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Original research

First-line treatment efficacy of anti-EGFR versus anti-VEGF antibodies in BRAF^{V600E}-mutated metastatic colorectal cancer according to primary tumor sidedness: A pooled analysis of seven clinical trials performed in the first-line treatment of mCRC (German AIO Study Group)☆

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ABSTRACT

Background: Both BRAF^{V600E}-mutation and right-sided primary tumor location (PTL), have been associated with poor prognosis in metastatic colorectal cancer (mCRC). The present pooled analysis of individual patient data evaluates the efficacy of first-line chemotherapy combined with anti-EGFR- or anti-VEGF-directed therapy in BRAF^{V600E}-mut mCRC together with PTL.

Methods: We conducted a pooled analysis of seven first-line AIO-studies (FIRE-3, FIRE-4, FIRE-4.5, CIOX, XELAVIRI, PANAMA, VOLFI) including patients with BRAF^{V600E}-mut and RAS-wild-type mCRC.

Results: Among 209 evaluable patients, left-sided primary tumors (LSPT) were observed in 98 (46.9 %) compared to 111 (53.1 %) patients with right-sided primary tumors (RSPT).

In the overall cohort, ORR was comparable (OR 0.85; 95 % CI 0.47–1.52), while median PFS was significantly shorter in patients receiving anti-EGFR-based therapy (HR 1.42; 95 % CI 1.05–1.91; P = 0.022), no major difference was observed with regard to OS (HR 0.96; 95 % CI 0.70–1.32; P = 0.80).

Patients with LSPT showed comparable PFS (HR 0.98; 95 % CI 0.63–1.51), but a numerical OS benefit (HR 0.71; 95 % CI, 0.45–1.14) with anti-EGFR- compared to anti-VEGF-based therapy. This effect was observed

☆ Trial identification number: NCT00433927 (FIRE-3), NCT02934529 (FIRE-4), NCT04034459 (FIRE-4.5), NCT01249638 (ML22011), NCT00254137 (CIOX), NCT01991873 (PANAMA), NCT01328171 (VOLFI) [clinicaltrials.gov]

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independent of sex. In contrast, patients with RSPT showed both, inferior PFS (HR 2.09; 95 % CI 1.35–3.22; P < 0.001) and OS (HR 1.31; 95 % CI, 0.84–2.05). These effects were observed in male and female patients.
Conclusions: The present analysis of BRAF^{V600E}-mut mCRC suggests a survival benefit from anti-EGFR- or anti-VEGF-directed antibodies in patients with LSPT. This effect was not observed in RSPT, where patients showed a clearly greater benefit from bevacizumab.

1. Introduction

Colorectal cancer (CRC) harboring an activating BRAF^{V600E}-mutation represents a biologically and clinically aggressive subtype [21]. BRAF^{V600E}-mutation is observed in approximately 8–10 % of metastatic CRC (mCRC) patients. It is more often diagnosed in mCRC with right-sided primary tumors and female patients and is generally associated with poor outcome [21,22].

The substitution of valine by glutamic acid at position 600 of the B-Raf protein leads to constitutive activation of the MAP kinase cascade (RAS–RAF–MEK–ERK) [24]. As a result, the signaling pathway becomes uncoupled from upstream input, promoting uncontrolled cellular proliferation, increased invasiveness, and resistance to therapy.

Targeted therapy with anti-EGFR agents has been a topic of controversial discussion in BRAF^{V600E}-mutant mCRC. Meta-analyses indicated either no or detrimental activity of cetuximab or panitumumab compared to standard therapy in this patient cohort [16,18]. Consequently, guidelines recommended not to use anti-EGFR agents in BRAF^{V600E}-mutant mCRC.

Since monotherapy with BRAF-inhibitors induces compensatory EGFR reactivation, combination with anti-EGFR agents is necessary to effectively shut down the EGFR-pathway [17]. In fact, dual blockade of BRAF and EGFR, effectively suppressed MAPK pathway reactivation [9].

PTL remains a relevant biological variable in the first-line treatment of mCRC. Right-sided primary tumors (RSPT) more frequently exhibit BRAF^{V600E}-mutations, microsatellite instability-high (MSI-H), a distinct inflammatory tumor microenvironment, and are more commonly seen in older female patients [10,23]. In contrast, left-sided primary tumors (LSPT) tend to be more often RAS-wild-type and are more responsive to anti-EGFR-based therapy [10,23]. Historically, treatment recommendations were stratified based on tumor sidedness [3,5,8]. However, these recommendations were primarily informed by retrospective subgroup analyses.

Emerging evidence now challenges the binary “left versus right” classification. Instead, recent molecular studies support a biological continuum along the colonic axis, with gradual shifts in mutation frequencies and consensus molecular subtypes (CMS) [7]. Studies have shown a continuous gradient in the hazard ratio for overall survival across tumor subsites, suggesting that primary tumor location holds prognostic and predictive value that extends beyond the traditional dichotomy [11].

The BREAKWATER study included patients with BRAF^{V600E}-mutated, RAS-wild-type mCRC and demonstrated superior efficacy of first-line treatment with encorafenib/cetuximab plus mFOLFOX6 compared to chemotherapy alone. The present evaluation investigates the efficacy of anti-EGFR therapy alone plus chemotherapy and addresses the question of to which extent treatment efficacy is dependent on exact primary tumor location or sex.

2. Methods

We conducted a pooled analysis of individual patient data from seven randomized first-line trials conducted by the German AIO study group: FIRE-3, FIRE-4, FIRE-4.5, CIOX, XELAVIRI, PANAMA, and VOLFI. These trials evaluated either chemotherapy doublet or triplet regimens in combination with targeted therapy, specifically anti-EGFR monoclonal antibodies (cetuximab or panitumumab) or the anti-VEGF antibody bevacizumab. Detailed study designs, inclusion criteria, treatment arms, and follow-up protocols are described in the original publications of the FIRE, CIOX, XELAVIRI, PANAMA and VOLFI trials [8,12,13,15,19,20].

2.1. Molecular status

Patients with an unknown RAS- or BRAF-mutational status, as well as those harboring RAS mutations, BRAF-wild-type tumors or BRAF non-^{V600E}-mutations were excluded. The assessment of RAS- and BRAF-mutational status was performed primarily in a decentralized manner within the individual clinical trials. As part of subsequent translational research projects, mutational status was re-evaluated and, where necessary, corrected. In the case of the FIRE-4.5 trial, BRAF status was assessed retrospectively.

An additional inclusion criterion was the availability of precise information on the primary tumor location. Patients for whom the primary site could not be clearly determined, or for whom the tumor extended across multiple colorectal segments, were excluded. Furthermore, patients who had not received targeted therapy within the respective clinical trial were not included in the present analysis.

Ultimately, 209 patients with confirmed RAS-wild-type and BRAF^{V600E}-mutated tumors met all eligibility criteria and were included in the pooled analysis. A detailed overview of the patient selection process is provided in the consort diagram (Fig. 1).

2.2. Primary tumor location

The anatomical location of the primary tumor was categorized into right-sided and left-sided tumors. RSPT comprised tumors located from the cecum to the transverse colon, while LSPT included those arising from the splenic flexure to the rectum. Classification was based on documented localization as reported in the original trial datasets. Subgroup analyses stratified by sex were conducted. Due to incomplete information on microsatellite status across all included studies, this parameter could not be systematically incorporated into the analysis.

2.3. Statistics

All statistical analyses were performed using SPSS software, version 29 (IBM Corp., Armonk, NY, USA). Survival data were analysed using the Kaplan–Meier method and reported as median values with 95 % confidence intervals (CI). Group comparisons were performed using the log-rank test, and treatment effects were expressed as hazard ratios (HR) with 95 % CI derived from proportional Cox regression models. A two-sided p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 209 patients with metastatic colorectal cancer, RAS-wild-type and BRAF^{V600E}-mutation were included in this exploratory, retrospective analysis. The median age was 63 years (range 31–81), with 53 % (N = 110) being male and 47 % (N = 99) female. A total of 132 patients received anti-EGFR-based therapy (cetuximab or panitumumab), 77 were treated with bevacizumab. The ECOG performance status was 0 in 54.5 % (N = 114) of the patients at baseline. Liver metastases were the most common site of disease (68.4 %), followed by

peritoneal- (28.7 %) and lymph node- (25.8 %) involvement. For more detailed information, please refer to Table 1.

3.2. Efficacy analysis in the total cohort based on tumor sidedness

In the overall cohort, ORR was comparable (52 % vs. 56 %) for patients treated with an anti-EGFR mAb versus those receiving bevacizumab (Table 2). Median PFS was significantly shorter in patients receiving anti-EGFR-based therapy (HR 1.42; 95 % CI 1.05–1.91; P = 0.022), while no major difference was observed with regard to OS (HR 0.96; 95 % CI 0.70–1.32; P = 0.80).

In the subgroup of patients with LSPT, median PFS was comparable between anti-EGFR and bevacizumab treatment groups (HR 0.98; 95 % CI 0.63–1.51). This was, however, accompanied by a numerically longer OS in the anti-EGFR-treated patients (17.8 vs. 11.8 months; HR 0.71; 95 % CI 0.45–1.14). Patients with RSPT receiving anti-EGFR mAbs had a significantly shorter PFS (HR 2.09; 95 % CI 1.35–3.22; P < 0.001) and a numerically shorter OS (HR 1.31; 95 % CI 0.84–2.05) compared to bevacizumab (Table 3).

3.3. Sex-specific subgroup analysis of survival

The sex-specific subgroup analysis of $BRAF^{V600E}$-mutant patients did not reveal major differences related to sidedness (Supplement 1 and 2). In LSPT, both male (HR 0.80; 95 % CI 0.44–1.44) and female patients (HR 0.55; 95 % CI 0.24–1.21) showed a numerical OS benefit when treated with anti-EGFR- versus bevacizumab-based therapy, while median PFS was comparable.

By contrast, female patients with RSPT experienced significantly worse PFS (HR 2.11; 95 % CI 1.24–3.57) and OS (HR 1.85; 95 % CI 1.05–3.25) in the anti-EGFR group. Comparably, a numerical disadvantage for PFS and OS (HR 0.76; 95 % CI 0.35–1.66) was also observed in male patients with RSPT (Table 4).

3.4. Relation between exact primary tumor location and outcome

This analysis was performed to understand the effect of exact primary tumor location on survival observed in the subgroups receiving either anti-EGFR mAbs or bevacizumab.

Median OS did not exceed 13 months in any of the right-sided tumors treated with anti-EGFR mAbs reaching the lowest value in the splenic flexure (4.9 months) and the transverse colon (5.7 months) (Table 5). By contrast, OS times greater than 20 months were mostly achieved in patients with RPTL receiving bevacizumab (apart from ascending colon with an OS of 13.4 months). A wave plot was used to visualize the hazard ratios along the anatomical location of the primary tumor. This analysis revealed the biological continuum of treatment effects (Figures 2 and 3). It indicates that also in $BRAF^{V600E}$-mutant patients sidedness plays a role and that the hazard ratio of 1.0 is reached or crossed in LSPT suggesting a potential benefit of anti-EGFR agents.

4. Discussion

In this pooled analysis of seven randomized first-line trials, we evaluated 209 individual patients with $BRAF^{V600E}$-mutated, RAS-wild-type metastatic colorectal cancer to assess the prognostic and predictive impact of primary tumor location in the context of targeted therapy. The present dataset was derived from a comprehensive integration of the German AIO study group trials FIRE-3, FIRE-4, FIRE-4.5, CIOX, XELAVIRI, PANAMA, and VOLFI [1,13–15,19,20,8]. Across these studies, different chemotherapy backbones were investigated in combination with either anti-EGFR monoclonal antibodies (cetuximab or panitumumab) or the VEGF-inhibitor bevacizumab, thereby allowing a comparative evaluation of targeted treatment strategies in the first-line setting.

The majority of data included in this analysis originated from post-hoc evaluations of the respective trials, in which patients harboring a $BRAF^{V600E}$-mutation represented a small minority. Importantly, however, the FIRE-4.5 study prospectively and exclusively enrolled patients with $BRAF^{V600E}$-mutated mCRC, thereby providing a dedicated dataset with predefined hypotheses regarding optimal targeted therapy [19]. By combining these datasets, the present pooled analysis substantially increased the number of evaluable patients and enabled a more granular investigation of treatment efficacy in relation to primary tumor location.

Our analysis suggests that the efficacy of anti-EGFR antibodies in $BRAF^{V600E}$-mutated mCRC patients is strongly influenced by the anatomical location of the primary tumor. While current clinical guidelines partly rely on a binary classification of colorectal cancer into left- versus right-sided disease, our findings support the concept of a biological continuum along the colonic axis. This notion has been highlighted in molecular studies demonstrating gradual shifts in genomic alterations, consensus molecular subtypes, and transcriptomic signatures from the cecum to the rectum [11,7]. In line with these observations, we observed a progressive change in treatment outcomes,

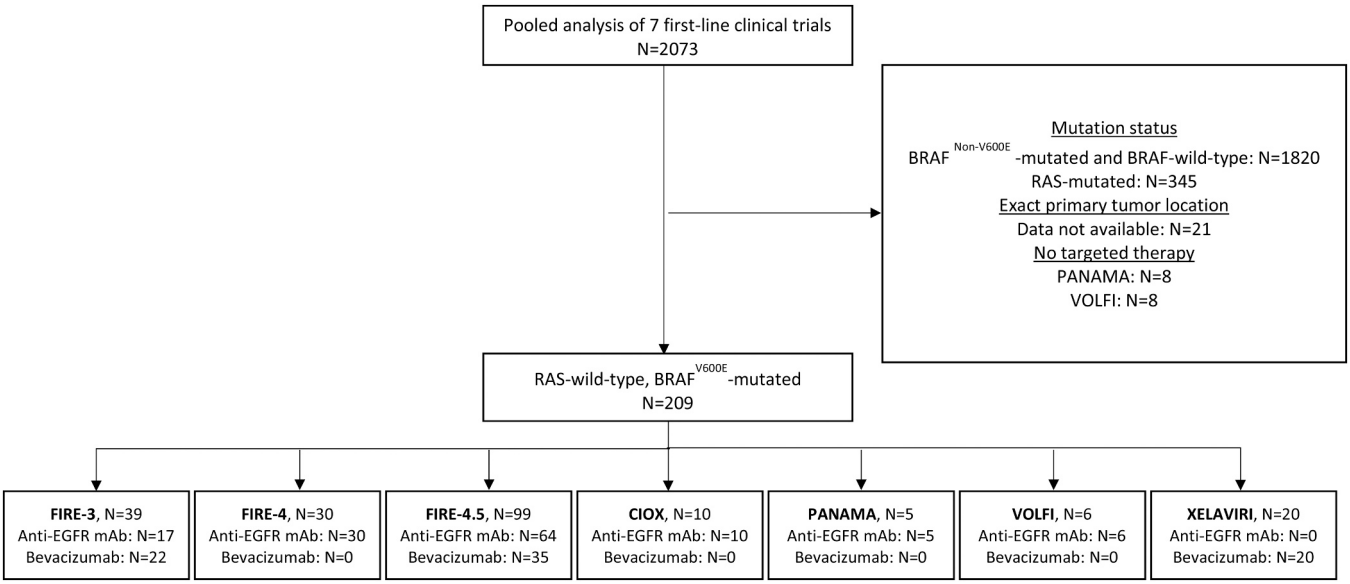

Fig. 1. Consort Diagram of $BRAF^{V600E}$-mut, RAS-wt Patients mAb: monoclonal antibody.

Table 1
Descriptive statistics of the analyzed $BRAF^{V600E}$-mut cohort.

	Total cohort	Coecum	Ascending colon	Hepatic flexure	Transverse colon	Splenic flexure	Descending colon	Sigmoid colon	Rectum
Number of Pat. [%]	209	29 [13.9]	49 [23.4]	17 [8.1]	16 [7.7]	13 [6.2]	11 [5.3]	37 [17.7]	37 [17.7]
Median Age in years, Range	63.0, 31–81	65.0, 33–79	69.0, 31–81	64.0, 46–78	69.0, 48–76	60.0, 31–76	63.0, 42–77	58.0, 38–74	63.0, 47–78
Sex [%]									
-Male-Female	110 [52.6] 99 [47.4]	14 [48.3]15 [51.7]	18 [36.7]31 [63.3]	6 [35.3]11 [64.7]	7 [43.8]9 [56.3]	8 [61.5]5 [38.5]	10 [90.9]1 [9.1]	20 [54.1]17 [45.9]	27 [73.0]10 [27.0]
Treatment [%]									
-Anti-EGFR mAb-Bevacizumab	132 [63.2] 77 [36.8]	23 [79.3]6 [20.7]	26 [53.1]23 [46.9]	10 [58.8]7 [41.2]	10 [62.5]6 [37.5]	10 [76.9]3 [23.1]	7 [63.6]4 [36.4]	24 [64.9]13 [35.1]	22 [59.5]15 [40.5]
ECOG [%]									
−0–1	114 [54.5] 95 [45.5]	17 [58.6]12 [41.4]	26 [53.1]23 [46.9]	11 [64.7]6 [35.3]	8 [50.0]8 [50.0]	5 [38.5]8 [61.5]	5 [45.5]6 [54.5]	24 [64.9]13 [35.1]	18 [48.6]19 [51.4]
Metastatic site [%]									
-Liver-Lung-Lymphnode-Peritoneal-Other	143 [68.4] 54 [25.8]89 [42.6]60 [28.7]33 [15.8]	21 [72.4]8 [27.6]13 [44.8]12 [41.4]6 [20.7]	31 [63.7]7 [14.3]29 [59.2] 13 [26.5]8 [16.3]	10 [58.8]3 [17.6]3 [17.6]6 [35.3]2 [11.8]	13 [81.3]7 [43.8]7 [43.8] 3 [18.8]-	11 [84.6]− 3 [23.1]5 [38.5]2 [15.4]	7 [63.6]3 [27.3] 4 [36.4]5 [45.5] 2 [18.2]	27 [73.0]10 [27.0]15 [40.5]12 [32.4]7 [18.9]	23 [62.2]16 [43.2]15 [40.5]4 [10.8]6 [16.2]

mAb: monoclonal antibody

Table 2
Response analysis in the $BRAF^{V600E}$-mut Cohort LSPT: Left-sided primary tumor; RSPT: Right-sided primary tumor; ORR: Objective Response Rate; OR: Odds Ratio.

	Anti-EGFR mAb ORR (%)	Bevacizumab	OR	P
Total cohort	52 [64/123]	56 [41/73]	0.85 [0.47–1.52]	0.66
LSPT	36 [22/60]	59 [19/32]	0.40 [0.16–0.95]	0.048
RSPT	67 [42/63]	54 [22/41]	1.73 [0.77–3.87]	0.22

with anti-EGFR therapy showing limited activity in right-sided tumors, but a potential benefit in left-sided tumors.

Importantly, the wave-like distribution of hazard ratios across exact tumor locations in our analysis provides clinical evidence for this continuum model. Rather than a sharp boundary at the splenic flexure, treatment efficacy appears to be modified gradually, reflecting underlying biological heterogeneity. These findings reinforce the idea that sidedness should not be considered a strict dichotomous variable but rather a surrogate for complex molecular gradients that modulate prognosis and treatment response.

When interpreting our findings, the role of exact primary tumor location warrants consideration. Although our analysis suggests marked

Table 3
Survival-Analysis of $BRAF^{V600E}$-mut patients receiving first-line therapy. mAb: monoclonar antibody; HR: Hazard ratio; LSPT: Left-sided primary tumor; RSPT: Right-sided primary tumor; PFS: Progression-free Survival; OS: Overall Survival.

		N	Anti-EGFR mAb		Bevacizumab		HR [95 % CI]	P
			N	Median survival, months	N	Median survival, months		
PFS	Total cohort	209	132	6.3	77	8.0	1.42 [1.05–1.91]	**0.022**
	LSPT	98	63	8.3	35	8.1	0.98 [0.63–1.51]	0.92
	RSPT	111	69	5.5	42	7.8	2.09 [1.35–3.22]	**< .001**
OS	Total cohort	209	132	13.7	77	15.4	0.96 [0.70–1.32]	0.80
	LSPT	98	63	17.8	35	11.8	0.71 [0.45–1.14]	0.16
	RSPT	111	69	11.6	42	17.1	1.31 [0.84–2.05]	0.23

Table 4
Survival of $BRAF^{V600E}$-mut patients according to sex. LSPT: Left-sided primary tumor; RSPT: Right-sided primary tumor; HR: Hazard ratio; PFS: Progression-free Survival; OS: Overall Survival.

			N	Anti-EGFR mAb		Bevacizumab		HR [95 % CI]	P
				N	Median survival, months	N	Median survival, months		
PFS	Male	TC	110	77	6.5	33	8.1	1.46 [0.94–2.26]	0.09
		LSPT	65	43	8.6	22	8.1	1.13 [0.66–1.93]	0.67
		RSPT	45	34	5.6	11	7.8	2.22 [0.96–5.16]	0.06
	Female	TC	99	55	5.9	44	7.7	1.41 [0.92–2.15]	0.11
		LSPT	33	20	8.3	13	7.7	0.73 [0.34–1.59]	0.43
		RSPT	66	35	4.2	31	8.0	2.11 [1.24–3.57]	**0.005**
OS	Male	TC	110	77	17.2	33	0.8	1.29 [0.50–1.27]	0.34
		LSPT	65	43	17.8	22	11.8	0.80 [0.44–1.44]	0.45
		RSPT	45	34	12.9	11	20.1	0.76 [0.35–1.66]	0.49
	Female	TC	99	55	11.6	44	14.7	1.16 [0.74–1.83]	0.52
		LSPT	33	20	16.4	13	11.6	0.55 [0.24–1.21]	0.13
		RSPT	66	35	10.2	31	17.1	1.85 [1.05–3.25]	**0.031**

Table 5
Survival of BRAF^{V600E}-mut patients according to exact primary tumor location. HR: Hazard ratio; PFS: Progression-free Survival; OS: Overall Survival.

		N	Anti-EGFR mAb		Bevacizumab		HR [95 % CI]	P
			N	Median Survival, months	N	Median Survival, months		
PFS	Coecum	29	23	4.1	6	8.9	4.25 [1.22–14.80]	**0.015**
	Ascending Colon	49	26	5.7	23	7.4	1.55 [0.85–2.81]	0.15
	Hepatic Flexure	17	10	5.4	7	26.0	3.95 [1.05–14.82]	**0.029**
	Transverse Colon	16	10	2.6	6	3.7	1.66 [0.50–5.44]	0.40
	Splenic Flexure	13	10	2.5	3	12.4	2.42 [0.52–11.19]	0.24
	Descending Colon	11	7	10.2	4	3.1	0.52 [0.11–2.40]	0.40
	Sigmoid Colon	37	24	9.0	13	8.1	0.99 [0.47–2.08]	0.98
	Rectum	37	22	8.2	15	7.7	0.90 [0.44–1.83]	0.77
OS	Coecum	29	23	13.0	6	22.6	1.64 [0.60–4.50]	0.33
	Ascending Colon	49	26	12.6	23	13.4	0.97 [0.50–1–88]	0.92
	Hepatic flexure	17	10	11.6	7	23.0	2.00 [0.51–7.88]	0.31
	Transverse Colon	16	10	5.7	6	4.0	1.38 [0.47–4.10]	0.56
	Splenic Flexure	13	10	4.9	3	15.7	1.27 [0.33–4.86]	0.73
	Descending Colon	11	7	30.7	4	8.2	0.32 [0.06–1.65]	0.15
	Sigmoid Colon	37	24	16.2	13	11.6	0.97 [0.43–2.16]	0.94
	Rectum	37	22	17.8	15	11.8	0.53 [0.25–1.13]	0.09

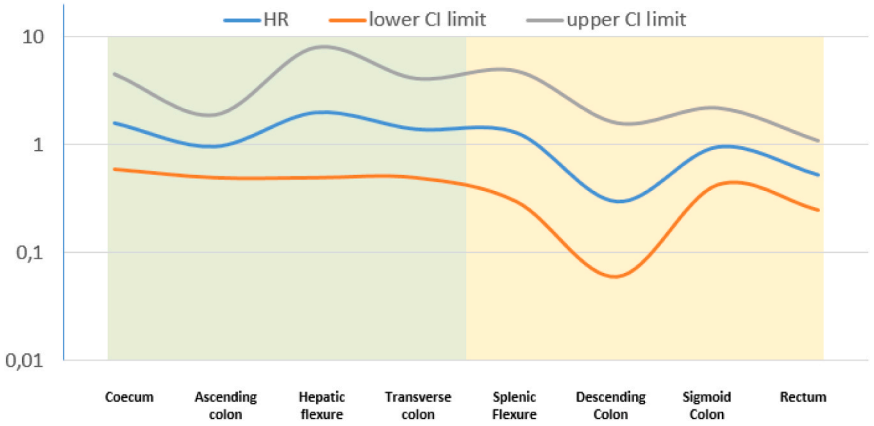

Fig. 2. Wave Diagram of PFS according to the Exact Primary Tumor Location. Blue: Hazard ratio, Orange: Lower limit of the CI, Gray: Upper limit of the CI, Yellow: Left-sided Primary Tumor, Green: Right-sided Primary Tumor. HR: Hazard ratio, CI: Confidence interval.

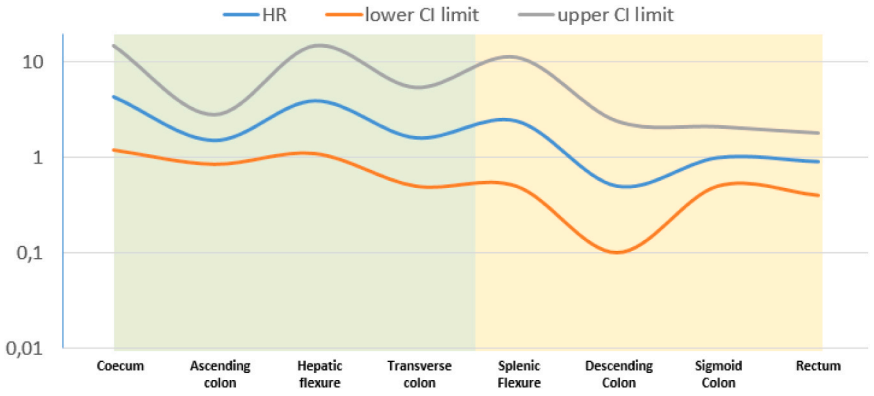

Fig. 3. Wave Diagram of OS according to the Exact Primary Tumor Location. Blue: Hazard ratio, Orange: Lower limit of the CI, Gray: Upper limit of the CI, Yellow: Left-sided Primary Tumor, Green: Right-sided Primary Tumor. HR: Hazard ratio, CI: Confidence interval.

prognostic heterogeneity across individual subsites, especially in tumors of the coecum and transverse colon where outcomes with anti-EGFR therapy were particularly poor, these results must be interpreted with caution. The limited number of patients in each subgroup introduces substantial statistical uncertainty, which prevents firm conclusions regarding treatment recommendations at the subsite level. Nevertheless, the consistently unfavorable prognosis of coecal and transverse colon tumors across treatment groups highlights the need for further dedicated studies to better characterize the biology and therapeutic vulnerabilities of these tumor locations [4].

Patients with BRAF^{V600E}-mutated, left-sided primary tumors derived a numerically longer overall survival from anti-EGFR-based therapy compared to bevacizumab, regardless of sex. This observation contrasts with later treatment guidelines, which generally recommended not to use anti-EGFR-based therapy for BRAF-mutant tumors [2]. Our results suggest that anti-EGFR-directed antibodies do show efficacy in patients

with left-sided $BRAF^{V600E}$-mutated tumors.

The effect of sex on treatment outcomes was also examined in this analysis. Overall, our findings do not suggest a clinically relevant interaction between sex and the efficacy of targeted therapy in $BRAF^{V600E}$-mutated mCRC. The apparent disadvantage observed in female patients with right-sided tumors should be interpreted with caution, as this subgroup was larger than the corresponding male cohort and therefore more likely to reach statistical significance. Importantly, both male and female patients with right-sided primaries experienced inferior outcomes with anti-EGFR therapy compared to bevacizumab, indicating that the observed sex-specific effect is most likely a reflection of sample size rather than a true biological interaction. These results reinforce the notion that sidedness, rather than sex, is the key variable guiding therapeutic decisions in this setting.

The present investigation needs to be discussed in the context of the BREAKWATER study, which recently established the triplet of mFOLFOX6, cetuximab plus encorafenib as the new standard of care in the first-line therapy of $BRAF^{V600E}$-mutated mCRC alone (Elez et al., [6]; [9]). A comparable OS benefit from triplet therapy as compared to chemotherapy alone was shown for left-sided (HR 0.48; 95 % CI: 0.32–0.72) and for right-sided tumors (HR 0.49; 95 % CI: 0.35–0.68) (Elez et al., 2025). Moreover, PFS (12.8 months), OS (30.3 months) and OR (65.7 %) observed with the triplet therapy in the BREAKWATER were clearly superior to the results obtained with the doublet of chemotherapy plus anti-EGFR agent in $BRAF^{V600E}$-mutated mCRC of the present analysis (Elez et al., [6]). As a result, the ESMO Living Guidelines now recommend this triplet regimen regardless of primary tumor location (V1.3 – July 2025) [2]. Since these results are in contrast to the negative effect of chemotherapy plus anti-EGFR mAbs observed in our analysis of patients with RSPT, it may be concluded that triplet therapy including the BRAF-inhibitor encorafenib overcomes resistance to chemotherapy plus anti-EGFR mAb alone in RSPT.

Despite the clinical relevance of our findings, this study has several limitations. The retrospective nature of the analysis, incomplete data regarding microsatellite status and limited sample sizes in certain subgroups reduce the statistical power of detailed stratified analyses. Furthermore, no data on later-line therapies were available, and their potential influence could not be assessed. In addition, no adjustment for potential confounding factors were performed. Nonetheless, to our knowledge, this dataset represents the largest pooled individual patient cohort to date derived from randomized first-line treatment trials, including a total of 209 patients. The use of data from over two decades of prospective, randomized trials lends strong clinical validity to our results.

5. Conclusion

In conclusion, this pooled analysis of individual patient data highlights the prognostic and predictive relevance of primary tumor location in patients with $BRAF^{V600E}$-mutated, RAS-wild-type metastatic colorectal cancer. While left-sided tumors may derive benefit from anti-EGFR–based therapy, right-sided primaries showed consistently inferior outcomes under this approach, irrespective of sex, when compared with bevacizumab. These results underscore sidedness as the clinically most relevant stratification variable, whereas exact tumor subsite and patient sex appear less robust due to limited sample sizes. Importantly, our findings support the concept of a biological continuum along the colonic axis rather than a strict dichotomy of left versus right sidedness.

Ethic

Ethical approval, Informed Consent and Consent to participate / to publish

Written informed consent for all the trial participation and for publication of trial results was obtained from each patient before any study specific procedure.

Informed consent was obtained from all subjects prior to participating in the study. The study was conducted in accordance with the Declaration of Helsinki.

Declarations

The author Volker Heinemann is an Editor of the EJC and was not involved in the editorial review or the decision to publish this article.

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Data/Supplement

Attached

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The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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(*continued on next page*)

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All remaining authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2025.116105.

Data availability

Available from the corresponding author upon reasonable request.

References

[1] Alig AHS, Modest DP, Stintzing S, Heinrich K, Geissler M, Fischer von Weikersthal L, et al. Impact of primary tumor sidedness and sex on prognosis and anti-epidermal growth factor receptor antibody efficacy in BRAF-mutant metastatic colorectal cancer: a pooled analysis of AIO studies FIRE-1, CIOX, FIRE-3, XELAVIRI, and VOLFI. ESMO Open 2024;9(9):103677. https://doi.org/10.1016/j.esmoop.2024.103677.
[2] Cervantes A, Martinelli E. Updated treatment recommendation for third-line treatment in advanced colorectal cancer from the ESMO metastatic colorectal cancer living guideline. Ann Oncol 2024;35(2):241–3. https://doi.org/10.1016/j.annonc.2023.10.129.
[3] Cremolini C, Antoniotti C, Rossini D, Lonardi S, Loupakis F, Pietrantonio F, et al. Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, randomised, controlled trial. Lancet Oncol 2020;21(4):497–507. https://doi.org/10.1016/S1470-2045(19)30862-9.
[4] Cremolini C, Benelli M, Fontana E, Pagani F, Rossini D, Fucà G, et al. Benefit from anti-EGFRs in RAS and BRAF wild-type metastatic transverse colon cancer: a clinical and molecular proof of concept study. ESMO Open 2019;4(2):e000489. https://doi.org/10.1136/esmoopen-2019-000489.
[5] Cremolini C, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol 2015;16(13):1306–15. https://doi.org/10.1016/s1470-2045(15)00122-9.

[6] Elez E, Yoshino T, Shen L, Lonardi S, Van Cutsem E, Eng C, et al. Encorafenib, cetuximab, and mFOLFOX6 in BRAF-mutated colorectal cancer. N Engl J Med 2025;392(24):2425–37. https://doi.org/10.1056/NEJMoa2501912.
[7] Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21(11):1350–6. https://doi.org/10.1038/nm.3967.
[8] Heinemann V, von Weikersthal LF, Decker T, Kiani A, Kaiser F, Al-Batran SE, et al. FOLFIRI plus cetuximab or bevacizumab for advanced colorectal cancer: final survival and per-protocol analysis of FIRE-3, a randomised clinical trial. Br J Cancer 2021;124(3):587–94. https://doi.org/10.1038/s41416-020-01140-9.
[9] Kopetz S, Yoshino T, Van Cutsem E, Eng C, Kim TW, Wasan HS, et al. Encorafenib, cetuximab and chemotherapy in BRAF-mutant colorectal cancer: a randomized phase 3 trial. Nat Med 2025;31(3):901–8. https://doi.org/10.1038/s41591-024-03443-3.
[10] Lee GH, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer? - a systematic review. Eur J Surg Oncol 2015;41(3):300–8. https://doi.org/10.1016/j.ejso.2014.11.001.
[11] Loree JM, Pereira AAL, Lam M, Willauer AN, Raghav K, Dasari A, et al. Classifying colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation profiles and consensus molecular subtypes. Clin Cancer Res 2018;24(5):1062–72. https://doi.org/10.1158/1078-0432.Ccr-17-2484.
[12] Modest DP, Fischer von Weikersthal L, Decker T, Vehling-Kaiser U, Uhlig J, Schenk M, et al. Sequential versus combination therapy of metastatic colorectal cancer using fluoropyrimidines, irinotecan, and bevacizumab: a randomized, controlled study-XELAVIRI (AIO KRK0110). PMID: 30388045 J Clin Oncol 2019;37(1):22–32. https://doi.org/10.1200/jco.18.00052.
[13] Modest DP, Karthaus M, Fruehauf S, Graeven U, Müller L, König AO, et al. Panitumumab plus fluorouracil and folinic acid versus fluorouracil and folinic acid alone as maintenance therapy in RAS wild-type metastatic colorectal cancer: the randomized PANAMA Trial (AIO KRK 0212). J Clin Oncol 2022;40(1):72–82. https://doi.org/10.1200/jco.21.01332.
[14] Modest DP, Martens UM, Riera-Knorrenschild J, Greeve J, Florschütz A, Wessendorf S, et al. FOLFOXIRI plus panitumumab as first-line treatment of RAS wild-type metastatic colorectal cancer: the randomized, open-label, phase II VOLFI study (AIO KRK0109). J Clin Oncol 2019;37(35):3401–11. https://doi.org/10.1200/jco.19.01340.
[15] Moosmann N, von Weikersthal LF, Vehling-Kaiser U, Stauch M, Hass HG, Dietzfelbinger H, et al. Cetuximab plus capecitabine and irinotecan compared with cetuximab plus capecitabine and oxaliplatin as first-line treatment for patients with metastatic colorectal cancer: AIO KRK-0104—a randomized trial of the German AIO CRC study group. J Clin Oncol 2011;29(8):1050–8. https://doi.org/10.1200/JCO.2010.31.1936.
[16] Pietrantonio F, Petrelli F, Coinu A, Di Bartolomeo M, Borgonovo K, Maggi C, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer 2015;51(5):587–94. https://doi.org/10.1016/j.ejca.2015.01.054.
[17] Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483(7387):100–3. https://doi.org/10.1038/nature10868.
[18] Rowland A, Dias MM, Wiese MD, Kichenadasse G, McKinnon RA, Karapetis CS, et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. Br J Cancer 2015;112(12):1888–94. https://doi.org/10.1038/bjc.2015.173.
[19] Stintzing S, Heinrich K, Tougeron D, Modest DP, Schwaner I, Eucker J, et al. FOLFOXIRI plus cetuximab or bevacizumab as first-line treatment of BRAF (V600E)-mutant metastatic colorectal cancer: the randomized phase II FIRE-4.5 (AIO KRK0116) study. J Clin Oncol 2023;41(25):4143–53. https://doi.org/10.1200/jco.22.01420.
[20] Stintzing S, Klein-Scory S, Fischer von Weikersthal L, Fuchs M, Kaiser F, Heinrich K, et al. Baseline liquid biopsy in relation to tissue-based parameters in metastatic colorectal cancer: results from the randomized FIRE-4 (AIO-KRK-0114) study. J Clin Oncol 2025;43(12):1463–73. https://doi.org/10.1200/jco.24.01174.
[21] Tabernero J, Ros J, Élez E. The evolving treatment landscape in BRAF-V600E-mutated metastatic colorectal cancer. Am Soc Clin Oncol Educ Book 2022;(42):1–10. https://doi.org/10.1200/edbk_349561.
[22] Tran B, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 2011;117(20):4623–32. https://doi.org/10.1002/cncr.26086.
[23] Waldstein S, Spengler M, Pinchuk IV, Yee NS. Impact of colorectal cancer sidedness and location on therapy and clinical outcomes: role of blood-based biopsy for personalized treatment. J Pers Med 2023;13(7).
[24] Weiss L, Zhang D, Kunz WG, Boeck S, Curigliano G, Subbiah V, et al. Exploring the pathway: clinical utility and open challenges of targeting BRAF alterations in biliary tract cancers and gastrointestinal malignancies. ESMO Gastrointest Oncol 2025;7. https://doi.org/10.1016/j.esmogo.2024.100129.