FIRST LINE 5-FU-BASED CHEMOTHERAPY WITH/ WITHOUT BEVACIZUMAB FOR METASTATIC COLORECTAL CANCER: ONE CENTER EXPERIENCE RESULTS

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ABSTRACT

Purpose: Colorectal cancer is the second leading cause of cancer mortality in the United States. According to the National Institute of Statistics in Bulgaria for 2012 there have been 2370 newly diagnosed colon cancer and 1664 rectal cancer cases and the total number of registered patients is 29995. Adding Bevacizumab to chemotherapy in patients with metastatic colorectal cancer improves progression-free survival but yet no predictive markers for patient selection have been described and proved in the clinical practice. In our study we examined two plasma biomarkers that may correlate with response to first line Bevacizumab containing chemotherapy in patients with metastatic colorectal cancer.

Patients and Methods: 54 patients with metastatic colorectal cancer were assigned to first line 5-Fu-based chemotherapy with/without Bevacizumab. The primary end point was progression-free survival, with additional determination of response and toxicity. Blood samples were collected at baseline from all 54 patients prior to initiation of chemotherapy and Bevacizumab. Plasma samples were stored at -80° C until analysis at the Immunology Laboratory at the University Hospital "St. Marina" (Varna, Bulgaria) by a multiple-step sandwich immunoassay Human ELISA VEGF121 and VEGF165 kits.

Results: The median progression-free survival for the group treated with CT/Bev was 8.8 months, compared with 5.4 months for the group treated with chemotherapy alone (95% CI, log-rank test P =0.003). The corresponding overall response rates were 19.3% and 10.2% respectively (P < 0.05 for CT/Bev vs CT).

Conclusion: The addition of Bevacizumab to 5-Fu based chemotherapy improves progression-free survival duration for patients with metastatic colorectal cancer. We could not find any association between pretreatment plasma levels of VEGF 121 and 165 and worse PFS.

Keywords: *colorectal cancer, Bevacizumab, VEGF121, VEGF 165, biomarkers, neuropillin-1*

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INTRODUCTION

The development of new blood vessels, termed angiogenesis, is a typical hallmark of cancer development. Four decades ago, angiogenesis was recognized as a therapeutic target for blocking cancer growth and antiangiogenic therapy showed broad clinical activity.1 The most important signaling molecule is the vascular endothelial growth factor or VEGF – it plays a central role in angiogenesis and is frequently highly expressed in cancers. Thus clinical efforts to develop antiangiogenic therapies have largely focused on inhibiting VEGF.2 However not all patients benefit from antiangiogenic therapy; the magnitude of response to this treatment also varies among patients, which makes identification of potential predictive biomarkers a crucial point in clinical practice.3 Identifying which tumors are most sensitive to anti-VEGF therapy would improve therapeutic outcome of patients and could provide insights into the mechanism of resistance to anti-VEGF therapy.

Multiple VEGF receptors are expressed on endothelial cells, including signaling receptor tyrosine kinases (VEGFR1 and VEGFR2) and the nonsignaling co-receptor Neuropilin-1. It is considered that the proangiogenic effect of VEGF is mediated predominantly via VEGFR2. 4,5 Neuropilin-1 binds only the isoform of VEGF responsible for pathological angiogenesis (VEGF165), and is thus a potential target for inhibiting VEGF signaling. VEGF121 is the predominant isoform, is considered by some to be the most proangiogenic and tumorigenic of the VEGF isoforms 6.

In our single center study we compared the efficacy of bevacizumab (anti-VEGF antibody) plus chemotherapy versus only chemotherapy as first line-treatment for patients with metastatic colorectal cancer (mCRC).

We tried to improve our understanding of the complexity of tumor angiogenesis with the evaluation of two serum biomarkers: circulating soluble VEGF (121 and 165 isoforms).

PATIENT SELECTION

We conducted a prospective non-experimental clinical study of 54 patients with histologically confirmed metastatic colorectal adenocarcinoma stage IV as per AJCC, 7th ed. All patients underwent surgery of the primary tumor; they had measurable disease as defined by the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1)7 and were eligible for Bevacizumab-containing 5-Fu based chemotherapy regimens as first line treatment. Their ECOG performance status was <2. Chemotherapy was performed at the University Hospital "St. Marina" and patients were subsequently followed for a period of up to 2 years – first patient was included on 15 February 2013 until follow up of last patient until 1 August 2015. Prior to inclusion in our study we obtained ICF for collection of biological material (plasma/serum) from all patients willing to participate.

Plasma/serum with K2EDTA as anticoagulant was collected at baseline. A second measurement of plasma/serum markers after 3 months (4-6 cycles) was done for 20 patients. Blood was centrifuged at 1000 g at 4°C for 15 minutes and plasma was stored in aliquots at -80°C until analysis. Plasma VEGF121 and VEGF165 levels were assessed by sandwich ELISA immunoassays with ready to use kits according to manufacturer's instructions. VEGF121 levels were assayed with Human VEGF121 ELISA Kit, catalog number CSB-E13709h (Cusabio, China) and VEGF165 with Human VEGF165 ELISA Kit, catalog number CSB-E13100h (Cusabio, China).

CLINICAL AND PATHOLOGIC FEATURES

We collected the following clinical data: demographical data (age at initial staging, sex, etc), date of surgery, extent of surgery, tumor localization and TNM classification, sites of

Table 1. Clinical and pathologic baseline patient characteristics.

Characteristic	5-FU-based CT + Bev (n = 31)	5-FU-based CT (n = 23)
Age at diagnosis		
Median	62.5	64.8
Range	37-81	59-81
Distribution by sex, %	females 45.1 males 54.9	females 65.2 males 34.8
Performance status, %		
0	41.9	49.2
1	49.9	44.0
2	8.2	5.8
Disease site		
Liver, %	70.9	65.4
Lung, %	19.4	16.3
Mutational status of KRAS, %	KRAS WT 35.4 KRAS M+ 48.3 Inadequate for genetic testing 16.3	KRAS WT 59.7 KRAS M+ 26.08 Inadequate for genetic testing 14.2

metastatic dissemination, ECOG performance status.

We collected the following pathologic data: tumor characteristics – histology, grade of differentiation and TNM classification, RAS-status determination.

Clinical and pathologic baseline patient characteristics are summarized in Table 1.

TREATMENT CHOICE AND DURATION

Patients received a minimum of 3 months of treatment. Chemotherapy regimens used are summarized in Table 2.

IMAGING ASSESSMENT, RESPONSE PATTERNS AND END POINTS DETERMINATION

Imaging the disease was performed at baseline and tumor response was assessed at regular intervals - every 4-6 cycles (3 months) for all cycles of CT/Bev till EOT or upon clinical symptoms. Imaging consisted of either CT of thorax, abdomen (and other areas if needed for additional lesion assessment) or PET/CT. Evaluation was performed using RECIST 1.1. During systemic treatment, disease free survival and response rate were assessed. Response was defined as either complete response (CR), partial response (PR) or stable disease (SD). Patients were followed for up to 2 years after start of first line treatment, death or August 1, 2015.

STATISTICAL DESIGN AND ANALYSIS

Descriptive statistics was used. Categorical features were summarized with frequencies and percentages. Our sample distribution was tested for normality with Kolmogorov-Smirnov and we used unpaired t-test to compare sample means.

Our statistical analysis included 54 patients treated with CT alone or CT/Bev. Our aim was to evaluate PFS and potential correlations and identification of good responders to Bevacizumab-containing treatment. PFS was defined as the time from assignment of treatment until progression. Survival curves were estimated by the Kaplan-Meier method,8 with differences assessed by the log-rank test.9 Differences of $P < 0.05$ were considered statistically different.

RESULTS

Efficacy

Our experience confirms that the addition of Bevacizumab to chemotherapy resulted in improvement in progression-free survival which remains a good surrogate for measurement of overall survival in patients with colorectal cancer. Our study demonstrate a significant improvement in PFS with the addition of Bevacizumab to chemotherapy (95% CI, log-rank test P =.003). Median PFS was 5.4 months (3.44-6.55) with CT versus 8.8 months (5.84-10.15) with CT/Bev.

Using the RECIST 1.1 criteria7 for response, 19.3% of patients treated with CT/Bev achieved a confirmed response to therapy (PR+SD) compared with 10.5% of patients treated with CT alone. Our results – PFS, response rates and toxicity profile of Bevacizumab are consistent with that documented in previous trials and the literature (10-12).

Toxicity

The toxicity profile of Bevacizumab was consistent with that documented in previous trials.10-12 As we expected all grade adverse events were registered at higher frequency in

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Table 2. Treatment regimens

Arm	Dosage	Administration	Schedule
FOLFOX4 (± Bevacizumab*)*			Every 14 days
Oxaliplatin	85 mg/m^2	IV 120 minutes	Day 1
Leucovorin	200 mg/m^2	IV 120 minutes	Days 1 + 2
Fluorouracil	400 mg/m^2	IV bolus, followed by	
Fluorouracil	600 mg/m^2	IV over 22 hours	Days 1 + 2
± Bevacizumab	10 mg/kg	30-90 minutes	Day 1
FOLFIRI (± Bevacizumab*)*			Every 14 days
Irinotecan	180 mg/m^2	IV 30-90 minutes	Day 1
Leucovorin	200 mg/m^2	IV 120 minutes	Days 1 + 2
Fluorouracil	400 mg/m^2	IV bolus, followed by	
Fluorouracil	600 mg/m^2	IV over 22 hours	Days 1 + 2
± Bevacizumab	10 mg/kg	30-90 minutes	Day 1
XELOX (± Bevacizumab*)*			Every 21 days
Oxaliplatin	85 mg/m^2	IV 120 minutes	Day 1
Capecitabine	2000-2500 mg/m^2	p.o.	Day 1 - 14
± Bevacizumab	15 mg/kg	30-90 minutes	Day 1
Capecitabine ± Bevacizumab			Every 21 days
Capecitabine	2000-2500 mg/m^2	p.o.	Day 1 - 14
± Bevacizumab	15 mg/kg	30-90 minutes	Day 1

Abbreviations: FOLFOX - oxaliplatin, fluorouracil, and leucovorin; FOLFIRI – irinotecan, fluorouracil, and leucovorin; XELOX – oxaliplatin, capecitabine; IV – intravenous; p.o. – per os.

the group of patients, treated with CT/Bev as compared to the only chemotherapy group. Most frequent AE as expected were nausea and vomiting, asthenia, neuropathy, neutropenia and thrombocytopenia. The occurrence of any grade 3 adverse event was greater for the individuals treated with the combination CT/Bev compared with patients treated with chemotherapy alone (49% vs 37%) with neuropathy, hypertension, bleeding, and vomiting. No AEs grade 4 were registered in our groups of patients.

Biomarker Levels at baseline

Median plasma levels of VEFG 121 were 201.4 pg/ml (range, 34 pg/ml to 1112 pg/ml) Median plasma levels of VEFG 165 were 205 pg/ml (range, 51 pg/ml to 731 pg/ml). There was no significant difference between the pretreatment VEGF 121 and VEGF 165 plasma levels in patients with stable disease and in patients who progressed on therapy (Figure 2). We could not detect any therapy benefit for Bevacizumab-treated patients according to their VEFG 121 and VEFG 165 pretreatment levels. Even patients with higher than median baseline plasma levels of both plasma markers showed no clear benefit of the addition of Bevacizumab to CT. Changes in plasma levels of VEFG 121 and 165 during treatment are unreliable because of interference by Bevacizumab (data not shown).

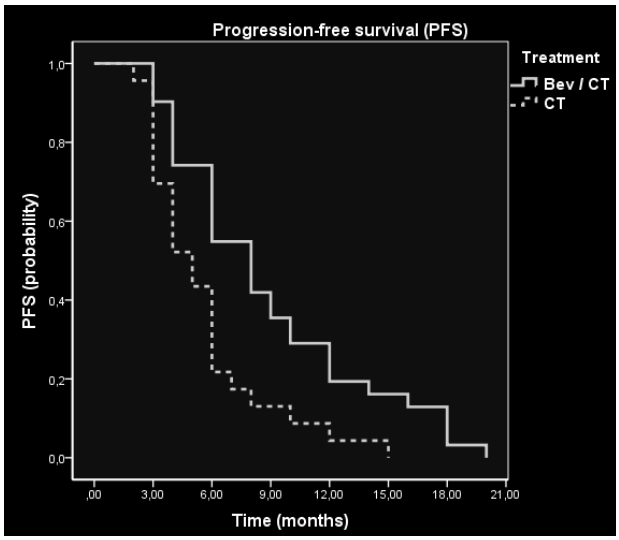

Fig. 1. Kaplan-Meier estimates of progression-free survival (PFS). The median PFS for the group treated with CT/Bev was 8.8 months, as compared with 5.4 months for the group treated with CT alone (95% CI, log-rank test P = .003).

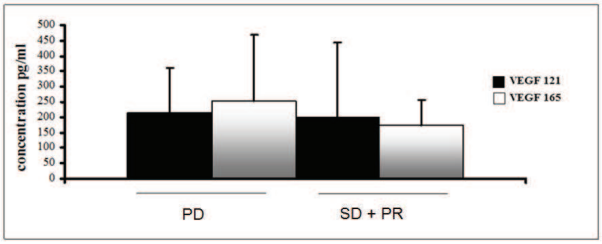

Fig. 2. Plasma concentration in pg/ml of VEGF 121 and VEGF 165 isoforms as measured and distributed according to response to chemotherapy. Response is classified as SD (SD + PR) and PD as no complete response were documented.

DISCUSSION

Antiangiogenic therapy with Bevacizumab in combination with chemotherapy prolongs survival and PFS for patients with metastatic colorectal cancer as this has been previously reported in clinical trials (13,14). Improvements in clinical outcome do not appear to be limited to a single chemotherapy regimen.

There are currently no validated surrogate markers of biological activity for anti-VEGF therapy. The reported mechanism of action of Bevacizumab and the potential for delayed efficacy had led to the speculation that PFS or overall survival may be more relevant measures of activity than objective response rate. Interestingly, improvements in objective response rate and PFS translated into better overall survival in patients with metastatic colorectal cancer receiving first-line chemotherapy plus Bevacizumab (10,11).

Pretreatment levels of circulating VEGF have been previously studied as a potential biomarker for VEGF-targeted therapy, but mainly so far they have been shown to be primarily prognostic rather than predictive (15-17).

In our relatively small cohorts of patients, we could not find any association between these two potential biomarkers – VEGF121 and VEGF165, with worse PFS. These data also showed that treatment outcome in patients receiving CT/Bev cannot be predicted by baseline levels of VEGF121 and VEGF165.

Given the biological complexity of tumor angiogenesis and the non-randomized study design, our results should be viewed with caution. So far, no serum markers were of significant predictive value in our pilot study - the data presented here suggest that baseline levels of neither VEGF121 nor VEGF165 are predictive for outcome on Bevacizumab treatment. Further research is warranted to clarify the predictive value of these markers.

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