ORIGINAL RESEARCH

Sequential therapy with bevacizumab and EGFR inhibitors for metastatic colorectal carcinoma: a national registry-based analysis

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Purpose: Although inhibitors of vascular endothelial growth factor and inhibitors of epidermal growth factor receptor (EGFRi) are commonly used for the treatment of metastatic colorectal cancer (mCRC), the optimal sequencing of these agents is currently unclear.

Methods: A national registry of targeted therapies was used to analyze baseline characteristics and outcomes of patients with mCRC and wild-type *KRAS* exon 2 status who received bevacizumab and EGFRi (cetuximab or panitumumab) as a part of first- and second-line treatment in either sequence.

Results: The cohort included 490 patients (181 patients treated with first-line EGFRi and second-line bevacizumab and 309 patients treated with first-line bevacizumab and second-line EGFRi). Median overall survival (OS) from the initiation on first-line therapy was similar for patients treated with either sequence, reaching 31.8 (95% CI 27.5–36.1) vs 31.4 months (95% CI 27.8–35.0) for EGFRi → bevacizumab vs bevacizumab → EGFRi cohort, respectively. Time from first-line initiation to progression on the second-line therapy [progression-free survival (PFS)] was 21.1 (95% CI 19.3–23.0) vs 19.3 months (95% CI 17.3–21.3) for bevacizumab → EGFRi vs EGFRi → bevacizumab cohort, respectively (*P*=0.016).

Conclusion: This retrospective analysis of real-world data of patients with wild-type *KRAS* exon 2 mCRC showed no differences in OS between cohorts treated with bevacizumab → EGFRi vs the reverse sequence while combined PFS favored the bevacizumab → EGFRi sequence.

Keywords: colorectal carcinoma, bevacizumab, panitumumab, cetuximab, sequence

Introduction

The monoclonal antibody (MoAb) against vascular endothelial growth factor (VEGF) bevacizumab and MoAbs directed against the epidermal growth factor receptor (EGFR) cetuximab and panitumumab are commonly used in the treatment of metastatic colorectal cancer (mCRC).

Three randomized trials have compared first-line treatment with VEGF inhibitors (VEGFi) vs EGFR inhibitors (EGFRi) added to a chemotherapy backbone. In the FIRE-3 trial, patients received 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) chemotherapy in combination with either cetuximab or bevacizumab. There were no differences in progression-free survival (PFS) or overall response rate (ORR); however, there was a significant difference in overall survival (OS) reflected in a late separation of survival curves favoring the cetuximab arm.[1] PEAK trial was a randomized phase II study using 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) chemotherapy with either bevacizumab or panitumumab in wild-type *KRAS* exon 2 mCRC. Similarly to FIRE-3, there was a numerical advantage in OS for the panitumumab cohort, although in the PEAK

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study, the difference was not statistically significant, probably due to the relatively small number of enrolled patients.[2] The largest trial specifically designed to compare between the two types of MoAbs in mCRC was the Cancer and Leukemia Group (CALGB) 80405 study. Patients were treated with either FOLFOX or FOLFIRI and randomized to bevacizumab or cetuximab. No differences in PFS or ORR were reported, and OS was also similar between patients receiving VEGFi and EGFRi therapy, contrasting with results of the FIRE-3 and the PEAK studies.[3] Three meta-analyses of these randomized studies have been carried out, all showing superior OS in patients who start with EGFRi as opposed to VEGFi.[4–6]

In the present retrospective study, a large clinical registry of cancer patients treated with targeted therapies was used to analyze and to compare the outcomes of patients treated with bevacizumab followed by EGFRi therapies including cetuximab or panitumumab vs patients treated with the reverse sequence of the targeted therapies.

Materials and methods
Study design
The CORECT (http://corect.registry.cz) registry was used as a data source for the present analysis. The registry is a prospective, non-interventional post-registration database that contains anonymized data on patients with mCRC treated with targeted therapy. Individual patient data including baseline characteristics, treatment type, response, survival, and adverse events are recorded. The data entries are updated twice a year. The therapy with targeted agents in the Czech Republic is reimbursed only in comprehensive cancer centers, and the registry provides data on ~90% of all patients treated with targeted therapy for mCRC in these centers.

All study procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the Declaration of Helsinki 1964 and its later amendments. The CORECT registry and the use of registry data for analysis were approved by the Multicenter Ethics Committee of the Masaryk Memorial Cancer Institute in Brno, Czech Republic. All patients included in the study signed informed consent with the inclusion and subsequent analysis of their data in the registry.

Patients
The cohort for analysis included all patients with colorectal adenocarcinoma and valid data in the registry as of October 10, 2016 who met the following inclusion criteria: 1) sequential therapy with first-line cetuximab or panitumumab and second-line bevacizumab OR first-line bevacizumab

and second-line cetuximab or panitumumab; 2) progression documented in the database between the first and the second line; 3) first-line therapy using FOLFOX or FOLFIRI regimens as chemotherapy backbone; 4) wild-type *KRAS* exon 2 status; 5) wild-type or unknown *KRAS* exon 3 and 4; and 6) wild-type or unknown *NRAS* status. The Consolidated Standards of Reporting Trials (CONSORT) diagram depicting patient selection is shown in Figure 1.

The interval of tumor assessments was not pre-specified. However, reimbursement conditions for targeted therapies stipulate radiological tumor assessment at least every 3 months or three cycles of therapy as applicable. Treatment response was assessed using the Response Evaluation Criteria in Solid Tumors version 1.1.[7]

Patients with primary tumor in the caecum, the colon ascendens, and transversum were considered to have a right-sided primary, while tumors in the descending and sigmoid colon or the rectum were classified as left-sided primary tumors.

Statistical analysis
Descriptive statistics and frequency tables were used to characterize the sample data set. Statistical significance of differences in categorical parameters was assessed using the Fisher's exact test or Pearson's test. For continuous variables, Mann–Whitney test was used. PFS was defined as the time from first-line treatment initiation to the date of documented progression on the second-line therapy or death of any cause. OS was defined as the time from first-line target therapy initiation to the date of death of any cause. PFS and OS were estimated using Kaplan–Meier method and all point estimates include 95% CI. Statistical significance of the differences in Kaplan–Meier estimates was assessed using the log-rank test. Univariate and multivariate Cox tests of proportional risks were used to calculate differences in survival for selected subgroups and select the principal prognostic factors. Point estimates of HR are shown with 95% CI. Statistical significance of HR was calculated using the Wald test. An alpha of 0.05 was used as the cutoff of significance for all statistical tests.

Results
Baseline characteristics
Overall, 490 patients, including 181 patients treated with first-line cetuximab or panitumumab and second-line bevacizumab and 309 patients treated with first-line bevacizumab and second-line cetuximab or panitumumab were included in the analysis (Figure 1).

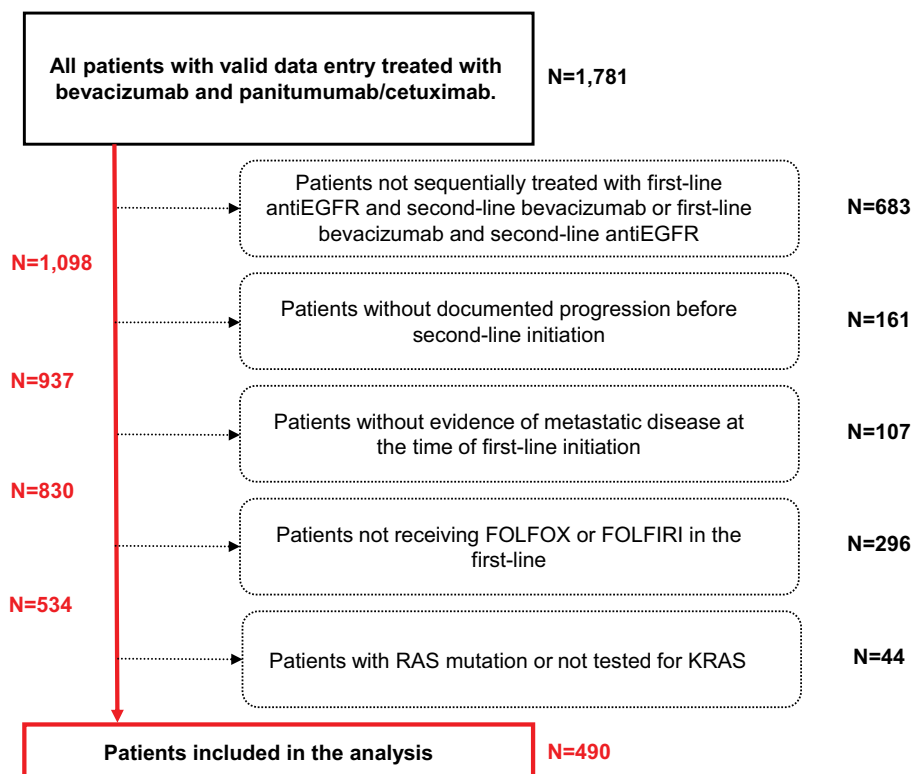Baseline patient characteristics are shown in Table 1. Patients treated with bevacizumab → EGFRi sequence

Figure 1 CONSORT diagram of selection of patient data from the CORECT database.
Abbreviations: EGFR, epidermal growth factor receptor; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; CONSORT, Consolidated Standards of Reporting Trials.

were significantly younger at the time of diagnosis and initiation of the first- and second-line treatment compared to patients receiving the reverse sequence, although the difference in median age was only 3 years and the proportion of patients >60 years of age was similar. A lower proportion of patients were tested for *NRAS* and *BRAF* mutations in the bevacizumab → EGFRi cohort. This cohort was also more likely to receive FOLFOX as a part of the first-line treatment (71% vs 63%, respectively, $P<0.001$).

An overview of patient characteristics at the time of second-line therapy initiation is provided in Table 2. Patients treated with the bevacizumab → EGFRi sequence were younger at the start of second-line treatment and tended to receive less intensive second-line backbone chemotherapy and 28.2% only received EGFRi monotherapy.

More than 98% of patients had Eastern Cooperative Oncology Group performance status of 0–1 at the time of initiation of the first and second line, in accordance with reimbursement conditions for targeted therapies.

Median follow-up from the initiation of the first-line therapy was 20.7 and 30.9 months for the EGFRi → bevacizumab and the bevacizumab → EGFRi cohort, respectively. Median follow-up from the initiation of the second-line therapy was 7.7 and 11.8 months, respectively.

Overall survival

Median OS from the initiation on first-line therapy was similar for patients treated with different sequences, reaching 31.4 (95% CI 27.8–35.0) vs 31.8 months (95% CI 27.5–36.1) for the bevacizumab → EGFRi vs the EGFRi → bevacizumab cohort, respectively ($P=0.940$) (Figure 2).

The Cox analysis of proportional risk showed that there was a trend bordering on statistical significance ($P=0.050$) in patients with wild-type *RAS* (ie, tested negative for both expanded *KRAS* and *NRAS* mutations, n=182) suggesting benefit from the bevacizumab → EGFRi sequence (Figure 3).

Multivariate Cox analysis revealed that male gender, left colon primary, M0 stage at diagnosis, and FOLFOX as first-line chemotherapy regimens were independently associated with favorable OS (Table 3)

PFS

PFS calculated as the time from the initiation of first-line therapy to progression on the second-line therapy was similar for the two sequences. Median PFS was 21.1 (95% CI 19.3–23.0) vs 19.3 months (95% CI 17.3–21.3) for the bevacizumab → EGFRi vs the EGFRi → bevacizumab cohort, respectively ($P=0.016$) (Figure 4). The time from the discontinuation of the first-line to the initiation of the

Table 1 Baseline patient characteristics

	Treatment sequence		P-value[a]
	EGFRi → bevacizumab (n=181)	Bevacizumab → EGFRi (n=309)	
Sex, n (%)			
Female	65 (35.9)	109 (35.3)	0.922
Male	116 (64.1)	200 (64.7)	
Age at diagnosis (years)			
Median (range)	63 (29–81)	60 (31–81)	0.003
Primary tumor site, n (%)			
Rectum	73 (40.3)	137 (44.3)	0.396
Colon	108 (59.7)	172 (55.7)	
Primary tumor side[b], n (%)			
Left colon	122 (82.4)	179 (89.1)	0.085
Right colon	26 (17.6)	22 (10.9)	
Not available	33	108	
Presence of metastases at diagnosis, n (%)			
M0	67 (37.0)	119 (38.5)	0.773
M1	114 (63.0)	190 (61.5)	
NRAS status, n (%)			
NRAS mutation	0 (0.0)	0 (0.0)	<0.001
NRAS wild-type	110 (60.8)	72 (23.3)	
Not done/not available	71 (39.2)	237 (76.7)	
BRAF status, n (%)			
BRAF mutation	4 (2.2)	2 (0.6)	0.006
BRAF wild-type	49 (27.1)	52 (16.8)	
Not done/not available	128 (70.7)	255 (82.5)	
Prior adjuvant therapy, n (%)			
Yes	65 (35.9)	108 (35.0)	0.845
No	116 (64.1)	201 (65.0)	
Age at first-line initiation (years)			
Median (range)	64 (29–82)	61 (31–83)	0.006
<60 years	58 (32.0)	125 (40.5)	0.067
>60 years	123 (68.0)	184 (59.5)	
BMI at first-line initiation[c] (kg/m²)			
Median (5–95 percentile)	26 (19–35)	26 (20–34)	0.882
<25	64 (37.9)	107 (39.3)	0.764
>25	105 (62.1)	165 (60.7)	
First-line targeted therapy, n (%)			
Bevacizumab	0 (0.0)	309 (100.0)	–
Cetuximab	100 (55.2)	0 (0.0)	
Panitumumab	81 (44.8)	0 (0.0)	
First-line backbone chemotherapy, n (%)			
FOLFOX	116 (64.1)	245 (79.3)	<0.001
FOLFIRI	65 (35.9)	64 (20.7)	
ECOG PS at first-line initiation, n (%)			
PS 0	80 (44.2)	153 (49.5)	0.191
PS 1	94 (51.9)	128 (41.4)	
PS 2	2 (1.1)	5 (1.6)	
Not available	5	23	

Notes: [a]Fisher's exact or Mann–Whitney test. [b]Right colon: cecum, ascendens, transversum; left colon: descendens, sigmoid, rectum. [c]BMI not available for 49 patients: EGFRi → bevacizumab (n=12), bevacizumab → EGFRi (n=37).
Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; EGFRi, inhibitors of epidermal growth factor receptor; PS, performance status; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; ECOG PS, Eastern Cooperative Oncology Group performance status.

second-line chemotherapy was 1.1 (95% CI 0.2–12.4) vs 1.7 months (95% CI 0.3–15.6) for the EGFRi → bevacizumab vs the bevacizumab → EGFRi (P=0.003). In multivariate analysis, bevacizumab → EGFRi and left colon primary were associated with better PFS (Table 3).

Discussion

A large cohort of patients with mCRC treated with EGFRi agents and bevacizumab in either sequence in the real-world setting was analyzed in this study. No statistically significant differences in OS were detected between patients treated with

Table 2 Patient characteristics at the start of second-line treatment

	Treatment sequence		P-value[a]
	EGFRi → bevacizumab (n=181)	Bevacizumab → EGFRi (n=309)	
Age at second-line initiation (years)			
Median (range)	65 (31–82)	62 (32–84)	0.022
BMI at second-line initiation[b] (kg/m^2)			
Median (5–95 percentile)	26 (20–35)	26 (19–34)	0.941
Second-line targeted therapy, n (%)			
Bevacizumab	181 (100.0)	0 (0.0)	–
Cetuximab	0 (0.0)	190 (61.5)	
Panitumumab	0 (0.0)	119 (38.5)	
Second-line chemotherapy[c], n (%)			
FOLFIRI	87 (48.1)	145 (46.9)	<0.001
Irinotecan monotherapy	4 (2.2)	58 (18.8)	
FOLFOX	29 (16.0)	4 (1.3)	
Capecitabine	20 (11.0)	1 (0.3)	
Other FU-based regimen	37 (20.4)	14 (4.5)	
Monotherapy	4 (2.2)	87 (28.2)	
ECOG PS at second-line initiation, n (%)			
PS 0	61 (35.1)	123 (42.6)	0.042
PS 1	113 (64.9)	161 (55.7)	
PS 2	0 (0.0)	5 (1.7)	
Not available	7	20	

Notes: [a]Fisher's exact or Mann–Whitney test. [b]BMI not available for 66 patients: EGFRi → bevacizumab (n=16), bevacizumab → EGFRi (n=50). [c]Pearson's test.
Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; EGFRi, inhibitors of epidermal growth factor receptor; PS, performance status; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; ECOG PS, Eastern Cooperative Oncology Group performance status.
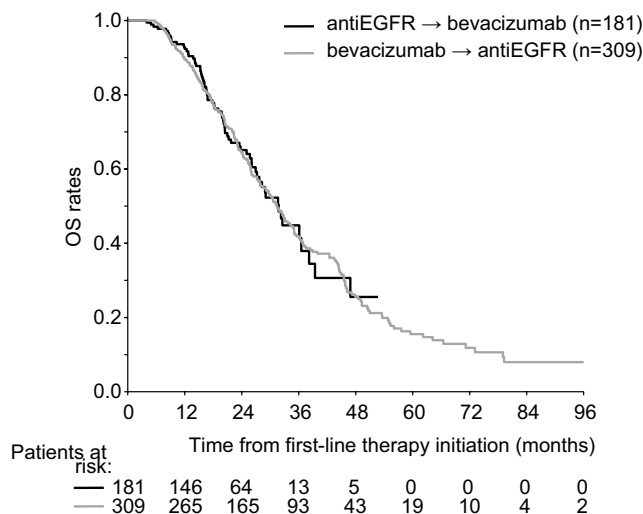
Figure 2 OS from first-line treatment initiation.
Abbreviations: EGFR, epidermal growth factor receptor; OS, overall survival.

the bevacizumab → EGFRi sequence and the reverse sequence for the whole cohort or for subgroups of patients defined by age, chemotherapy backbone, the presence of synchronous/metachronous metastatic disease, anatomic location of the primary tumor, *RAS* gene status, and body mass index. PFS but not OS was better in the bevacizumab–EGFRi cohort even though less intensive backbone chemotherapy regimens were used with second-line EGFRi therapies and 28% of the patients received second EGFRi treatment only as monotherapy.

Proper sequencing of targeted agents in mCRC is currently a matter of substantial controversy. Two approaches have been used to explain the discrepancies between the results of FIRE3 and PEAK studies on one hand, and CALGB 80405 on the other. First, there are inter-trial differences in off-study second-line therapies, particularly in the exposure to second-line biologicals. In the CALGB trial, almost 90% patients crossed over to the other type of antibody, while this proportion in the FIRE3 trial was approximately 50%.[8,9] In the PEAK trial, 50% of patients subsequently received the other type of inhibitor, while approximately one-third continued on the same type of inhibitor.[2] In this respect, CALGB 80405 is similar to the present study, which confirms its findings in the real-world setting. Tumor sidedness has been associated with relative benefits of different sequences in post-hoc analyses of FIRE-3 and CALGB 80405. The results suggest that first-line EGFRi are optimal for patients with left-sided primary tumors, while patients with right-sided primary tumor might benefit from the bevacizumab as a part of the first-line regimen.[10,11]

Another possible explanation postulates an interaction between the two types of therapies administered sequentially. It has been hypothesized that EGFRi therapy given as a part of first-line treatment enhances the effect of post-progression VEGFi therapy.[9] This theory has been formulated to explain the late divergence of OS curves in the FIRE-3 trial.[1,9] There is also some evidence from preclinical studies indicating that
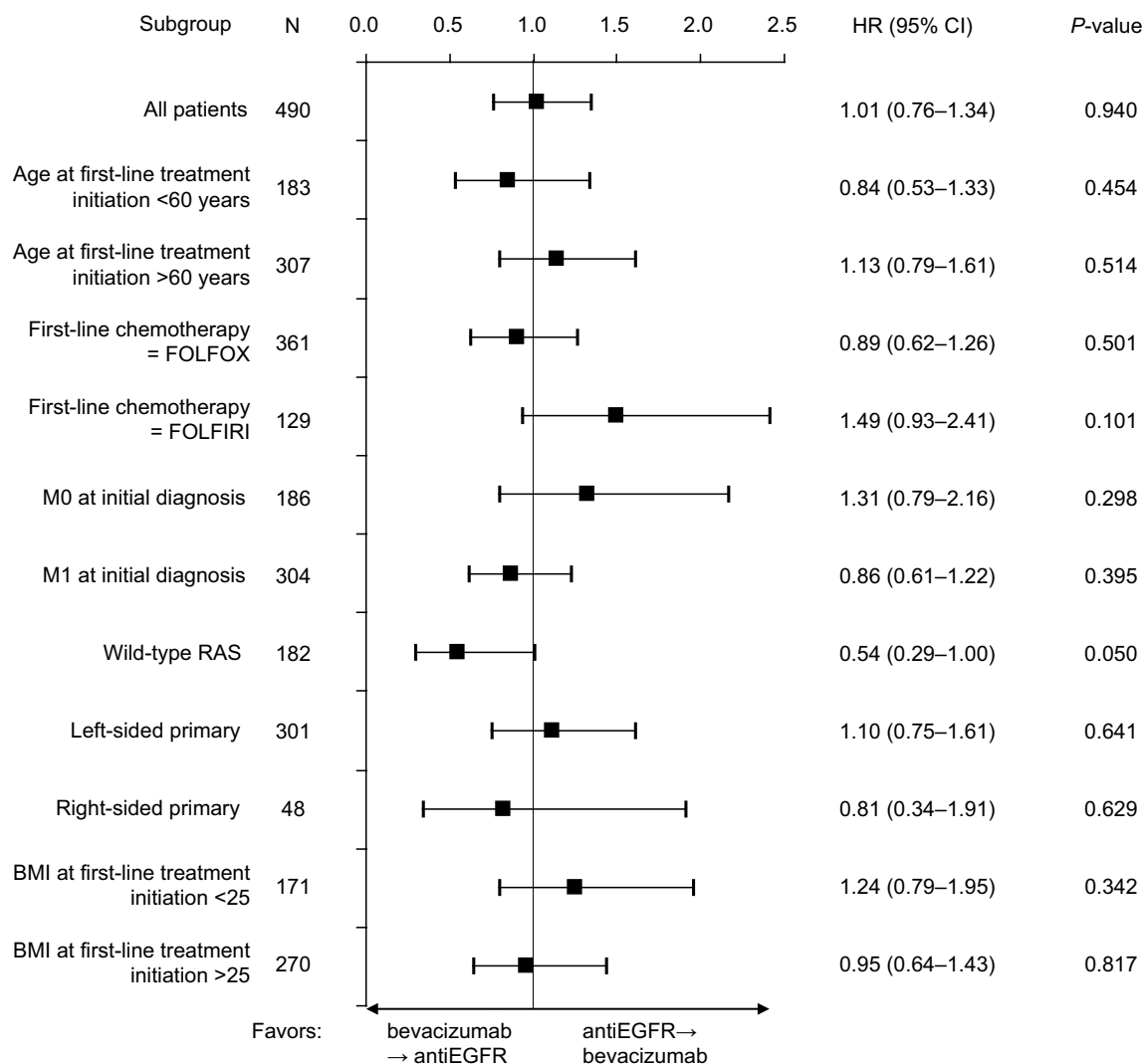
Figure 3 Forest plot of OS for selected patient subgroups.
Note: Wild-type *RAS* subgroup includes patient with available result of extended *KRAS* and *NRAS* testing.
Abbreviations: BMI, body mass index; OS, overall survival.

Table 3 Multivariable Cox-proportional hazards model for overall and PFS

Characteristic	Category	n	OS		PFS	
			HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Treatment sequence	EGFRi → bevacizumab	139	1.00	–	1.00	–
	Bevacizumab → EGFRi	176	1.12 (0.77–1.65)	0.552	0.68 (0.53–0.87)	0.002
Sex	Female	103	1.00	–	1.00	–
	Male	212	0.70 (0.50–0.99)	0.041	0.86 (0.67–1.10)	0.220
Primary tumor site	Left colon	82	1.00	–	1.00	–
	Rectum	190	1.58 (1.02–2.44)	0.040	1.12 (0.86–1.47)	0.401
	Right colon	43	3.12 (1.74–5.62)	<0.001	1.50 (1.03–2.18)	0.035
Presence of metastases at diagnosis	M0	125	1.00	–	1.00	–
	M1	190	1.62 (1.14–2.29)	0.007	0.86 (0.67–1.10)	0.238
Age at first-line initiation	<60 years	110	1.00	–	1.00	–
	>60 years	205	1.18 (0.84–1.65)	0.336	1.15 (0.90–1.47)	0.264
BMI at first-line initiation	<25	120	1.00	–	1.00	–
	>25	195	0.73 (0.52–1.02)	0.064	0.80 (0.63–1.02)	0.068
First-line backbone chemotherapy	FOLFOX	230	1.00	–	1.00	–
	FOLFIRI	85	1.57 (1.07–2.31)	0.020	1.00 (0.75–1.33)	0.976

Abbreviations: BMI, body mass index; EGFRi, inhibitors of epidermal growth factor receptor; OS, overall survival; PFS, progression-free survival.
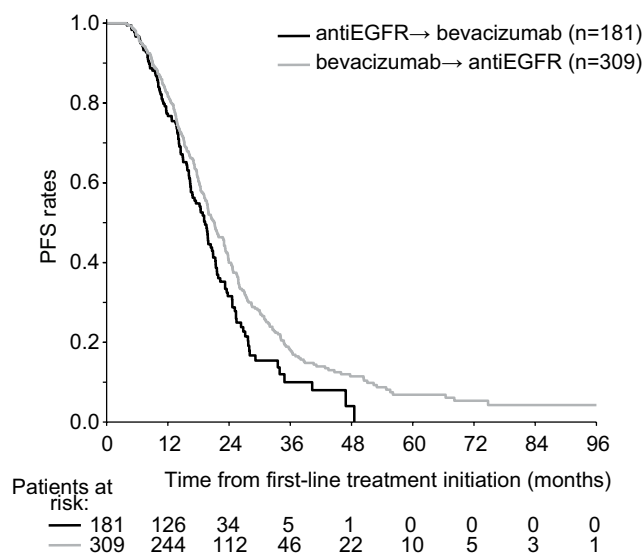
Figure 4 Time from treatment initiation to progression on second-line treatment.
Abbreviations: EGFR, epidermal growth factor receptor; PFS, progression-free survival.

chronic VEGF inhibition produces changes in phenotype of tumor cells that lead to increased invasiveness and metastatic potential.[12,13] The biological hypotheses on the potential temporal interaction between EGFRi, VEGFRi and subsequent disease course and response to sequential treatments have been extensively reviewed by Wainberg and Drakaki.[14]

Several recent retrospective studies also support the hypothesis that the carryover effect of VEGFi therapy negatively influences subsequent treatment efficacy. Derangère et al found difference in PFS on EGFRi depending on previous treatment with bevacizumab, although there was no difference in OS.[15] In a study published by Moehler et al, patients treated with EGFRi → VEGFi sequence had better OS than those treated with reverse sequence.[16] Sato et al reported that patients not receiving bevacizumab prior to cetuximab had better OS, although there were imbalances in age and treatment intensity, which could have caused or contributed to the findings.[17] No patients pretreated with bevacizumab responded to subsequent EGFRi in a small cohort analyzed by Norguet et al.[18] Conversely, prior EGFRi therapy had no influence of the efficacy of subsequent bevacizumab-containing regimens in another retrospective analysis.[19] The influence of VEGFi on subsequent EGFRi may depend on interval between the two therapies, the effect decreasing over time.[20]

In contrast, several prospective studies identified no adverse impact of VEGFi therapy on subsequent outcomes. Price et al reported similar survival of patients previously treated vs untreated with bevacizumab-containing therapy in the randomized ASPECCT trial comparing cetuximab

to panitumumab.[21] There was even a trend to improved OS in patients who continued on VEGFi in the Prodige 18 randomized phase II study assessing the efficacy and safety of crossover chemotherapy plus bevacizumab or cetuximab after progression on bevacizumab-containing regimen.[22] Price et al found that outcomes of EGFRi treatment were independent of previous use of VEGFi.[23] The efficacy of continued VEGF blockade was confirmed by the results of the ML18147 randomized phase III study.[24] An ongoing clinical trial called STRATEGIC-1 is aimed at optimizing the sequential approach to targeted therapies in mCRC, with one of the arms testing a three-line strategy with continued bevacizumab after progression on bevacizumab and a switch to EGFRi only after the second progression and the other arm the EGFRi → VEGFi approach.[25]

The prognostic and predictive role of the primary tumour location has been recently reported in several studies. It is evident that the different clinical and molecular characteristics of right-sided vs left-sided tumors translate into different clinical outcomes, with right-sided tumors displaying a markedly worse prognosis.[26–32] The results of recently published meta-analysis of 13 randomized clinical trials by Holch et al confirmed that right-sided tumors are associated with significantly shorter OS compared to left-sided tumors.[33] In our study, the sidedness of primary tumour was not associated with benefit of either sequence as all patients were exposed to both EGFRi and VEGFRi. However, the negative prognostic impact of right-sided primary tumors was confirmed in multivariate analysis (Table 3).

The strength of the present study includes the exposure of all patients to both types of treatment, enabling to test the hypothesis on the possible interactions between EGFRi and VEGFi strategies given sequentially.

The limitations are inherent in the retrospective, registry-based approach with retroactive cohort identification. In a prospective study, certainly less than 100% of patients receiving any first-line therapy would be able to proceed to second-line therapy. The protocol of the CORECT registry prevented direct comparison of individual lines of treatment, as it permits only combined analyses rather than comparisons of individual drugs. Only a small number of patients had *BRAF* analysis, precluding us from analyzing the impact of this important biomarker.

There are imbalances in baseline characteristics of patients that can be mostly linked to changing preferences and reimbursement for the respective therapies in different time periods, resulting in selection bias. Prior to 2012, a higher proportion of patients treated with bevacizumab outside of clinical studies in the Czech Republic continued

to EGFRi than vice versa. The possibility of selection of early patients with favorable disease course in the bevacizumab → EGFRi cohort cannot be excluded, and the inclusion of these patients could have biased the results in favor of the bevacizumab → EGFRi. Many patients in the bevacizumab → EGFRi cohort had been treated before the predictive role of *NRAS* mutations and exon 3+4 *KRAS* mutations were recognized.[34–36] Full *RAS* testing could potentially improve the outcomes of the bevacizumab → EGFRi cohort (Figure 3). The fact that *RAS* wt population appears to derive a benefit from bevacizumab–antiEGFR sequence over the reverse sequence is of interest, although the finding needs to be prospectively verified. There are actually few published data on the efficacy of second-line EGFRi given in combination with fluorouracil-based chemotherapy, with a single trial showing improved PFS on FOLFIRI/panitumumab in patients pretreated with a fluoropyrimide-based regimen.[37] Previous therapy with bevacizumab was not allowed in this trial.[38] However, a biological explanation is also possible. Correale et al published a study showing that exposure to chemotherapy increased the membrane expression of EGFR on cancer cells and sensitized them to cetuximab-mediated antibody-dependent cell cytotoxicity.[39] In a recently published study, we report that limited pretreatment with chemotherapy before the start of EGFRi could be associated with modestly improved outcomes.[40]

Conclusion

The present retrospective analysis of real-world data of patients with mCRC without *KRAS* mutation treated with EGFRi agents and bevacizumab in either sequence found no differences in OS between cohorts treated with bevacizumab → EGFRi vs the reverse sequence. There was a modest but statistically significant improvement in PFS for the bevacizumab → EGFRi sequence. Tumor sidedness was not significantly associated with benefit of either sequence.

Acknowledgments

Disclosure

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