

Bevacizumab in combination with chemotherapy in the first-line treatment of metastatic colorectal carcinoma

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Colorectal carcinoma (CRC) is a malignancy of worldwide increased incidence. The vast majority of all CRC cases occur in patients older than age 50. The initial stage at the time of diagnosis has a strong influence on the overall survival (OS). According to AJCC sixth edition system, 5-year stage-specific survivals are over 90% in stage I, but only approximately 8% in stage IV [1]. Chemotherapy in combination with biological treatment has improved response rates (RR), with prolongation of progression free survival (PFS) and OS. Important role in treatment of metastatic colorectal carcinoma (mCRC) plays surgical resection of metastases. Multidisciplinary cooperation between medical oncologist, surgeon, radiologist and radiotherapist is necessary to achieve the best therapeutic results. The aim of our analysis was to describe the efficacy of bevacizumab used in combination with chemotherapy in the first-line setting and to evaluate frequency of thromboembolic complications during the treatment. The analysis included 58 patients with mCRC, who have been treated with first-line chemotherapy in combination with bevacizumab at the St. Elizabeth Cancer Institute in Bratislava since 2006 and first assessed for the first therapeutic results in October 2010. The clinical benefit after the treatment represented by overall response rate (ORR) and stable disease (SD) was achieved in 87.93% of patients, and surgical resection of metastases after therapy underwent 12.07% of patients. Median time to progression (TTP) was 8 months and median OS evaluated in October 2011 was 27 months. Mutation status of *KRAS* gene had no influence on the effectiveness of treatment and *BRAF* mutations exhibited a strong negative prognostic significance. Thromboembolic complications were present in 17.24%.

Key words: metastatic colorectal carcinoma, first-line treatment, biological treatment, molecular markers, bevacizumab-related toxicity

Colorectal carcinoma is one of the leading causes of death from cancer worldwide [2]. Interestingly, the localization of primary tumor varies depending on age. Some studies revealed, that young patients (<40) tend to develop right-sided colon tumors, whereas the tumors in patients >40 are more often localized in the left colon [3]. The incidence and mortality rates are slightly higher in men than in women. The most frequent localizations for metastatic spread are liver and lung. Approximately one quarter of CRC patients present with distant metastases at initial diagnosis, and almost 50% will develop them later, which has a strong influence on overall prognosis [4]. Only radical surgery offers the chance of long-term survival. After complete resection of single metastasis, the 5-year survival may exceed 50%, and in case of less than four lesions, the 5-year survival reaches 30-50% [5,6]. In

certain cases of initially unresectable disease, the metastases can become suitable for resection throughout a so-called conversion therapy.

For more than 40 years the only drug available for mCRC was 5-fluorouracil (5-FU), the use of which gives RR of 10-15% and median survival of 10 months. Important change in treatment of mCRC brought the addition of new cytostatics (irinotecan and oxaliplatin) and several studies confirmed that it is possible to achieve higher RR (40-50%), PFS (7-9 months) and OS (16-20 months) with combined regimens [7,8,9]. Another important progress in treatment of mCRC represents targeted biological therapy using monoclonal antibodies – anti-VEGF (bevacizumab) and anti-EGFR (cetuximab, panitumumab). Combined cytostatic therapy together with targeted biological treatment and improved

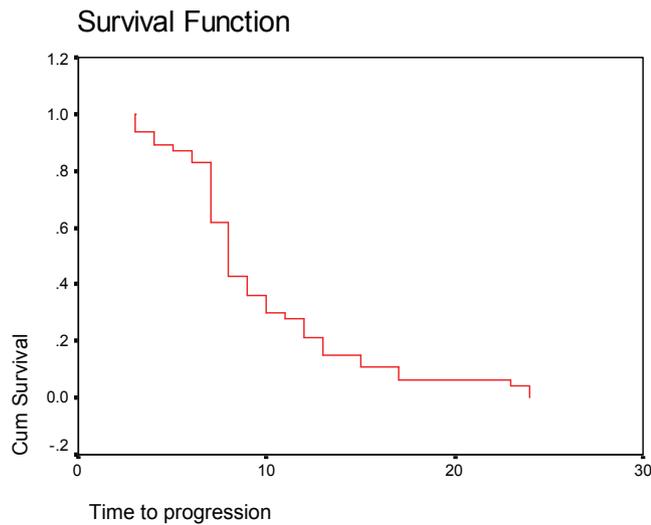


Figure 1. Kaplan-Meier analysis of TTP in 58 patients with mCRC. Median TTP was 8 months.

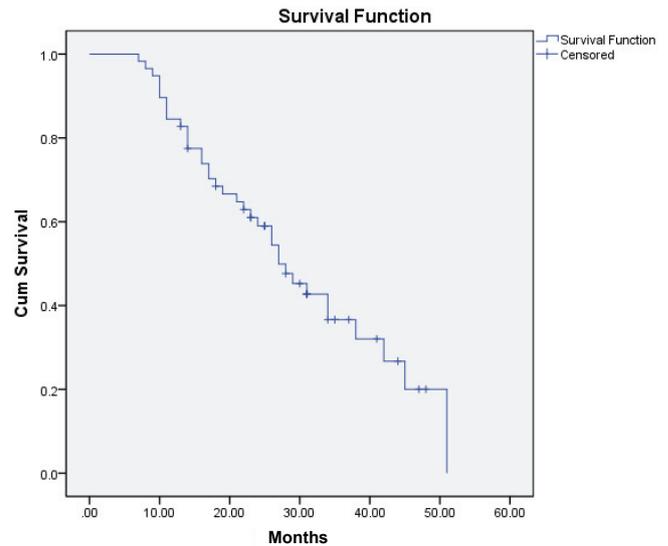


Figure 2. Kaplan-Meier analysis of OS in 58 patients with mCRC. Median survival was 27 months.

techniques to resect metastatic disease have improved the median survival of patients with mCRC from 6 months to 2 years. Treatment strategy depends on the treatment aim. Aggressive approach is indicated in patients with potentially resectable metastases, with symptomatic disease in whom tumor regression is needed and in patients with the risk of rapid deterioration due to the aggressive tumor biology and/or extensive disease. In patients with clearly unresectable disease, without tumor-related symptoms and low risk of rapid progression as well as comorbidity, a sequential therapy starting with a fluoropyrimidine monotherapy in combination with biological agent bevacizumab is the valid option.

Before starting the treatment by biological agent, the assessment of relevant predictive and prognostic molecular markers may be of interest. While prognostic markers identify patients with different outcome of the disease regardless of treatment strategy, predictive marker helps us to predict efficiency of a particular therapy [10]. The most important predictive marker for anti-EGFR therapy is *KRAS* mutational status. Many retrospective and prospective studies have shown, that *KRAS* mutations that occur approximately in 40% of patients with CRC are associated with resistance to anti-EGFR antibodies cetuximab and panitumumab [11-17]. This resistance is presented when anti-EGFR antibodies are used as a single agent or in combination with chemotherapy, in pretreated patients as well as in early lines of treatment. However, only 40-60% of *KRAS* wild-type patients respond to treatment suggesting that other molecular markers have to be considered in the future. One of them is *BRAF* gene that may be mutated up to 6-10% of patients with mCRC [18,19]. Interestingly, *BRAF* and *KRAS* mutations are mutually exclusive. Several studies have confirmed, that presence of the *BRAF* mutation corre-

lates with resistance to anti-EGFR therapy in chemorefractory mCRC [19-22]. However the available data on the strength of *BRAF* status for predicting response to anti-EGFR therapy are limited by retrospective analyses and small numbers of evaluated patients. Moreover, updated data from CRYSTAL study suggest that *KRAS* wild-type/*BRAF* mutant patients may actually respond to anti-EGFR therapy [18]. On the other hand, many studies have confirmed, that *BRAF* is a poor prognostic marker, because patients with this mutation have shorter PFS and OS, regardless of the type of treatment [18,19,23].

Anti-VEGF therapy is represented by monoclonal antibody bevacizumab. Bevacizumab is a recombinant humanized monoclonal IgG₁ antibody that specifically binds all isotopes of VEGF-A. VEGF-A is a member of the VEGF platelet-derived growth factor gene family and represents one of the most potent positive regulators of angiogenesis, including tumor angiogenesis. Its biological effects are mediated by binding to two tyrosine kinase receptors (VEGFR-1 and VEGFR-2) [24]. Since angiogenesis is essential for the growth, progression and metastasis of solid tumors, both VEGF-A and its receptors have been evaluated as potential therapeutic targets.

The problem with bevacizumab is that there are currently no clear predictive molecular markers for anti-VEGF therapy. According to the so far published studies, the presence of *KRAS* mutation does not seem to influence the outcome of anti-VEGF therapy probably because VEGF and RAS signaling pathways are independent of each other.

The toxicity of anti-VEGF and anti-EGFR therapy is different. Most frequent toxicity associated with cetuximab is acne-like rash. The frequency of acne-like rash in large, phase III studies of cetuximab in mCRC was 78-88%, with most events of grade 1 or 2 intensity [25]. Other adverse effects include infusion reactions and electrolyte dysbalances. Toxic-

ity profile of bevacizumab was assessed in several prospective randomised clinical trials as well as in observational studies [26-30]. Most adverse events associated with bevacizumab (hypertension, proteinuria and bleeding) are mild–moderate in severity and are manageable using standard therapies [26,31-33]. To more serious adverse events reported with bevacizumab belong thromboembolic events, gastrointestinal (GI) perforations and wound-healing complications. But serious toxic effects related to bevacizumab have generally the incidence of <5% [34].

The aim of our study was to perform a descriptive, retrospective analysis of patients with mCRC treated with chemotherapy and bevacizumab in the first line.

Patients and methods

Our analysis included 58 patients >18 years old with histologically confirmed mCRC who were treated with first-line fluoropyrimidine-based chemotherapy in combination with bevacizumab from January 2006. The first part of results was assessed in October 2010, OS was evaluated in October 2011. The chemotherapy regimens contained single agent fluoropyrimidine or fluoropyrimidine plus irinotecan (regimens IFL – irinotecan, 5-fluorouracil bolus injection, leucovorin (LV); XELIRI – irinotecan, capecitabine; FOLFIRI – irinotecan, 5-FU continuous infusion, LV) or oxaliplatin (regimens XELOX – oxaliplatin, capecitabine; FOLFOX 4 – oxaliplatin, 5-FU continuous infusion, LV). Bevacizumab dose was 5 mg/kg every 2 weeks or 7.5 mg/kg every 3 weeks. Bevacizumab was administered i.v., initially over 90 min. If the first infusion was well tolerated, the second was delivered over 60 min; if the 60 min infusion was well tolerated, all subsequent infusions were delivered over 30 min. Bevacizumab doses were not reduced or escalated; in cases of serious bevacizumab-related toxicity, drug was temporarily or permanently suspended. The analysis included also those patients who underwent adjuvant treatment for colorectal cancer in the past. The aim of our study was to evaluate following parameters in the cohort: age structure and gender of patients, localization of primary tumor and metastases, resectability of metastases, RR, OS, TTP and frequency of thromboembolic events during the treatment. We have also assessed genetic profile of the patients – frequency of *KRAS* and *BRAF* mutations in the tumor and importance of *BRAF* as a negative prognostic factor. We didn't performed VEGF staining because it is not demanded to check it before starting the treatment of mCRC with bevacizumab and it is not being evaluated in our institute.

RR were evaluated according to RECIST criteria [35] and the used imaging modality was computed tomography (CT). As a complete response (CR) we considered disappearance of all target lesions and each pathological lymph node had to have a short axis reduction to <10 mm. Partial response was defined as at least 30% decrease in the sum of diameters of target lesions, taking the baseline sum diameters as a reference value. Progressive disease (PD) was defined as at least 20%

increase in the sum of diameters of target lesions, taking the smallest sum as a reference value and the sum must also demonstrate an absolute increase of at least 5 mm. In case that the response was not PR neither PD we have assigned it as a SD. All patients were monitored equally and we have not offered any investigations above the framework of routine clinical practice. TTP and OS were calculated using Kaplan-Meier analysis.

KRAS and BRAF mutational analysis. The most common *KRAS* and *BRAF* point mutations were identified by focusing our analysis on exons 2 and 15, respectively (exon 2 includes codons 12 and 13 of *KRAS*, and exon 15 included codon 600 of *BRAF*, which represent the mutational hot spots leading to gain-of-function mutations in the respective proteins). *KRAS* exon 2 was PCR-amplified from tumor DNA as a 173 bps fragment, whereas exon 15 of *BRAF* was PCR-amplified as 103 bps fragment. PCR products were purified by ExoSAP-IT[®] kit (USB Corporation, Cleveland, Ohio) at 37°C for 45 min and subsequently at 80°C for 15 min. Mutations were then assessed by ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA) at the relevant nucleotides using single base extension SNaPshot primers (the sequences are available on request). The multiplex SNaPshot reaction was performed in a final volume of 10 µl, containing 1 µl of each PCR product, 2 µl of the SNaPshot Multiplex Ready Reaction Mix, 1 µl of 5x sequencing buffer of Big Dye V3.1 Terminator Kit and SNaPshot primers at 0.01-0.06 µM concentration. Cycling conditions were carried out according to the manufacturer's kit recommendation and SNaPshot products were subsequently purified by treatment with 2 U of shrimp alkaline phosphatase (USB Corporation, Cleveland, Ohio) at 37°C for 45 min. After heat inactivation of the enzyme at 80°C for 15 min, the fluorescently labeled products were separated using a 7 min run on ABI Prism 3130 DNA sequencer and data were analyzed using GeneMapper Analysis Software version (Applied Biosystems).

Results

Our analysis included 58 patients with median age 55.65 years, CRC was more often diagnosed in men (n=34) than in women (n=24). The median age was 57.53 in men and 55.17 in women. Primary tumor was more frequently localized in the left colon and typical localization of metastatic spread was the liver (Table 1).

Twenty-five patients (43,10%) underwent adjuvant chemotherapy for CRC in the past. Bevacizumab was used in first-line

Table 1. Localization of metastases

Metastases	Total	Men	Women
Peritoneum	10 (17,24%)	5	5
Liver	28 (48,27%)	15	13
Lungs	8 (13,79%)	5	3
Multiorgan	12 (20,68%)	9	3

Table 2.1 Used chemotherapy regimens

Name of Regimen	Schedule
XELOX	Oral capecitabine (1000 mg/m ²) twice a day for 14 days plus oxaliplatin (130 mg/m ²) on day 1 every 3 weeks
FOLFOX 4	Oxaliplatin (85 mg/m ²) administered as a 2-hour infusion on day 1; leucovorin (200 mg/m ²) administered as a 2-hour infusion on day 1 and day 2; followed by a loading dose of 5-FU (400 mg/m ²) IV bolus, then 5-FU (600 mg/m ²) administered via pump for a period of 22 hours on day 1 and day 2, every 2 weeks
IFL	Irinotecan (100-125 mg/m ²), 5-FU (425-500 mg/m ²) IV bolus, and leucovorin (20 mg/m ²) IV bolus administered weekly for 4 out of 6 weeks
XELIRI	Irinotecan (200 mg/m ²) administered on day one and capecitabine (800 mg/m ²) twice a day for 14 days 2-15, every 21 days
FOLFIRI	Irinotecan (180 mg/m ²) administered as a 2-hour infusion on day 1 only; leucovorin (200 mg/m ²) administered as a 2-hour infusion on days 1,2; 5-FU (400 mg/m ²) IV bolus administered on day 1 and day 2, 5-FU (600 mg/m ²) administered via pump for a period of 22 hours on day 1 and day 2, every 2 weeks
Fluoropyrimidine alone	capecitabine (1250 mg/m ²) twice a day for 14 days, every 21 days

Table 2.2 Chemotherapy regimens in combination with bevacizumab and TTP

Chemotherapy (+ bevacizumab)		Men	Women	Total	Time to progression
Oxaliplatin-based regimens	XELOX	6	4	15	8,7
	FOLFOX 4	2	3		
Irinotecan-based regimens	IFL	16	14	42	10
	XELIRI	6	2		
	FOLFIRI	4	-		
Other	Fluoropyrimidine alone	-	1	1	7

in combination with chemotherapy. Exact schedules of used chemotherapy regimens are described in Table 2.1. Irinotecan-based regimens (IFL, FOLFIRI, XELIRI) were used more frequently than oxaliplatin-based regimens (XELOX, FOLFOX 4) while fluoropyrimidine as a single agent was used only in one case (Table 2.2).

Clinical benefit represented by ORR (CR and PR) plus SD was achieved in 51 patients (87.93%). The rest of the patients have experienced PD after treatment. CR was achieved in

6 patients (11.76%), PR in 13 patients (25.49%) and SD in 32 patients (62.75%).

Twenty-two patients underwent resection of metastases and 7 of them achieved operability of metastases after the treatment with chemotherapy in combination with bevacizumab, here of 6 patients had liver and 1 patient had lung metastases. The data about response rates and resection of metastases are summarized in Table 3 and Table 4. OS in patients who underwent resection of metastases are shown on Figure 3.

Median TTP in patients treated with the first-line chemotherapy in combination with bevacizumab was 8 months as shown on Figure 1 and in Table 5 and was longer in patients treated with irinotecan-based regimens (Table 2.2). Median OS was evaluated in October 2011 and achieved 27 months as demonstrated on Figure 2 and in Table 6. OS according to used chemotherapy regimen and BRAF status are displayed on Figure 4 and 5.

Thromboembolic complications were observed in 10 patients (17.24%). Most frequent complication was a deep thrombophlebitis which was observed in 7 patients. Pulmonary embolism was present in 3 patients and in these cases bevacizumab was permanently suspended.

We have not seen any postoperative complications (bleeding or wound-healing complications) in 7 patients who underwent surgical resection. This was probably reached by preservation of the right time interval (surgery at least 6 weeks after the last applied dose of bevacizumab).

Majority of tumor samples of the patients enrolled in the study could be analyzed for the presence of *KRAS* and

Table 3. Objective tumor response rates

Therapeutic effect	Type of response	Total	Men	Women
Clinical benefit	Total	51 (87,93%)	30	21
	Complete response	6 (11,76%)	4	2
	Partial response	13 (25,49%)	6	7
	Stable disease	32 (62,75%)	20	12
Progressive disease		7 (12,06%)	4	3

Table 4. Resection of metastases

	Resection achieved with treatment	
	Liver	Other (lungs)
Men (n= 34)	3	0
Women (n=24)	3	1
Total (n=58)	6 (10,34%)	1 (1,72%)

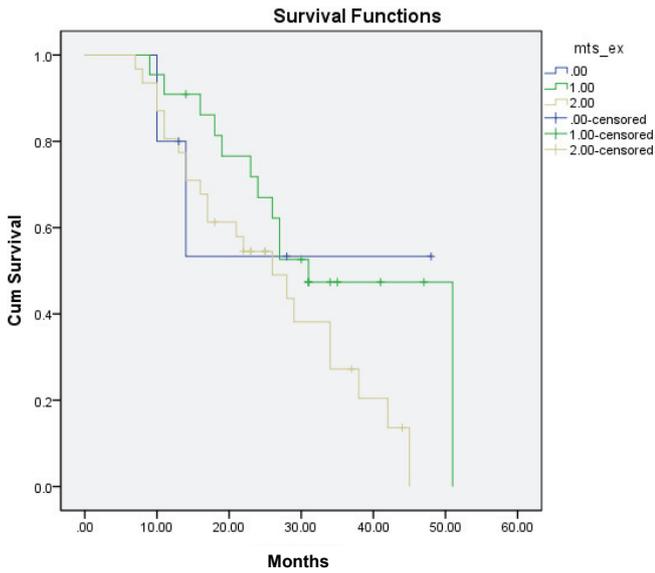


Figure 3. OS in mCRC patients who underwent resection of metastases as initial treatment (0), after bevacizumab treatment (1) and patients who did not have metastases resection (2).

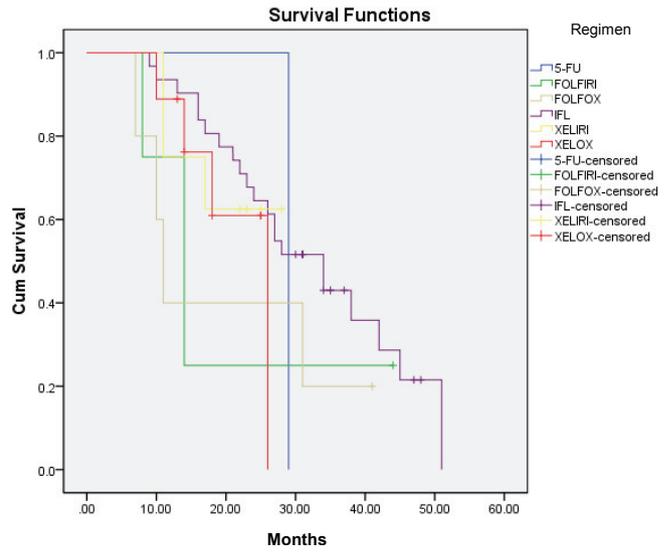


Figure 4. OS in mCRC patients on different chemotherapy regimens.

BRAF mutations. The data from these analyses together with results from EGFR immunohistochemical staining are summarized in Table 7. The presence of *KRAS* mutation was confirmed in 22 out of 56 patients (39.29%) and *BRAF* mutation was detected in other 3 out of 47 analysed patients (6.38%).

We have found that the presence of *KRAS* mutations had not influenced the response to treatment. However, slightly more than would be expected, (6 out of 7) patients who had progression on chemotherapy in combination with bevacizumab were *KRAS* wild-type. In the groups of patients with CR, PR or SD, the cases with or without *KRAS* mutation occurred with similar ratio (data not shown). All patients with *BRAF* mutations have fallen into PD group.

Discussion

The purpose of our retrospective analysis was to evaluate the efficacy and safety of bevacizumab when added to first-line cytostatic chemotherapy in patients with histologically confirmed mCRC. We compared the results of our analysis with data from large well-known multicentric studies.

Bevacizumab was used in our analysis in combination with fluoropyrimidine alone or with fluoropyrimidine combined with either oxaliplatin (regimens XELOX, FOLFOX 4) or irinotecan (regimens IFL, XELIRI, FOLFIRI).

The phase III trial AVF2107g, which compared first-line IFL regimen with and without bevacizumab in patients with mCRC revealed, that OS and PFS were longer in the IFL-be-

Table 5. Means and medians for time to progression

Mean				Median			
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
9.66	0.73	8.23	11.09	8.00	0.38	7.26	8.74

Table 6. Means and medians for survival time

Mean ^a				Median			
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
29.843	2.159	25.611	34.074	27.000	3.010	21.100	32.900

a. Estimation is limited to the largest survival time if it is censored.

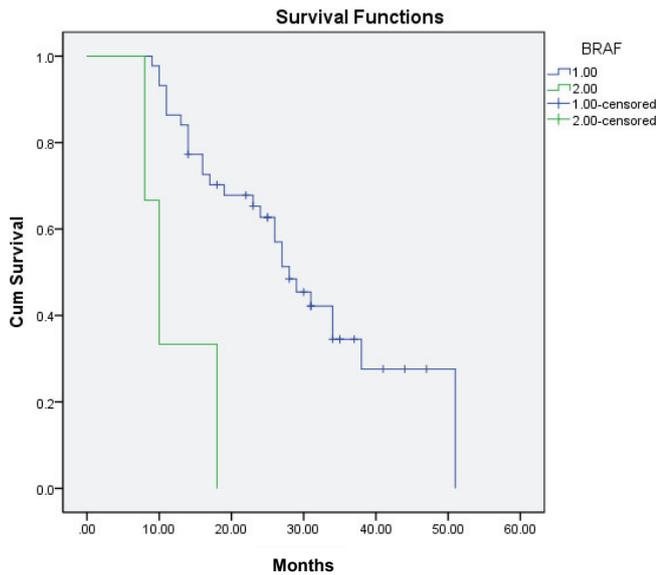


Figure 5. OS in mCRC patients with wild-type (1) and mutated (2) form of BRAF gene.

vacizumab arm [26]. These results led to the initial approval of bevacizumab by the FDA. The phase III BICC-C clinical trial evaluated bevacizumab in combination with regimen FOLFIRI and IFL. The results revealed that the patients in the FOLFIRI–bevacizumab arm had a longer median OS time than those in the IFL–bevacizumab arm and therefore FOLFIRI has gradually replaced IFL [36,37]. Another possibility, largely used especially in Europe is regimen XELIRI.

The efficacy of bevacizumab combined with a fluoropyrimidine and oxaliplatin has been evaluated in several randomized trials [28,33]. In the phase III NO16966 study [33] the addition of bevacizumab to chemotherapy (regimen XELOX and FOLFOX 4) resulted in a longer median OS time, 21.3 months versus 19.9 months in comparison to chemotherapy-placebo arm, but this difference was not statistically significant ($p=0.077$). The response rates were similar but there was a statistically significant longer median PFS interval for

patients receiving bevacizumab than for those given placebo ($p=0.002$). Regardless of the type of chemotherapy, patients treated with first-line chemotherapy plus bevacizumab consistently experience a median PFS interval in the range of 9–12 months and a median OS time of approximately 2 years. These results have been reached in several large trials, including CAIRO2, PACCE, HORIZON III trials [38,39,40]. Similar OS and PFS results were observed also in large observational studies, including BEAT, BRITE and ARIES trials [30,41,42]. In the ARIES study patients receiving FOLFOX plus bevacizumab in the first line had a median TTP and OS of 9.7 and 23.5 months, respectively [43] and patients receiving FOLFIRI plus bevacizumab had a median TTP and OS of 9.3 months and 26.3 months respectively.

Median TTP in our patients treated with the first-line chemotherapy in combination with bevacizumab was 8 months and was longer in patients treated with irinotecan-based regimens. Median OS achieved in our analysis 27 months. Our analysis included 58 patients, but achieved results are comparable to those presented in the larger studies mentioned above. This underlines the importance of monitoring and evaluation of treatment efficacy also in smaller groups of patients and these results can be used as a basis for larger pooled analyses. Approximately one quarter of CRC patients with distant metastases at initial diagnosis and one third of patients with mCRC have disease confined to the liver [44]. In these cases only radical surgery offers the chance of long-term survival. The safety and efficacy of bevacizumab in preoperative setting were evaluated in a post hoc analysis of the NO16966 and First BEAT clinical trials [45]. In the group of patients with metastases limited to the liver, 12.3% of patients treated with chemotherapy plus bevacizumab (26 of 211) eventually received a R0 resection, compared with 11.6% of patients (24 of 207) treated with chemotherapy plus placebo. Seven of 58 patients (12.07%) in our analysis achieved operability following the first-line treatment with chemotherapy in combination with bevacizumab, 6 of them had metastases in the liver and 1 in lungs. Percentage of operability achieved in our study is comparable to the results of analysis mentioned above. Additional studies have assessed conversion therapy with bevacizumab in patients with liver-only metastases, with encouraging results [46,47,48].

Most adverse events associated with bevacizumab are hypertension, proteinuria and bleeding, more serious are thromboembolic events, gastrointestinal perforations and wound-healing complications. In our analysis we evaluated the incidence of thromboembolic complications, which were observed in 10 patients (17.24%). Deep thrombophlebitis occurred in 7 patients (12.07%) and pulmonary embolism in 3 patients (5.17%). On the other hand, arterial thromboembolism was not observed in our patients. Although AVF2107g study did not show an association between bevacizumab and the risk for venous thromboembolism (VTE), a meta-analysis of four placebo-controlled studies of chemotherapy with and without bevacizumab suggested a potential risk for VTE in patients receiving antiangiogenic therapy [49].

Table 7. Genetic profile of patients

		Men	Women	Total
EGFR	Present	24	20	44
	Absent	7	4	11
	Uninvestigated	3	0	3
KRAS	Wild-type	21	13	34
	Mutated	12	10	22
	Uninvestigated	1	1	1
BRAF	Wild-type	25	19	44
	Mutated	2	1	3
	Uninvestigated	7	4	11

Another large pooled analysis of 10 placebo-controlled trials of chemotherapy with and without bevacizumab found no difference in the risk for VTE in patients receiving bevacizumab compared with placebo [50]. Therefore VTE still remains an important topic in all mCRC patients [51].

We separately evaluated the genetic profile of patients. Current consensus guidelines recommend that the presence of *KRAS* mutations has to be determined in all patients diagnosed with stage IV colorectal cancer. The *KRAS* mutation data were achievable for most of the patients included in our study. In addition, at our institute *KRAS* is now simultaneously analyzed together with *BRAF*, as the mutation detection system that had been introduced is enabling an easy and cost-effective parallel analysis of both genes. In our patients cohort only 3 of 47 patients analyzed displayed mutation V600E of *BRAF*, which represents 6.38%, a frequency similar to those published elsewhere [18,19]. *KRAS* mutations were seen in 22 cases of 56 analyzed and also this frequency of *KRAS* changes in mCRC (39.29%) is common. Our data basically support observations that *KRAS* status of colorectal cancer does not interfere with the efficacy of bevacizumab treatment [52]. In regard to mutations in *BRAF* gene, the positive patients occurred exclusively in the group of patients with progression and represented a very high percentage of the cases in this group (42.8%). Our results indirectly support the notion that mutated *BRAF* has a strong negative prognostic significance.

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