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Retrospective Analysis of Treatment Pathways in Patients With BRAF^{V600E}-mutant Metastatic Colorectal Carcinoma – MORSE^{CRC}

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Abstract. *Background/Aim: Metastatic colorectal cancer (mCRC) is a heterogeneous disease with distinct molecular subtypes. The BRAF^{V600E}-mutation found in approximately 8-12% of mCRC patients is associated with poor prognosis. Guideline recommendations for this population are mostly based on small cohorts due to lack of clinical data. This retrospective analysis was designed to evaluate (approved) therapeutic approaches and algorithms in BRAF^{V600E}-mutant mCRC prior to approval of the targeted combination encorafenib plus cetuximab in Germany, Austria, and Switzerland. Patients and Methods: Anonymized data from BRAF^{V600E}-mutant mCRC patients were analyzed retrospectively regarding 1st-, 2nd- and 3rd-line treatment using descriptive statistics. Results: Forty-two patients were eligible for analysis (mean age 62.1 years, 47.6% female). At initial diagnosis, 20 patients (47.6%) were documented with right-sided tumors. Most patients (81.0%) were tested for BRAF before 1st-line. Four patients (9.5%) showed high microsatellite instability (MSI-H). Based on 94 treatment lines, chemotherapy combined with targeted therapy (TT) was used mostly (61.7%),*

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followed by chemotherapy alone (19.1%). Backbone therapies were most frequently FOLFOXIRI (27.7%), FOLFOX/CAPOX (22.3%), or FOLFIRI (20.2%). Anti-VEGF/VEGFR and anti-EGFR-treatments were used in 45.7% and 23.4% of patients, respectively. Across all treatment lines and types, the predominantly documented reason for discontinuation was lack of efficacy. Conclusion: Combined chemotherapy+TT (anti-VEGF/VEGFR and anti-EGFR) played a predominant role in BRAF^{V600E}-mutated mCRC treatment prior to approval of the targeted combination encorafenib plus cetuximab. Since lack of efficacy was the major reason for treatment discontinuation, newly approved therapies including encorafenib plus cetuximab and – for MSI-H tumors – pembrolizumab represent urgently needed options for future mCRC patients.

At an incidence of approximately 140,000 new cases for both men and women in 2020, colorectal cancer (CRC) is among the three leading cancer entities in Western Europe (1) and represents a leading cause of cancer death in Germany (ca. 24,800 in 2016) (2). Austria (ca. 2,100 in 2018) (3), and Switzerland (ca. 1,700 in 2013-2017) (4). Across all stages, the five-year survival rate is approximately 60% (5). However, at diagnosis approximately 20% of the patients present with distant metastases (6, 7) and almost 50% develop metastatic disease (mCRC) during disease course (8). Within this population, the median overall survival (mOS) is around 30 months (9) with a five-year survival rate below 10% (6). In the current European Society for Medical Oncology (ESMO) guidelines, combination regimens with cytotoxic and targeted therapy are recommended for the treatment of patients with unresectable mCRC. These include folinic acid plus 5-fluoruracil (FOLF), irinotecan (IRI), oxaliplatin (OX), anti-*VEGF/VEGFR* agents (bevacizumab, aflibercept, ramucirumab) and anti-*EGFR* antibodies (cetuximab and panitumumab) (9).

The *BRAF*^{V600E}-mutation, which leads to constitutive activation of the *BRAF*-kinase and sustained *RAS/RAF/MEK/ERK* pathway signaling, resulting in increased cell proliferation, is described in approximately 8-12% of mCRC-cases (10-12). The presence of a *BRAF*^{V600E} mutation as the predominant *BRAF* alteration is considered a marker of poor prognosis in patients with mCRC and is associated (in the 1st-line setting of metastatic disease) with a mOS of approximately 12 months (13, 14). In addition, in 2nd- and later lines, treatment outcomes with current therapy options are poor in patients with *BRAF*-mutated mCRC, with overall response rates (ORR) ≤11%, a median progression-free survival (mPFS) between 1.8 and 2.8 months and a mOS between 4.1 and 6.2 months (15-23).

Factors that are associated with an increased probability of mutated *BRAF* include female sex, age above 60 years, right-sided tumor localization, higher-grade anaplasia, metastases, and microsatellite instability (9, 24-27). The ESMO guidelines recommend to molecularly assess the *BRAF* mutation status in all patients at the time of mCRC diagnosis (9).

For the treatment of *BRAF*-mutant mCRC patients, the ESMO consensus guidelines recommend a poly-chemotherapy based regimen of FOLF, OX and IRI (FOLFOXIRI) with or without bevacizumab as preferred choice in the 1st-line setting (9). Recently, however, a meta-analysis of five randomized trials (n=1.697) concluded that in the *BRAF*-mutated subgroup (6.8%) there is rather no increased benefit from intensified 1st-line combination therapy (28). Second and third choices in the 1st-line setting consist of a cytotoxic doublet together with bevacizumab or FOLFOXIRI alone, respectively. In the 2nd-line, the use of a cytotoxic doublet with bevacizumab is recommended as first choice, followed by FOLF plus IRI (FOLFIRI) together with aflibercept or ramucirumab as second choice. In the 3rd-line, regorafenib or trifluridine/tipiracil are recommended.

Of note, if patients are "unfit" and, thus, not eligible for these treatment regimens, no alternative recommendations are provided. In general, the ESMO guidelines state that mCRC patients should receive all three available cytotoxic agents (fluoropyrimidine, OX and IRI) and all targeted treatments [anti-vascular endothelial growth factor (Receptor) (*VEGF(R)*) and, if *RAS* wild-type, anti-epidermal growth factor receptor (*EGFR*)] treatment while concluding that the optimal therapy sequence remains elusive (9).

BRAF and *RAS* mutations are reportedly almost mutually exclusive in mCRC (9). Against this background, the effectiveness of anti-*EGFR*-based therapy in *BRAF*-mutant mCRC is currently under debate. Two meta-analyses from 2015 are drawing different conclusions. Pietrantonio *et al.* stated that addition of cetuximab or panitumumab in the *BRAF*-mutant subgroup did not significantly improve progression-free survival (PFS) (HR=0.88, p=0.33) or OS (HR=0.91, p=0.63) (29). However, Rowland *et al.* concluded that there is insufficient evidence to definitively state that *BRAF*-mutant mCRC patients attain a different treatment benefit from anti-*EGFR*-based therapy than the *BRAF* wild-type population (30). More recently, a meta-analysis with pooled data from two studies directly comparing chemotherapy plus anti-*EGFR* against chemotherapy plus anti-*VEGF* in 1st-line mCRC showed that regarding the *BRAF*-mutant subgroup there is no relevant difference in overall survival between bevacizumab- and cetuximab-based treatment (n=138; HR=1.01, 95%CI=0.69-1.48) (31).

Objective. This retrospective data analysis was designed to evaluate the approved therapeutic approaches and treatment pathways in *BRAF*^{V600E}-mutant mCRC at specialized colon-cancer centers before approved availability of the targeted combination of encorafenib plus cetuximab in Germany, Austria, and Switzerland.

Further objectives were: i) Descriptive rationale for treatment decision making in the 1st, 2nd- and 3rd-line setting; ii) Description of treatment algorithms.

Patients and Methods

In this retrospective, cross-sectional documentation based on anonymized data from fourteen specialized colon-cancer centers in Germany, Austria, and Switzerland regarding male or female patients, at least 18 years of age, with metastatic *BRAF^{V600E}*-mutated colorectal cancer according to the 8th American joint Commission on Cancer (AJCC) classification and confirmed by valid test method treated between January 2016 and May 2020 in the 1st-, 2nd- and 3rd-line setting with substances registered in the respective country at the time of treatment was collected retrospectively and analyzed using descriptive statistics. To be eligible for data documentation, at least the 2nd-line systemic treatment as per local standard had to be initiated.

The exclusion criteria were: i) *BRAF* test performed after initiation of 2nd-line treatment, ii) other secondary stage III-IV tumor diseases or concomitant systemic treatment of any secondary tumor disease, iii) pregnant or lactating patient during the documented treatment lines, and iv) participation in a clinical trial during the documented treatment lines.

The analysis was carried out using epidemiological statistical methods. All data were summarized by frequency tables and sample statistics and interpreted descriptively only. All analyses were performed with the Analysis Set, which contained all documented patients who meet all inclusion criteria and none of the exclusion criteria and for whom at least the 1st- and 2nd-treatment line were documented. The datasets included demographic and baseline characteristics, effectiveness and safety observations.

Most analyses were performed overall, by treatment line, by treatment type (all treatment lines and separately for each treatment line), by treatment type of targeted therapy (all treatment lines and separately for each treatment line), and by treatment regimen (all treatment lines and separately for each treatment line).

Before the start of the study, the project received a positive ethics committee (EC) vote in Germany (reference number: EA4/044/20) and Austria (reference number: 32-233 ex 19/20) and was confirmed to be no subject for EC evaluation in Switzerland (reference number: 2020-00142). An informed consent process was not required in this anonymized, retrospective data analysis. Moreover, before project start, the study was registered in the publicly accessible German Clinical Trials Register (number DRKS00020982). The clinical investigations detailed in the manuscript submitted were conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Results

Study sites. Half of the patients (21, 50.0%) were documented in Germany, 9 patients (21.4%) in Austria and 12 patients (28.6%) in Switzerland. All patients in Germany were documented in private practices; all patients in Austria and Switzerland were documented in hospitals.

Patient population. Treatment sequences of 49 patients were documented. Forty-two patients met all inclusion and none of the exclusion criteria and had started at least two lines of therapy with five 2nd-line treatments not being considered in the analysis as off-label treatments. Fifteen patients were eligible for analysis in the 3rd-line setting, accounting for a total of 94 treatment lines. At initiation of the 1st-line treatment, 22 patients (52.4%) were male and 20 were female (47.6%). The mean age for both sexes was 62.1 years at the time when the 1st-line treatment was initiated. At the time of the initial diagnosis, the primary tumor was located in the colon/cecum in 37 patients (88.1% of total with most diagnoses in colon ascendens: 16 patients, 38.1%) and in 5 patients (11.9%) rectal carcinoma was diagnosed. In terms of tumor sidedness, 20 patients (47.6%) presented with right sided colorectal cancer and 8 patients (19.0%) with left sided colorectal cancer. Three or more organs were involved in 4 patients (9.8%), *i.e.*, in the majority of patients (37 patients, 90.2%; one patient with missing data) a maximum of two organs were affected by the tumor disease. At this point, 17 patients (40.5%) presented with stage IV-M1c, *i.e.*, with peritoneal metastasis. Liver metastases were present in 27 patients (64.3%). As required by the project inclusion criteria, all patients eligible for data documentation were tested positive for *BRAF^{V600E}* mutation. Most of the patients [34 (81.0%)] were tested prior to the start of 1st-line treatment. At the time of metastatic stage diagnosis, 31 and 29 patients (73.8% *vs*. 69.0%) underwent molecular testing for *KRAS* and/or *NRAS* mutations, respectively. Most of these tests were performed simultaneously to *BRAF* testing (87.1% for *KRAS*, 93.1% for *NRAS*). One out of all patients tested (3.2%) presented with additionally mutated *KRAS*. The test on microsatellite instability (MSI) status was performed in 24 patients (57.1%) at time of diagnosis of the metastatic stage and in 2 patients (5.4%) at start of 2nd-line treatment. Four patients (9.5% based on all 42 patients) displayed high microsatellite instability (MSI-H). Key patient characteristics at the time of initiation of each treatment line are presented in Table I.

Treatments administered. Among all 94 treatment lines, chemotherapy in combination with TT was the treatment type chosen in most cases (58 treatment lines) representing 61.7% of the regimens administered followed by chemotherapy alone (18 lines; 19.1%) and other treatment types (13 lines, 13.8%) (Figure 1). (OF NOTE: Treatments that were summarized as "other treatment type" (2nd-line: 8 patients, 21.6%; 3rd-line: 5 patients, 33.3%) included in the 2nd-line "regorafenib alone" in 1 patient, "unknown treatment" in 6 patients and "unknown targeted therapy" in 1 patient and in the 3rd-line "other treatment type" comprised "unknown treatment" in 4 patients and "unknown targeted therapy" in 1 patient).

Overall, chemotherapy backbones were mostly FOLFOXIRI (26 treatments, 27.7%) or doublet (overall: 40 treatments, 42.6%; FOLFOX/CAPOX: 21 treatments, 22.3%; FOLFIRI: 19 treatments, 20.2%). The frequency of chemotherapy alone decreased with later treatment lines (Figure 1).

Table I. *Key patient characteristics at the time of initiation of 1st-line treatment (if not stated otherwise).*

	Total (N=42)	Patients with 2nd-line started (N=37)	Patients with 3rd-line started (N=15)
Sex, n (%)			
Male	22 (52.4)	18 (48.6)	5 (33.3)
Female	20 (47.6)	19 (51.4)	10 (66.7)
Age, years			
Mean (range)	62.1 (29.0-82.0)	62.3 (29.0-82.0)	58.3 (29.0-78.0)
Median	64.5	64.0	61.0
ECOG, n (%)			
0	23 (57.5)	20 (57.1)	9 (60.0)
1	15 (37.5)	13 (37.1)	5 (33.3)
2	2 (5.0)	2 (5.7)	1 (6.7)
Missing	2	2	0
Primary localization[1], n (%)			
Coecum	7 (16.7)	7 (18.9)	2 (13.3)
Colon ascendens	16 (38.1)	15 (40.5)	6 (40.0)
Colon transversum	4 (9.5)	3 (8.1)	1 (6.7)
Colon descendens	4 (9.5)	3 (8.1)	1 (6.7)
Colon sigmoideum	6 (14.3)	5 (13.5)	4 (26.7)
Rectum	5 (11.9)	4 (10.8)	1 (6.7)
Tumor sidedness[1,2], n (%)			
Right	20 (47.6)	19 (51.4)	8 (53.3)
Left	8 (19.0)	5 (13.5)	1 (6.7)
Both	2 (4.8)	1 (2.7)	1 (6.7)
Unknown	12 (28.6)	12 (32.4)	5 (33.3)
Microsatellite instability (MSI) status at the time of metastatic stage[3], n (%)			
MSI-H	4 (16.7)	4 (20.0)	2 (20.0)
MSS	20 (83.3)	16 (80.0)	8 (80.0)
Not tested	18	17	5
Involvement of ≥3 organs at the time of metastatic stage, n (%)			
Yes	4 (9.8)	4 (11.1)	1 (6.7)
No	37 (90.2)	32 (88.9)	14 (93.3)
Missing	1	1	0
Liver metastases at the time of metastatic stage, n (%)			
Yes	27 (64.3)	24 (64.9)	11 (73.3)
No	15 (35.7)	13 (35.1)	4 (26.7)
M-category of stage IV at the time of metastatic stage[4], n (%)			
Stage IV-M1a	16 (38.1)	15 (40.5)	9 (60.0)
Stage IV-M1b	8 (19.0)	7 (18.9)	1 (6.7)
Stage IV-M1c	17 (40.5)	14 (37.8)	4 (26.7)
Unknown	1 (2.4)	1 (2.7)	1 (6.7)
Time of testing for $BRAF^{V600E}$, n (%)			
Prior to 1st-line treatment	34 (81.0)		
During 1st-line treatment	2 (4.8)		
After 1st-line treatment	6 (14.3)		
Type of tissue sent to the laboratory[5], n (%)			
Archived tissue from primary tumor	28 (68.3)		
Tissue from local recurrence	1 (2.4)		
Tissue from metastases	12 (29.3)		
Missing values	1		
$BRAF^{V600E}$ test method[5], n (%)			
Immune-histochemistry	5 (16.1)		
Single gene sequencing	11 (35.5)		
Next generation sequencing (NGS)	15 (48.4)		
Missing values	11		

Table I. *Continued*

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	Total (N=42)	Patients with 2nd-line started (N=37)	Patients with 3rd-line started (N=15)
Turnaround time for BRAF^{V600E} testing[5] [days]			
Median	6.5		
Range	1.0-16.0		
KRAS mutation at the time of metastatic stage, n (%)[6]			
Test performed	31 (73.8)		
Test performed simultaneously to BRAF^{V600E} testing	27 (87.1)		
Result positive	1 (3.2)		
NRAS mutation at the time of metastatic stage, n (%)[6]			
Test performed	29 (69.0)		
Test performed simultaneously to BRAF^{V600E} testing	27 (93.1)		
Result positive	0 (0.0)		

ECOG: Eastern Cooperative Oncology Group. [1]At the time when the initial diagnosis was established. [2]As per local assessment. [3]MSI-H: high microsatellite instability; MSS: MS-stable. [4]AJCC version 8. [5]At the time of BRAF^{V600E} testing. [6]Percentages for 'Test performed' are calculated in relation to all patients in the analysis set. Percentages for the other results are calculated in relation to the number of tests performed N and n refer to the number of patients.

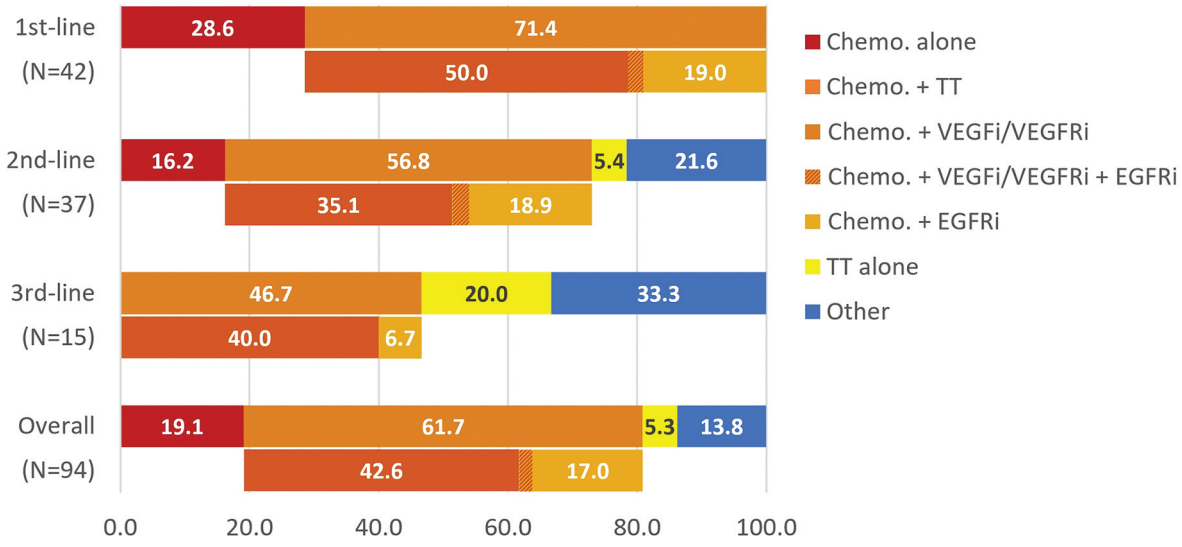

Figure 1. *Treatment types administered. The percentage of treatment types are shown by treatment line and overall. The bars underneath the chemo/TT combinations break down to the two combination partners, VEGF(R)i and EGFRi, respectively. In this group, one patient in the 1st-line setting (2.4%) and one patient in the 2nd-line (2.7%) were treated with chemo. +VEGF(R)i+EGFRi (triple combination) depicted by the hatched area (overall: 2.1%). Chemo.: Chemotherapy; TT: targeted therapy; VEGF(R)i: vascular endothelial growth factor (Receptor) inhibitor; EGFRi: epidermal growth factor receptor inhibitor.*

Targeted agents –either in combination with chemotherapy or in monotherapy – were used in approximately two thirds of the treatment lines administered [63 treatment lines (67.0%)] Among these patients, the majority received a *VEGF(R)*i treatment (43 treatment lines, 45.7%). An *EGFR* inhibitor (*EGFR*i) was chosen in 22 treatment lines (23.4%). Among these, one patient in both, the 1st and the 2nd treatment line (2.4% and 2.7%, respectively), was treated with *VEGF(R)*i plus *EGFR*i plus chemotherapy. In monotherapy, *EGFR*i was used in four patients (4.3%) and *VEGF(R)*i in one patient (1.1% - bevacizumab). Accordingly, when administered in combination, *VEGF(R)*i therapy was chosen more frequently than *EGFR*i (Figure 1) and *EGFR*i was the preferred choice when TT was administered in monotherapy [*EGFR*i: 4 patients (4.3%); *VEGF(R)*i: 1 patient (1.1%)]. Bevacizumab was the preferred *VEGF(R)* targeted therapy when combined with chemotherapy (over all treatment lines: 40.4%) and the only compound chosen

Table II. *Treatment types, regimen, and active combinations/substances, overall and by treatment line.*

Overall Active combinations/substances	1st-line (N=42) n (%)	2nd-line (N=37) n (%)	3rd-line (N=15) n (%)	Overall (N=94) n (%)
Chemotherapy				
FOLFOX	12 (28.6)	4 (10.8)	2 (13.3)	18 (19.1)
FOLFIRI	7 (16.7)	10 (27.0)	2 (13.3)	19 (20.2)
FOLFOXIRI	19 (45.2)	5 (13.5)	2 (13.3)	26 (27.7)
CAPOX	2 (4.8)	1 (2.7)	0 (0.0)	3 (3.2)
Capecitabine alone	2 (4.8)	0 (0.0)	1 (6.7)	3 (3.2)
5-Fluorouracil/folinic acid alone	2 (4.8)	2 (5.4)	0 (0.0)	4 (4.3)
Irinotecan alone	0 (0.0)	2 (5.4)	0 (0.0)	2 (2.1)
Targeted therapy				
Combination				
VEGF(R)i				
Aflibercept (comb.)	0 (0.0)	2 (5.4)	1 (6.7)	3 (3.2)
Bevacizumab (comb.)	22 (52.4)	12 (32.4)	4 (26.7)	38 (40.4)
Ramucirumab (comb.)	0 (0.0)	0 (0.0)	1 (6.7)	1 (1.1)
EGFRi				
Cetuximab (comb.)	2 (4.8)	3 (8.1)	0 (0.0)	5 (5.3)
Panitumumab (comb.)	7 (16.7)	5 (13.5)	1 (6.7)	13 (13.8)
Monotherapy				
VEGF(R)i				
Bevacizumab (mono)	0 (0.0)	0 (0.0)	1 (6.7)	1 (1.1)
EGFRi				
Cetuximab (mono)	0 (0.0)	1 (2.7)	0 (0.0)	1 (1.1)
Panitumumab (mono)	0 (0.0)	1 (2.7)	2 (13.3)	3 (3.2)
Kinase inhibitor				
Regorafenib (mono)	0 (0.0)	1 (2.7)	0 (0.0)	1 (1.1)

VEGF(R)i: Vascular endothelial growth factor (Receptor) inhibitor; EGFRi: epidermal growth factor receptor inhibitor. One patient in the 1st-line setting (2.4%) and one patient in 2nd-line (2.7%) was treated with chemotherapy+VEGF(R)i+EGFRi (triple combination) (overall: 2.1%). Due to the nature of the combination treatment regimens, multiple answers were possible. Accordingly, percentages and totals do not always result in 100%.

when *VEGF(R)*i was administered in monotherapy. Regarding 1st-line treatment, 22 patients (52.4%) received the *VEGF*i bevacizumab (all of them in combination with chemotherapy), while 7 patients (16.7%) and 2 patients (4.8%) were treated with the anti-*EGFR* antibodies panitumumab and cetuximab, respectively (all of them in combination with chemotherapy). Table II provides details on the treatment types, regimen, and active combinations/substances, overall and by treatment line.

Most of the patients were treated consecutively with a combination regimen chemotherapy plus TT in both, the 1st- and the 2nd-line setting, changing one or more of the combination compounds within the types administered (n=16, 53.3% of the 30 patients having been treated with chemotherapy+TT in 1st-line). In the rest of the cases, the treatment types were usually switched to the remaining treatment types. Out of the twelve patients that started treatment with chemotherapy alone, three patients (25%) continued to be treated with chemotherapy alone changing one of the combination partners; the remaining of the patients switched to other treatment types. The treatment sequences (from the 1st-line to 2nd-line to 3rd-line treatment) are shown in Figure 2.

Main reasons for treatment choices. To enquire about the main reasons for the treatment choice, a list of six specific options to choose from was provided to the centers (Table III). The most frequently indicated reason to choose any treatment type was – over all treatment lines – remission pressure, *i.e.*, after rapid tumor progress or based on the patients' tumor load [36 treatment lines (38.3%) followed by *BRAF* mutation (25 treatment lines, 26.6%] and the physician's preferences (18 treatment lines, 19.1%). Remission pressure was more prominent in later treatment lines amounting from 28.6% (12 patients) in the 1st-line setting and 45.9% (17 patients) in 2nd-line up to 46.7% (7 patients) in the 3rd treatment line compared to *BRAF* mutation that played a more dominant role for the treatment choice in the 1st-line setting (35.7%, 15 patients) *versus* 18.9% (7 patients, 2nd-line) *versus* 20% (3 patients, 3rd-line)). The decision to choose a specific treatment was motivated by the physician's preference in around 20% of the patients in each of the treatment lines (1st-line: 8 patients, 19.0%; 2nd-line: 7 patients, 18.9%; 3rd-line: 3

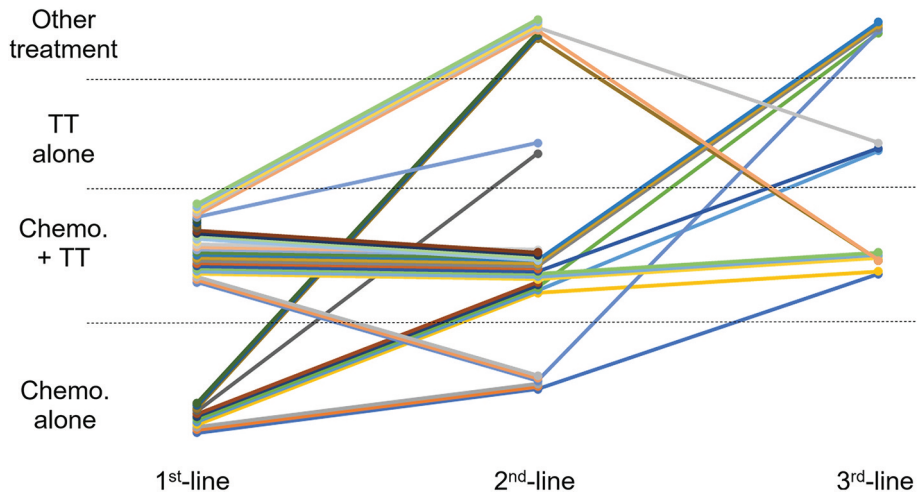

Figure 2. *Treatment sequences. The treatment sequences (1st-line to 2nd-line to 3rd-line treatment) for all patients are depicted. Each patient is represented by a single, colored line or dot in case only the 1st-line treatment was considered. Therefore, the therapy sequence of each patient can be followed from left to right with the width of the bar (consisting of several colored lines) indicating the proportion of patients receiving a specific therapy sequence. Chemo.: Chemotherapy; TT: targeted therapy.*

Table III. *Main reasons for choosing specific treatment type.*

	Chemo. alone (N=18) n (%)	Chemo.+TT (N=58) n (%)	Chemo.+ VEGF(R)i (N=42) n (%)	Chemo.+ EGFRi (N=18) n (%)	TT alone (N=5) n (%)	Other (N=13) n (%)	Overall (n=94)
Remission pressure (rapid PD, tumor load)	6 (33.3)	22 (37.9)	13 (31.0)	9 (50.0)	4 (80.0)	4 (30.8)	36 (38.3)
Toxicity profile	2 (11.1)	4 (6.9)	2 (4.8)	2 (11.1)	1 (20.0)	1 (7.7)	8 (8.5)
Patient's preference	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)
Physician's preference	6 (33.3)	11 (19.0)	10 (23.8)	2 (11.1)	0 (0.0)	1 (7.7)	18 (19.1)
Comorbidities	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
BRAF mutation	3 (16.7)	16 (27.6)	14 (33.3)	3 (16.7)	0 (0.0)	6 (46.2)	25 (26.6)
Other (overall)	0 (0.0)	5 (8.6)	3 (16.7)	2 (11.1)	0 (0.0)	1 (7.7)	6 (6.4)
Other: allergic reaction to eloxatine	0 (0.0)	1 (1.7)	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)
Other: right-sided colon carcinoma	0 (0.0)	1 (1.7)	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)
Other: unknown	0 (0.0)	3 (5.2)	1 (2.4)	2 (11.1)	0 (0.0)	0 (0.0)	3 (3.2)
Other: MSI-H	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	1 (1.1)

Chemo.: Chemotherapy; TT: targeted therapy; VEGF(R)i: vascular endothelial growth factor/receptor inhibitor; EGFRi: epidermal growth factor receptor inhibitor; MSI-H: high microsatellite instability. The column "Chemo.+TT" comprise of the sub-groups "Chemo.+VEGF(R)i" and "Chemo.+EGFRi".

patients, 20.0%). Regimen specific toxicity profiles influenced the treatment decision making in 8 lines (8.5%) in total without trend towards early or later treatment stage.

Specific toxicity profiles of the substances administered played a less prominent role in decision making. The main reasons for prescribing a specific treatment type are summarized in Table III.

Treatment discontinuation. Most treatment lines (81 lines, 86.2%) were discontinued by the time of data documentation. To enquire about the main reasons for the treatment discontinuation, a list of nine specific options to choose from was provided to the centers (Table IV; interpretation of any of these options was at the discretion of the study sites).

Within each treatment line as well as for all treatment types across treatment lines, the predominantly documented reason for discontinuation was lack of efficacy (42 treatment lines in total, 51.9%). Planned treatment discontinuation as the number of pre-planned cycles was reached and toxicities (7 treatment lines each, 8.6%) were the next most frequent reasons to discontinue treatment. Best benefit reached was a prominent reason for discontinuation in patients being treated in 1st-line

Table IV. *Main reasons for treatment discontinuation by treatment line.*

Overall Active combinations/substances	1st-line (N=42) n (%)	2nd-line (N=37) n (%)	3rd-line (N=15) n (%)	Overall (N=94) n (%)
Treatment discontinuation				
Yes	41 (97.6)	31 (83.8)	9 (60.0)	81 (86.2)
No	1 (2.4)	6 (16.2)	6 (40.0)	13 (13.8)
Main reason for treatment discontinuation				
Any reason	41 (100.0)	31 (100.0)	9 (100.0)	81 (100.0)
Lack of efficacy	18 (43.9)	20 (64.5)	4 (44.4)	42 (51.9)
Progression	4 (9.8)	2 (6.5)	0 (0.0)	6 (7.4)
Toxicity	4 (9.8)	1 (3.2)	2 (22.2)	7 (8.6)
Best benefit reached	5 (12.2)	0 (0.0)	0 (0.0)	5 (6.2)
Number of planned cycles reached	7 (17.1)	0 (0.0)	0 (0.0)	7 (8.6)
Patient's decision	1 (2.4)	1 (3.2)	0 (0.0)	2 (2.5)
Lost to follow-up	0 (0.0)	1 (3.2)	0 (0.0)	1 (1.2)
Death	0 (0.0)	4 (12.9)	2 (22.2)	6 (7.4)
Other	2 (4.9)[1]	2 (6.5)[2]	1 (11.1)[3]	5 (6.2)[4]

Each of the following reasons was named once: [1]Selective internal radiation therapy (SIRT), amputation of the fifth toe because of gangrene; [2]Subileus and abscess after toe amputation; reduced condition; [3]Obstructive kidney failure; [4]SIRT, obstructive kidney failure, amputation of the fifth toe because of gangrene, Subileus and abscess after toe amputation, reduced condition, obstructive kidney failure.

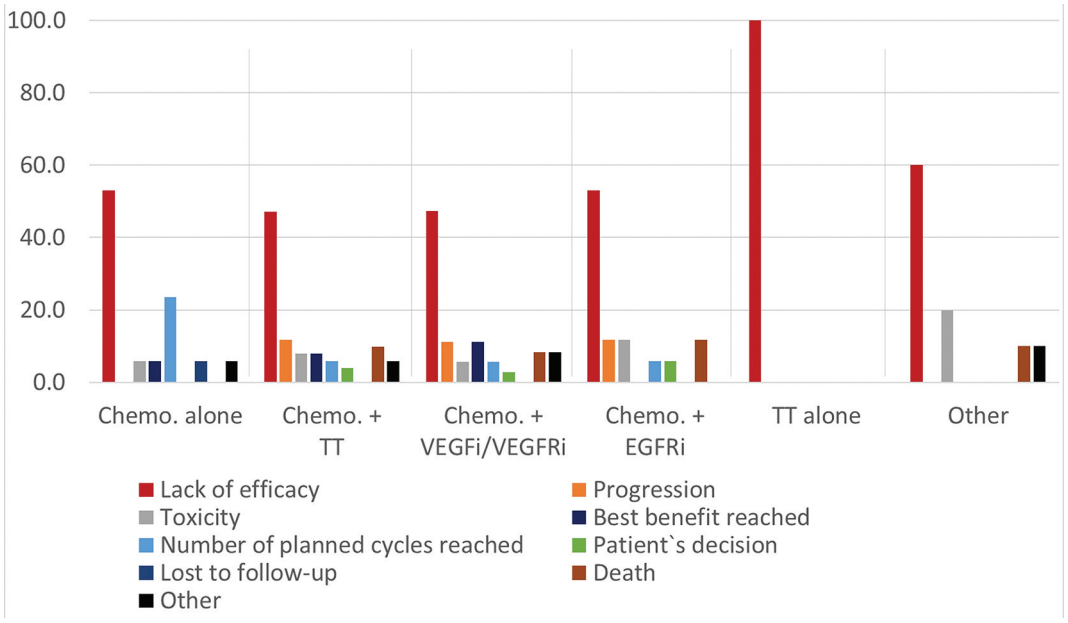

Figure 3. *Reasons for discontinuation. The percentages for the reasons to discontinue treatment according to pre-defined response options in all treatment lines by treatment type are shown. Chemo.: Chemotherapy; TT: targeted therapy; VEGF(R)i: vascular endothelial growth factor (Receptor) inhibitor; EGFRi: epidermal growth factor receptor inhibitor.*

only (5 patients, 12.2%) and death leading to treatment discontinuation became a more prominent reason in later treatment lines (1st-line: 0 patients, 0.0%; 2nd-line: 4 patients, 12.9%; 3rd-line: 2 patients, 22.2%). Table IV and Figure 3 summarize the main reasons for treatment discontinuation by treatment line and treatment type, respectively.

Allergic reaction (2 cases) was the most frequent cause of treatment discontinuation due to toxicities. Both patients were treated with chemotherapy in combination with TT (grade 2: FOLFOX plus cetuximab, and grade 3: FOLFOX plus bevacizumab, in this case associated with bronchospasm). In another patient being treated with

chemotherapy plus *VEGF*i (FOLFOXIRI plus bevacizumab) increased bilirubin, grade 3, required treatment discontinuation. In another case when chemotherapy was combined with an *EGFR*i, the treatment was discontinued upon occurrence of hydronephrosis due to ureter stone, grade 3 (FOLFOXIRI plus panitumumab). Capecitabine used in monotherapy was discontinued due to grade 2 coronary spasm in a single case. Toxicities occurring during therapy with other treatment types that were not otherwise specified required treatment discontinuation due to fever of unknown cause, grade 2, and grade 2 emesis.

Six patients died (three patients while being treated with chemotherapy+*VEGF(R)*i, two patients while in treatment with chemotherapy+*EGFR*i and one patient under treatment with other treatment types).

Discussion

In this study, we examined the real-world treatment of metastatic *BRAF^{V600E}*-mutated colorectal cancer in patients treated at fourteen sites in Germany, Austria, and Switzerland before approved availability of the targeted combination of encorafenib plus cetuximab. The aim of our data analysis was to better understand the treatment landscape and decision-making process. The results of this study include data on patient profiles, treatment patterns and outcomes of patients treated in the real-world setting.

In the three countries *involved*, the majority of the patients were tested for *BRAF*, *KRAS*, and *NRAS* mutations. *BRAF* testing usually was performed before systemic 1st-line therapy and simultaneously to *KRAS* and *NRAS* testing. This testing strategy is in compliance with the recommendations of applicable clinical practice guidelines as the results of the molecular evaluation constitute a critical requirement to choose the optimal therapy within the given treatment algorithms (9, 10, 32, 33).

In the 1st-line setting, all patients received a chemotherapy regimen with or without TT. Most of these patients were treated with a cytotoxic, platinum-based doublet or triplet. The targeted agent predominantly used as combination partner was bevacizumab. In the 2nd-line setting, the treatment type was either switched or – in case the therapy was continued with the same treatment type – one or more of the combination partner/s within the type was/were replaced. Clinical study results investigating 1st-line regimens have shown that combination chemotherapy with a fluoropyrimidine (such as FOLF or capecitabine) plus OX and/or IRI (FOLFOX or FOLFIRI or FOLFOXIRI) provides higher response rates and better progression-free and (partly) overall survival than a fluoropyrimidine alone (34-37). In addition, monoclonal antibodies such as the *VEGF*i bevacizumab or the *EGFR* inhibitors cetuximab and panitumumab have demonstrated an improved clinical outcome of patients with mCRC when combined with chemotherapy regimens in the 1st-line setting (38-47). Accordingly, those intensive 1st-line treatment triplet- or doublet-based regimens are recommended in the treatment algorithms of current national and international clinical practice guidelines in patients in good general health condition, limiting the recommended regimens to FOLF or capecitabin in combination with bevacizumab in patients in deteriorated condition (9, 10, 32). Moreover, according to the ESMO consensus guidelines, in patients in whom the initial chemotherapy backbone has failed, the chemotherapy backbone should be changed and biologics should be considered in 2nd-line if not used in the 1st-line treatment (9).

Our data show that both, the testing strategy and treatment principles in the treatment of mCRC patients within the three countries involved, reflected the recommendations laid out in these guidelines.

Across all treatment lines and types, however, lack of efficacy represented the leading cause for treatment discontinuation. Toxicities played a minor role when the decision was made to discontinue treatment. In general, up to 30% of the patients with mCRC experience disease- and treatment-related toxicities CTC grade III-IV, in particular diarrhea, nausea and vomiting, mucositis/stomatitis, constipation and neuropathy (19). While toxicities were not a major cause for treatment discontinuation in this study, most toxicities reported and attributed to a specific treatment regimen are typical for the substances or combinations used (48-58).

Against this background, molecularly defined approaches including the registration of encorafenib in combination with cetuximab for treatment of *BRAF^{V600E}*-positive mCRC after systemic therapy as well as the registration of pembrolizumab provide further promising options for future mCRC patients.

Methodological limitations. A general limitation is introduced by the nature of this study with its retrospective, uncontrolled, open design, non-standardized treatment allocations and conditions, as well as its observational character. Therefore, the study data is presented in a descriptive way only, showing the real-life situation during the specified documentation period. Observed treatment trends and tendencies must be interpreted with caution, as these might be influenced by underlying patient and disease characteristics.

Conclusion

Based on the MORSE^{CRC} patient cohort, current treatment practice of *BRAF^{V600E}*-mutant mCRC patients in Germany, Austria, and Switzerland typically includes combination chemotherapy plus targeted agents- thus reflecting the current clinical practice guidelines. While both anti-*VEGF*- and anti-

EGFR-targeted therapy play a relevant role in this treatment setting, lack of efficacy including progression is the leading cause for treatment discontinuation. In line with the current guideline recommendations for upfront molecular testing at the time of mCRC diagnosis, recently approved molecularly defined therapies represent promising treatment options for mCRC patients.

Conflicts of Interest

Armin Gerger disclosed a consulting role for Pierre Fabre; Dieter Koeberle has disclosed a consulting role with Pierre Fabre; Manfred Welslau has disclosed advisory roles for Amgen, Bristol-Myers Squibb, Celgene, Gilead, Hexal, Janssen, Lilly, medac, Novartis, Roche, and Sanofi. He received honoraria from Amgen, astellas, AstraZeneca, Celgene, Gilead, Hexal, Janssen, Lilly, Novartis, Roche, And Sanofi. Jens Uhlig has disclosed advisory roles in Ad Boards and workshops with Roche, Amgen, Servier, MSD, Bristol-Myers Squibb, Sanofi, Merck, Celgene, Novartis, Janssen-Cilag, Boehringer-Ingelheim, and Bayer. Richard Greil has disclosed a consulting role with Celgene, Novartis, Roche, BMS, Takeda, Abbvie, AstraZeneca, Janssen, MSD, Merck, Gilead, Daiichi Sankyo. He has received honoraria from Celgene, Roche, Merck, Takeda, AstraZeneca, Novartis, Amgen, BMS, MSD, Sandoz, Abbvie, Gilead, and Daiichi Sankyo. Eyck von der Heyde has disclosed consulting roles with Bristol-Myers Squibb, AstraZeneca, Merck, Novartis, and Boehringer Ingelheim. Dominik Paul Modest has disclosed a consulting role or honoraria for lectures with Amgen, Merck, Pierre Fabre, Servier, Lilly, MSD, BMS, onkowissen, Taiho, Incyte, AstraZeneca, G1, Transgene and Sanofi. He is currently receiving research grants from Servier and Amgen. Ralph Fritsch has disclosed a consulting role and is on the speaker`s bureau for Pierre Fabre.

Wolfgang Eisterer, Stefan Fuxius, Sara Bastian, Dilara Akhoundova Sanoyan, Christian Maas; Klaus Fenchel, Gaëlle Rhyner Agocs, Rudolf Weide, and Monika Schwager declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Frank Reichenbach is an employee at Pierre Fabre Pharma GmbH.

The Authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Authors' Contributions

This project and the analysis were designed and conducted by Pierre Fabre. All named Authors participated in the development of this manuscript and in the decision to submit this manuscript for publication. The Authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the accuracy and integrity of any part of the work as a whole and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, and have given their approval for this version to be published.

Armin Gerger: Conceptualization, methodology, resources (patients), investigation, writing-original draft preparation, writing-review, and editing. Wolfgang Eisterer, Stefan Fuxius, Sara Bastian, Dieter Köberle, Manfred Welslau, Dilara Akhoundova Sanoyan, Christian Maas, Jens Uhlig, Klaus Fenchel, Richard Greil, Eyck von der Heyde, Gaëlle Rhyner, and Rudolf Weide: Resources (patients), investigation, writing-review, and editing. Monika Schwager: Resources (computing), data curation, formal analysis, statistical analysis, software, visualization. Frank Reichenbach: Conceptualization, methodology, project administration, writing-original draft preparation, writing-review and editing, visualization, validation. Dominik Paul Modest: Conceptualization, methodology, writing-original draft preparation, writing-review, and editing. Ralph Fritsch: Conceptualization, methodology, resources (patients), investigation, writing-original draft preparation, writing-review, and editing.

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References

1 World Health Organization – International Agency for Research on Cancer (IARC) WHO Region EURO, 2022. Available at: https://gco.iarc.fr/today/fact-sheets-cancers [Last accessed on July 15, 2022]

2 Bericht zum Krebsgeschehen in Deutschland 2016, 2022. Available at: https://www.krebsdaten.de/Krebs/DE/Content/Krebsarten/Darmkrebs/darmkrebs_node.html [Last accessed on July 15, 2022]

3 StatistikAustria, 2022. Available at: https://www.statistik.at/statistiken/bevoelkerung-und-soziales/gesundheit/krebserkrankungen [Last accessed on July 15, 2022]

4 Schweizerische Eidgenossenschaft – Bundesamt für Statistik, 2022. Available at: https://www.bfs.admin.ch/bfs/de/home/statistiken/gesundheit/gesundheitszustand/krankheiten/krebs/spezifische.assetdetail.14816241.html [Last accessed on July 15, 2022]

5 Cancer Research UK Bowel cancer incidence statistics, 2022. Available at: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/survival#heading-Zero [Last accessed on July 15, 2022]

6 Cancer Research UK Bowel cancer incidence statistics, 2022. Available at: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/incidence#heading-Three [Last accessed on July 15, 2022]

7 Modified from: American Cancer Society colorectal-cancer-facts-and-figures-2017-2019, 2022. Available at: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/colorectal-cancer-facts-and-figures/colorectal-cancer-facts-and-figures-2017-2019.pdf [Last accessed on July 15, 2022]

8 Van Cutsem E, Cervantes A, Nordlinger B, Arnold D and ESMO Guidelines Working Group: Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol *25 Suppl 3*: iii1-iii9, 2014. PMID: 25190710. DOI: 10.1093/annonc/mdu260

9 Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, Aranda Aguilar E, Bardelli A, Benson A, Bodoky G, Ciardiello F, D'Hoore A, Diaz-Rubio E, Douillard JY, Ducreux M, Falcone A, Grothey A, Gruenberger T, Haustermans K, Heinemann V, Hoff P, Köhne CH, Labianca R, Laurent-Puig P, Ma B, Maughan T, Muro K, Normanno N, Österlund P, Oyen WJ, Papamichael D, Pentheroudakis G, Pfeiffer P, Price TJ, Punt C, Ricke J, Roth A, Salazar R, Scheithauer W, Schmoll HJ, Tabernero J, Taïeb J, Tejpar S, Wasan H, Yoshino T, Zaanan A and Arnold D: ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol *27(8)*: 1386-1422, 2016. PMID: 27380959. DOI: 10.1093/annonc/mdw235

10 Leitlinienprogramm Onkologie. S3-Leitlinie Kolorektales Karzinom. Langversion 2.1 – AWMF-Registernummer 021/007OL. January 2019. Available at: https://www.leitlinienprogramm-onkologie.de/leitlinien/kolorektales-karzinom/ [Last accessed on July 15, 2022]

11 Tran B, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, Agarwal A, Maru DM, Sieber O and Desai J: Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer *117(20)*: 4623-4632, 2011. PMID: 21456008. DOI: 10.1002/cncr.26086

12 Tveit KM, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, Sigurdsson F, Kure E, Ikdahl T, Skovlund E, Fokstuen T, Hansen F, Hofsli E, Birkemeyer E, Johnsson A, Starkhammar H, Yilmaz MK, Keldsen N, Erdal AB, Dajani O, Dahl O and Christoffersen T: Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) *versus* FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. J Clin Oncol *30(15)*: 1755-1762, 2012. PMID: 22473155. DOI: 10.1200/JCO.2011.38.0915

13 Van Cutsem E, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P and Ciardiello F: Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol *29(15)*: 2011-2019, 2011. PMID: 21502544. DOI: 10.1200/JCO.2010.33.5091

14 Sorbye H, Dragomir A, Sundström M, Pfeiffer P, Thunberg U, Bergfors M, Aasebø K, Eide GE, Ponten F, Qvortrup C and Glimelius B: High BRAF mutation frequency and marked survival differences in subgroups according to KRAS/BRAF mutation status and tumor tissue availability in a prospective population-based metastatic colorectal cancer cohort. PLoS One *10(6)*: e0131046, 2015. PMID: 26121270. DOI: 10.1371/journal.pone.0131046

15 Seymour MT, Brown SR, Middleton G, Maughan T, Richman S, Gwyther S, Lowe C, Seligmann JF, Wadsley J, Maisey N, Chau I, Hill M, Dawson L, Falk S, O'Callaghan A, Benstead K, Chambers P, Oliver A, Marshall H, Napp V and Quirke P: Panitumumab and irinotecan *versus* irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. Lancet Oncol *14(8)*: 749-759, 2013. PMID: 23725851. DOI: 10.1016/S1470-2045(13)70163-3

16 Loupakis F, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, Santini D, Masi G, Stasi I, Canestrari E, Rulli E, Floriani I, Bencardino K, Galluccio N, Catalano V, Tonini G, Magnani M, Fontanini G, Basolo F, Falcone A and Graziano F: KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer *101(4)*: 715-721, 2009. PMID: 19603018. DOI: 10.1038/sj.bjc.6605177

17 Kopetz S, McDonough SL, Lenz H-J, Magliocco AM, Atreya CE, Diaz LA, Allegra CJ, Raghav KPS, Morris VK, Wang SE, Lieu CH, Guthrie KA and Hochsteret HS: Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406). J Clin Oncol *35(suppl)*: abstract No. 3505, 2017. DOI: 10.1200/JCO.2017.35.15_suppl.3505

18 Mitani S, Taniguchi H, Honda K, Masuishi1 T, Narita1 Y, Kadowaki1 S, Ura1 T, Ando1 M, Tajika M and Muro K: Analysis of efficacy and prognostic factors in second-line chemotherapy for BRAF V600E mutant metastatic colorectal cancer. Ann Oncol *28(5s)*: abstract No. 532P, 2017. DOI: 10.1093/annonc/mdx393.058

19 Morris V, Overman MJ, Jiang ZQ, Garrett C, Agarwal S, Eng C, Kee B, Fogelman D, Dasari A, Wolff R, Maru D and Kopetz S: Progression-free survival remains poor over sequential lines of systemic therapy in patients with BRAF-mutated colorectal cancer. Clin Colorectal Cancer *13(3)*: 164-171, 2014. PMID: 25069797. DOI: 10.1016/j.clcc.2014.06.001

20 Peeters M, Oliner KS, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, Andre T, Chan E, Lordick F, Punt CJA, Strickland A, Wilson G, Ciuleanu TE, Roman L, Van Cutsem E, Yu H, Jung AS, Sidhu R and Pattersonet SD: Updated analysis of KRAS/NRAS and BRAF mutations in study 20050181 of panitumumab (pmab) plus FOLFIRI for second-line treatment (tx) of metastatic colorectal cancer (mCRC). J Clin Oncol *32(15_Suppl)*: abstract No. 3568, 2014. DOI: 10.1200/jco.2014.32.15_suppl.3568

21 Saridaki Z, Tzardi M, Sfakianaki M, Papadaki C, Voutsina A, Kalykaki A, Messaritakis I, Mpananis K, Mavroudis D, Stathopoulos E, Georgoulias V and Souglakos J: BRAF^{V600E} mutation analysis in patients with metastatic colorectal cancer (mCRC) in daily clinical practice: correlations with clinical characteristics, and its impact on patients' outcome. PLoS One *8(12)*: e84604, 2013. PMID: 24367680. DOI: 10.1371/journal.pone.0084604

22 Ulivi P, Capelli L, Valgiusti M, Zoli W, Scarpi E, Chiadini E, Rosetti P, Bravaccini S, Calistri D, Saragoni L, Casadei Gardini A, Ragazzini A, Frassineti GL, Amadori D and Passardi A: Predictive role of multiple gene alterations in response to cetuximab in metastatic colorectal cancer: a single center study. J Transl Med *10*: 87, 2012. PMID: 22569004. DOI: 10.1186/1479-5876-10-87

23 De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M and Tejpar S: Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol *11(8)*: 753-762, 2010. PMID: 20619739. DOI: 10.1016/S1470-2045(10)70130-3

24 Kudryavtseva AV, Lipatova AV, Zaretsky AR, Moskalev AA, Fedorova MS, Rasskazova AS, Shibukhova GA, Snezhkina AV, Kaprin AD, Alekseev BY, Dmitriev AA and Krasnov GS: Important molecular genetic markers of colorectal cancer. Oncotarget *7(33)*: 53959-53983, 2016. PMID: 27276710. DOI: 10.18632/oncotarget.9796

25 Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, Wolff RK and Slattery ML: Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res *65(14)*: 6063-6069, 2005. PMID: 16024606. DOI: 10.1158/0008-5472.CAN-05-0404

26 Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B and Velculescu VE: Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature *418(6901)*: 934, 2002. PMID: 12198537. DOI: 10.1038/418934a

27 Oikonomou E, Makrodouli E, Evagelidou M, Joyce T, Probert L and Pintzas A: BRAF(V600E) efficient transformation and induction of microsatellite instability *versus* KRAS(G12V) induction of senescence markers in human colon cancer cells. Neoplasia *11(11)*: 1116-1131, 2009. PMID: 19881948. DOI: 10.1593/neo.09514

28 Cremolini C, Antoniotti C, Stein A, Bendell J, Gruenberger T, Rossini D, Masi G, Ongaro E, Hurwitz H, Falcone A, Schmoll HJ and Di Maio M: Individual patient data meta-analysis of FOLFOXIRI plus bevacizumab *versus* doublets plus bevacizumab as initial therapy of unresectable metastatic colorectal cancer. J Clin Oncol: JCO2001225, 2020. PMID: 32816630. DOI: 10.1200/JCO.20.01225

29 Pietrantonio F, Petrelli F, Coinu A, Di Bartolomeo M, Borgonovo K, Maggi C, Cabiddu M, Iacovelli R, Bossi I, Lonati V, Ghilardi M, de Braud F and Barni S: Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer *51(5)*: 587-594, 2015. PMID: 25673558. DOI: 10.1016/j.ejca.2015.01.054

30 Rowland A, Dias MM, Wiese MD, Kichenadasse G, McKinnon RA, Karapetis CS and Sorich MJ: 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 *112(12)*: 1888-1894, 2015. PMID: 25989278. DOI: 10.1038/bjc.2015.173

31 Karapetis CS, Liu H, Sorich M, Fiskum J, Grothey A, Adams RA, Venook A, Heinemann V, Lenz HJ, Yoshino T, Zalcberg JR, Chibaudel B, Buyse ME, De Gramont A and Shi Q: Impact of molecular markers status on treatment effects comparing EGFR and VEGF monoclonal antibodies (mAbs) in untreated metastatic colorectal cancer (mCRC): Pooled individual patient data (IPD) analysis of randomized trials from the ARCAD database. Ann Oncol *31(Suppl 4)*: 409-461, 2020. DOI: 10.1016/j.annonc.2020.08.545

32 NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Colon Cancer. Version 4.2020. Available at: https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1428 [Last accessed on July 18, 2021]

33 Hofheinz RD, Arnold D, Borner M, Folprecht G, Ghadimi BM, Graeven U, Hebart H, Hegewisch-Becker S, Heinemann V, Meybier T, Pritzkuleit R, Rödel C, Scheithauer W, Thaler J, Wörmann B, in cooperation with AIO: Onkopedia Leitlinie Kolonkarzinom.2018, 2022. Available at: https://www.onkopedia.com/de/onkopedia/guidelines/kolonkarzinom/@@guideline/html/index.html [Last accessed on July 15, 2022]

34 de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F and Bonetti A: Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol *18(16)*: 2938-2947, 2000. PMID: 10944126. DOI: 10.1200/JCO.2000.18.16.2938

35 Douillard JY and V-303 Study Group: Irinotecan and high-dose fluorouracil/leucovorin for metastatic colorectal cancer. Oncology (Williston Park) *14(12 Suppl 14)*: 51-55, 2000. PMID: 11200150.

36 Fuchs CS, Marshall J, Mitchell E, Wierzbicki R, Ganju V, Jeffery M, Schulz J, Richards D, Soufi-Mahjoubi R, Wang B and Barrueco J: Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. J Clin Oncol *25(30)*: 4779-4786, 2007. PMID: 17947725. DOI: 10.1200/JCO.2007.11.3357

37 Köhne CH, De Greve J, Hartmann JT, Lang I, Vergauwe P, Becker K, Braumann D, Joosens E, Müller L, Janssens J, Bokemeyer C, Reimer P, Link H, Späth-Schwalbe E, Wilke HJ, Bleiberg H, Van Den Brande J, Debois M, Bethe U and Van Cutsem E: Irinotecan combined with infusional 5-fluorouracil/folinic acid or capecitabine plus celecoxib or placebo in the first-line treatment of patients with metastatic colorectal cancer. EORTC study 40015. Ann Oncol *19(5)*: 920-926, 2008. PMID: 18065406. DOI: 10.1093/annonc/mdm544

38 Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J and Rougier P: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med *360(14)*: 1408-1417, 2009. PMID: 19339720. DOI: 10.1056/NEJMoa0805019

39 Van Cutsem E, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P and Ciardiello F: Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol *29(15)*: 2011-2019, 2011. PMID: 21502544. DOI: 10.1200/JCO.2010.33.5091

40 Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zubel A and Koralewski P: Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol *27(5)*: 663-671, 2009. PMID: 19114683. DOI: 10.1200/JCO.2008.20.8397

41 Bokemeyer C, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M and Koralewski P: Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol *22(7)*: 1535-1546, 2011. PMID: 21228335. DOI: 10.1093/annonc/mdq632

42 Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R and Patterson SD: Panitumumab-FOLFOX4 treatment and

RAS mutations in colorectal cancer. N Engl J Med *369(11)*: 1023-1034, 2013. PMID: 24024839. DOI: 10.1056/NEJMoa1305275

43 Van Cutsem E, Lenz HJ, Köhne CH, Heinemann V, Tejpar S, Melezínek I, Beier F, Stroh C, Rougier P, van Krieken JH and Ciardiello F: Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol *33(7)*: 692-700, 2015. PMID: 25605843. DOI: 10.1200/JCO.2014.59.4812

44 Bokemeyer C, Köhne CH, Ciardiello F, Lenz HJ, Heinemann V, Klinkhardt U, Beier F, Duecker K, van Krieken JH and Tejpar S: FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. Eur J Cancer *51(10)*: 1243-1252, 2015. PMID: 25937522. DOI: 10.1016/j.ejca.2015.04.007

45 Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M and Gansert J: Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) *versus* FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol *28(31)*: 4697-4705, 2010. PMID: 20921465. DOI: 10.1200/JCO.2009.27.4860

46 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R and Kabbinavar F: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med *350(23)*: 2335-2342, 2004. PMID: 15175435. DOI: 10.1056/NEJMoa032691

47 Saltz LB, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzén F and Cassidy J: Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol *26(12)*: 2013-2019, 2008. PMID: 18421054. DOI: 10.1200/JCO.2007.14.9930

48 Pfizer Pharma PFE GmbH. Leucovorin Summary of Product Characteristics, 2022. Available at: https://www.fachinfo.de/suche/fi/003571 [Last accessed on May 27, 2022]

49 Medac GmbH. 5-Fluorouracil Summary of Product Characteristics, 2022. Available at: https://www.fachinfo.de/api/fachinfo/pdf/001835 [Last accessed on May 27, 2022]

50 Fresenius Kabi Deutschland GmbH. Oxaliplatin Summary of Product Characteristics, 2022. Available at: https://www.fachinfo.de/api/fachinfo/pdf/012120 [Last accessed on May 27, 2022]

51 Onkovis GmbH. Irinotecan Summary of Product Characteristics, 2022. Available at: https://www.fachinfo.de/api/fachinfo/pdf/012070 [Last accessed on May 27, 2022]

52 Roche Pharma AG. Xeloda Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/xeloda-epar-product-information_en.pdf [Last accessed on May 27, 2022]

53 Bayer AG. Eylea® Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/eylea-epar-product-information_en.pdf [Last accessed on May 27, 2022]

54 Roche Pharma AG. Avastin Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/avastin-epar-product-information_en.pdf [Last accessed on May 27, 2022]

55 Lilly, S.A. Cyramza Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/cyramza-epar-product-information_en.pdf [Last accessed on May 27, 2022]

56 Merck Healthcare KGaA. Erbitux Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/erbitux-epar-product-information_en.pdf [Last accessed on May 27, 2022]

57 Amgen Europe B.V. Vectibix Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/vectibix-epar-product-information_en.pdf [Last accessed on May 27, 2022]

58 Bayer AG. Stivarga Summary of Product Characteristics, 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/stivarga-epar-product-information_en.pdf [Last accessed on May 27, 2022]

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