningful clinical benefit and improved quality of life remains to be further established.

Biomarker studies have shown that pretreatment plasma concentrations of angiogenic factors are associated with the response of patients with GC to treatment with bevacizumab.[15] In the AVAGAST population, non-Asian patients with high plasma VEGF-A levels and/or low neuropilin-1 levels were found to have a significant OS benefit from treatment with bevacizumab (HR, 0.6 [95% CI, 0.43-0.82] and HR, 0.6 [95% CI, 0.41-0.86], respectively).[15] Similar consistent findings have been demonstrated in patients with breast cancer,[15,16] thereby suggesting the validity of VEGF-A inhibition at least in subsets of patients with GC and breast cancer.

The validity of VEGF inhibition as an anticancer strategy in patients with GC is also demonstrated by the efficacy of ramucirumab and apatinib, 2 other drugs that inhibit the VEGF/VEGF receptor axis. Both drugs improve OS in later lines of treatment.[17,18] Of interest is the finding that antiangiogenic drugs have demonstrated a beneficial effect almost exclusively in later lines of treatment. The finding that VEGF-A levels in patients with GC increase with progressing disease, and the indications that VEGF-A levels are predictive of the effect of bevacizumab, might suggest that benefit from antiangiogenic drugs is greater when used in patients with later stages of the disease. Of note in this respect is the report of a randomized phase 2 study of ramucirumab as the first-line treatment of GC in combination with FOLFOX (folinic acid, 5-FU, and oxaliplatin), which indicated that ramucirumab did not improve PFS in this setting (HR, 0.98).[19]

Maintenance treatment with bevacizumab and capecitabine may have further contributed to the observed improvement in PFS. Continuation of bevacizumab and capecitabine after induction with B-DOC could theoretically result in a delay in tumor regrowth. In patients with colorectal cancer, this theory is supported by data from the CAIRO3 study (Treatment with Capecitabine and Bevacizumab Versus Observation), which demonstrated that maintenance significantly delayed PFS and was associated with a trend toward longer OS compared with observation after induction chemoimmunotherapy.[10] In this study, maintenance treatment was found to be feasible and did not lead to excessive toxicity, a finding that is in keeping with 2 previous studies.[11,20] Feasibility of the B-DOC regimen was further demonstrated by adequate RDI for the administered drugs. The achieved RDI for capecitabine during the first 6 cycles was somewhat lower than expected (75%-80%), but is in keeping with a recent study that demonstrated that the RDI was 74% in patients treated with a similar DOC regimen.[21]

Administering B-DOC on an outpatient basis was found to be feasible in a majority of cycles (98%).

The results of translational studies indicated that patients with detectable CTCs had significantly inferior PFS (HR, 3.8; $P = .007$) and OS (HR, 3.4; $P = .014$) compared with patients without detectable CTCs at baseline, thereby confirming the potential usefulness of CTCs as a prognostic marker in patients with GC.[22] The *MTHFR* 677C>T polymorphism was found to be associated with outcome, with the TT genotype conferring inferior PFS and OS. The T allele is associated with a reduction in MTHFR activity, which leads to higher concentrations of 5,10-Methylenetetrahydrofolate and therefore would be expected to lead to a greater cytotoxic effect of 5-FU as a result of stabilization of the ternary complex between thymidylate synthase and 5-fluorodeoxyuridine monophosphate.[23,24] However, the T allele also has been found to be associated with an increased risk of GC development, suggesting that *MTHFR* 677C>T might have both a prognostic and a predictive effect on outcome.[25] The findings in the current study therefore could be explained by the fact that *MTHFR* has both a prognostic and a predictive effect, which act in opposing directions and could have a different magnitude depending on the population studied.

A limitation of the current study is that we compared outcome with a historical cohort of patients. Although an indirect comparison, the population treated with DOC was highly similar (treated in the same country only several years earlier, without any major changes in clinical practice occurring during this period) and therefore we believe the risk of selection bias as a result of the historical comparison is relatively low. The fact that we selected HER2-negative patients for this study did introduce selection bias because the patients treated with DOC were not selected based on HER2 status. However, the risk of positively affecting outcome by enrichment with HER2-negative tumors is most likely low because only a percentage of the patients were HER2 positive and because a recent meta-analysis indicated that HER2 status was not associated with outcome in patients with GC (HR, 0.97 [$P = .63$] for OS).[26]

The results of the current study confirm that DOC is a safe and efficacious treatment for patients with GC, and can be combined with nonchemotherapeutic anticancer drugs such as bevacizumab. B-DOC chemotherapy followed by maintenance treatment with bevacizumab plus capecitabine was found to confer a modest improvement in PFS in comparison with DOC administered in a historical cohort of patients. The therapeutic value of bevacizumab and of maintenance treatment need to be confirmed in a randomized controlled study.

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CONFLICT OF INTEREST DISCLOSURES

Jan Willem B. de Groot reports personal fees from Bristol-Myers Squibb, GlaxoSmithKline, Roche, MSD, Merck, Celgene, Nordic, Amgen, and Pfizer for work performed outside of the current study. Steven A.L.W. Vanhoutvin reports an educational grant from Roche to the institute for work performed as part of the current study. Jos H. Beijnen has a patent on a Pharmaceutical formulation for oral treatment of taxanes. The patent has been licensed to Modra Pharmaceuticals (spinout/start-up of The Netherlands Cancer Institute and Slotervaart Hospital). Spinout has no turnover. Potential royalties (if they arise in the future) are coming to the institute and inventors (Beijnen). The current study does not deal with oral taxanes nor the products of the abovementioned patent. This disclosure is given for completeness. Annemieke Cats reports personal fees from Hoffman-La Roche, Merck Serono, and Lilly and nonfinancial support from Hoffman-La Roche and Lilly for work performed outside of the current study.

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