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Mutational profiles of metastatic colorectal cancer treated with FOLFIRI plus cetuximab or bevacizumab before and after secondary resection (AIO KRK 0306; FIRE-3)

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Abstract

Secondary resection of metastases is recommended in metastatic colorectal cancer (mCRC). Data describing changes in mutational profiles of corresponding primary tumor and metastatic tissue after conversion treatment are limited. Next generation sequencing was performed in formalin-fixed mCRC samples from patients of the FIRE-3 trial (FOLFIRI plus cetuximab or bevacizumab) before treatment start (baseline) and after secondary resection of metastases (post baseline). Changes of mutational profiles and tumor mutational burden (TMB) were assessed within a post-hoc analysis. Median overall survival (OS), progression-free survival (PFS) and objective response rate (ORR) were compared between treatment arms. Paired tumor samples were obtained from 25 patients (19 *RAS* wild-type, 6 *RAS* mutant by pyrosequencing). ORR (92.0% vs 58.0%) and OS (60.8 vs 35.4 months, hazard ratio = 0.39 [95% CI 0.14-1.12], $P = .08$) were higher for patients receiving cetuximab. After conversion therapy, 56 alterations (42 in the cetuximab and 14 in the bevacizumab arm) were newly observed in 18 patients (9 each treated with cetuximab or bevacizumab). Gains (n = 21) and losses (n = 21) of alterations occurred during cetuximab-based treatment, while mainly gains of alterations occurred during bevacizumab (n = 10). Three of nine patients treated with cetuximab that presented a change of mutational profiles, developed resistance to cetuximab.

Abbreviations: 5-FU, 5-fluorouracil; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CI, confidence interval; CNA, copy number alteration; DFS, disease free survival; DNA, desoxyribonucleinacid; EGFR, epidermal growth factor receptor; FFPE, formalin fixed paraffin embedded; FOLFIRI, 5-fluorouracile, leucovorin, irinotecan; GNAS, guanine nucleotide binding protein subunit alpha; HER2/neu, ERB-B2 receptor tyrosine kinase 2; HR, hazard ratio; KRAS, Kirsten rat sarcoma; LV, leucovorin; MAF, mutation allele frequency; MAPK, mitogen activated protein kinase; mCRC, metastatic colorectal cancer; MSS, microsatellite stable; MUT, mutation; NF1, neurofibromin 1; NGS, next generation sequencing; OR, odds ratio; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PIK3CA, phosphatidylinositol-3-kinase; PSS, post-surgical survival; RAS, rat sarcoma; SRC, proto-oncogene tyrosine-protein kinase rous sarcoma; TMB, tumor mutational burden; VEGF, vascular endothelial growth factor; WT, wild-type.

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Mutational profiles were largely comparable before and after treatment with anti-*VEGF* or anti-*EGFR* directed monoclonal antibodies after secondary resection. Mutations associated with resistance to anti-*EGFR* antibodies were observed in only one-third of patients.

KEYWORDS
bevacizumab, cetuximab, metastatic colorectal cancer, NGS, paired samples

What's New?

Secondary resection for initially unresectable metastatic colorectal cancer (mCRC) is associated with improved prognosis. Predicting opportunities for secondary resection, however, depends on the discovery of molecular changes in primary tumor and corresponding metastatic tumor tissue. Here, mutational profiles were investigated for mCRC patients in the FIRE-3 trial, a study of FOLFIRI plus cetuximab or bevacizumab as first-line therapy for irresectable mCRC. Of nine mCRC patients undergoing cetuximab therapy who experienced changes in tumor mutational profile, one-third became resistant to cetuximab. For patients treated with anti-VEGF and anti-EGFR antibodies, mutational profiles were similar before and after treatment and following secondary resection.

1 | INTRODUCTION

Prognosis of patients with primarily unresectable metastatic colorectal cancer (mCRC) has markedly improved over the past decades by introduction of monoclonal antibody treatment according to molecular tumor characteristics.[1,2] After cytotoxic conversion therapy of initially unresectable lesions, multidisciplinary treatment approaches (eg, surgery and local-ablative treatment) are recommended to improve long-term overall survival.[3,4] Although previous investigations mainly focused on R0 resection rate, even R1 resection of metastatic lesions has been associated with improved outcome in both liver-limited and non-liver-limited disease.[5-10] It is, therefore, essential to identify patients that benefit from multidisciplinary treatment and to evaluate biomarkers for increasing their frequency. Various factors influence secondary resectability, such as surgeons' experience, location of metastases, but also surrogate parameters of response like early tumor shrinkage or depth of response. The presence of *BRAF V600E* mutations (MUT) was associated with a lower likelihood of secondary resectability.[11] However, little is known about dynamics of molecular tumor characteristics in paired samples of primary tumors and corresponding metastases after conversion treatment.

FIRE-3 was an open-label multicenter randomized controlled phase III trial that evaluated the combination of FOLFIRI plus cetuximab or bevacizumab as first-line regimen in irresectable *KRAS* wild-type (WT) mCRC patients.[12] Of these, 29% of patients underwent secondary resection after conversion.[5] Additionally, a subgroup of 373 patients provided formalin-fixed paraffin embedded (FFPE) samples for targeted next generation sequencing (NGS) analysis of 315 genes (FoundationOne, Roche).[13] We were able to re-perform NGS in FFPE specimens of metastases from patients who underwent secondary resection and to correlate this analysis with data from corresponding primary tumors. Our aim was to assess dynamic changes in molecular characteristics of paired specimen (primary tumor and metastases) after conversion treatment with cytotoxic agents (5-FU, LV, Irinotecan) and biologicals (cetuximab or bevacizumab).

2 | MATERIAL AND METHODS

2.1 | Experimental design and patients

FIRE-3 compared FOLFIRI plus cetuximab or bevacizumab for first line treatment of *KRAS* WT mCRC patients within an open-label, multicenter, randomized phase III trial concept. Details on treatment protocol, safety and efficacy in all patients and molecular subgroups were reported elsewhere.[12]

This retrospective analysis investigated a subgroup of patients with available, paired DNA sequencing data from tumor FFPE specimens prior to systemic treatment and after resection of metastases. Details on methods of DNA sequencing (Foundation One, Foundation Medicine, Penzberg, Germany), quality assessment and type of data were reported previously,[2,12-14] and are briefly summarized in the Appendix S1, Material and Methods section. The 315 genes that were investigated by the above-mentioned assay are listed in the Table S1. The sequencing coverage and quality statistics for each sample are summarized in Table S2.

2.2 | Objectives

Main objective of this analysis was the exploratory comparison of DNA mutational profiles in paired samples of patients with metastatic colorectal cancer at baseline (eg before treatment start) and after secondary resection of metastases (post baseline). Further objectives were the assessment of mutated allele frequency changes of mutations, copy number alterations (CNA), tumor mutational burden (TMB) and *RAS* status (WT to MUT and vice versa). Objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) were defined as previously published, starting from randomization.[12] Furthermore, two additional exploratory survival endpoints were defined in this analysis. Disease-free survival (DFS) involved the time from secondary resection of metastases to the subsequent progression of disease. The postsurgical survival (PSS) was

defined as the period from secondary resection of metastases to death by any cause. Patients alive were censored at the last patient contact, the last update on patient survival was performed in February 2021.[15]

2.3 | Statistical analysis

Statistical analyses were pre-specified in a protocol analysis plan before start of the analysis. Logistic regression was applied to estimate the relative treatment benefit on ORR and the odds ratio (OR) of cetuximab versus bevacizumab and was calculated together with the 95% confidence intervals (CI). The Kaplan-Meier method and Cox proportional hazard models were used to estimate the relative treatment benefit on OS, PFS, DFS and PSS. Median survival as well as hazard ratios (HR) together with the 95% confidence intervals (CI) was provided. The *t*-test for paired samples compared median exon coverage and tumor mutational burden of matching samples analyzed at two timepoints. All *P*-values <.05 (two-sided) were considered significant. However, none of the analyses was powered for the comparisons made, as the sample size in this post-hoc analysis resulted from available tumor samples. Due to the exploratory nature of our study, no adjustment for multiple testing was applied. SAS 9.4 (SAS Institute, Cary, North Carolina) and R version 3.2.3 software were used for statistical analyses.

3 | RESULTS

3.1 | Baseline characteristics of paired samples subset

In FIRE-3, DNA sequencing was performed successfully in FFPE tumor tissue of 373 patients at baseline and of 57 patients

TABLE 1 Baseline characteristics of patients with a paired set of FFPE samples before and after resection of metastases

Variable	Treatment		
	FOLFIRI + cetuximab n = 13	FOLFIRI + bevacizumab n = 12	Total n = 25
Gender			
Male [n (%)]	10 (76.9%)	7 (58.3%)	17 (68.0%)
Female [n (%)]	3 (23.1%)	5 (41.7%)	8 (32.0%)
Age			
Median, years [range]	64.0 [38.0-76.0]	61.0 [31.0-74.0]	63.0 [31.0-76.0]
>65 years [n (%)]	4 (30.8%)	5 (41.7%)	9 (36.0%)
≤65 years [n (%)]	9 (69.2%)	7 (58.3%)	16 (64.0%)
ECOG performance index			
0 [n (%)]	7 (53.8%)	7 (58.3%)	14 (56.0%)
1 [n (%)]	6 (46.2%)	5 (41.7%)	11 (44.0%)
Primary tumor side			
Right [n (%)]	4 (30.8%)	2 (16.7%)	6 (24.0%)
Left [n (%)]	9 (69.2)	10 (83.3%)	19 (76.0%)
Number of involved organs			
1 [n (%)]	7 (53.8%)	6 (50.0%)	13 (52.0%)
2 [n (%)]	6 (46.2%)	3 (25.0%)	9 (36.0%)
3 [n (%)]	0 (0.0%)	3 (25.0%)	3 (12.0%)
Liver limited disease			
Yes [n (%)]	7 (53.8%)	6 (50.0%)	13 (52.0%)
No [n (%)]	6 (46.2%)	6 (50.0%)	12 (48.0%)
Diagnosis of metastases			
Synchronous [n (%)]	10 (76.9%)	9 (75.0%)	19 (76.0%)
Metachronous [n (%)]	3 (23.1%)	3 (25.0%)	6 (24.0%)
Alkaline phosphatase			
<300 U/L [n (%)]	13 (100.0%)	10 (83.3%)	23 (92.0%)
≥300 U/L [n (%)]	0 (0.0%)	2 (16.7%)	2 (8.0%)
Leucocytes			
<8/nL [n (%)]	7 (53.8%)	6 (50.0%)	13 (52.0%)
≥8/nL [n (%)]	6 (46.2%)	6 (50.0%)	12 (48.0%

Abbreviation: FOLFIRI, 5-fluorouracil, leucovorin and irinotecan.

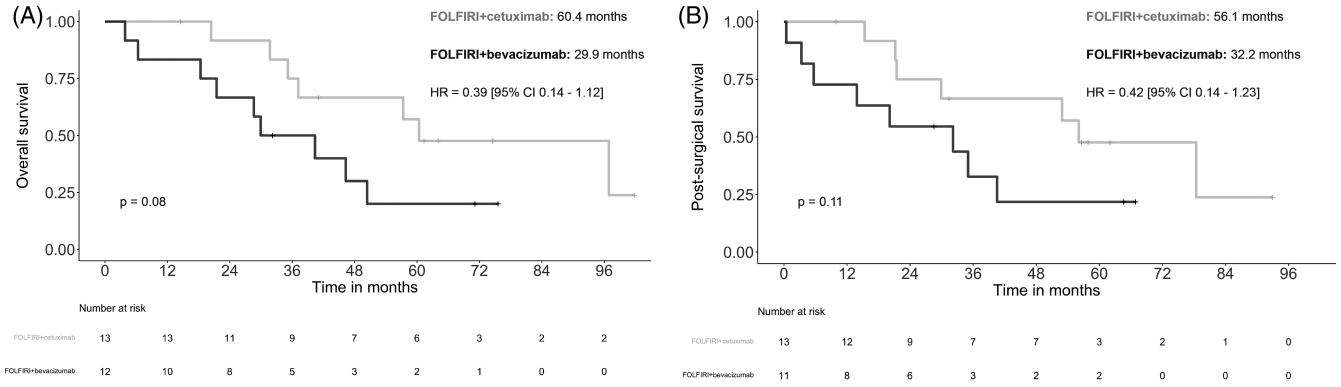

FIGURE 1 Overall survival (A) and post-surgical survival (B) according to first line treatment of 25 patients with metastatic colorectal cancer in FIRE-3 who underwent conversion treatment, resection and DNA sequencing of primary tumors obtained prior to treatment and corresponding metastases after resection

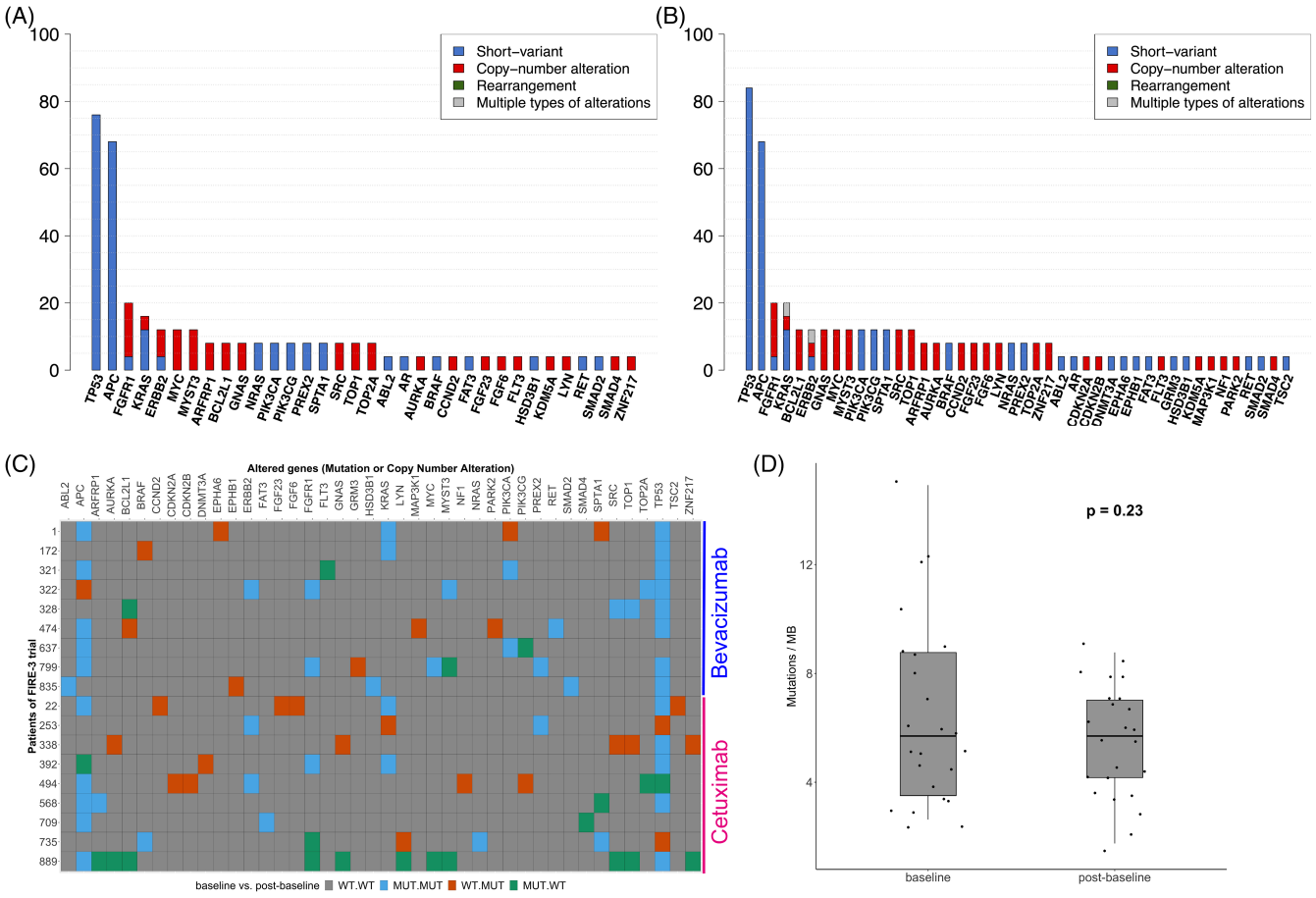

FIGURE 2 (A) Mutational profile of tumors from FIRE-3 patients with metastatic colorectal cancer prior to systemic treatment (baseline) and (B) of corresponding metastases after conversion treatment and resection (post-baseline). (C) Change of alterations during conversion treatment with either cetuximab or bevacizumab and (D) change of tumor mutational burden from baseline to post baseline. Legend: WT.WT, wild-type at baseline and wild-type post baseline; MUT.MUT, mutated/altered at baseline and mutated/altered post baseline; WT.MUT, wild-type at baseline and mutated/altered post baseline; MUT.WT, mutated/altered at baseline and wild-type post baseline

post-baseline. Paired tumor samples (ie, patients with primary tumor tissue before treatment start and corresponding metastatic specimen after resection) were provided by 25 patients (cetuximab, n = 13; bevacizumab, n = 12). Nineteen of twenty five tumors (76.0%) were classified *RAS/BRAF* WT and six tumors *RAS* MUT (24.0%) by pyrosequencing, respectively. Microsatellite stability (MSS) was shown in all tumors. Data regarding the median exon coverage of paired samples is

TABLE 2 Summary of changes per gene in patients of FIRE-3 post baseline compared to baseline

Gene	Gain of mutation n (%)	Loss of mutation n (%)	Gain of CNA n (%)	Loss of CNA n (%)
ARFRP1	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8%)
AURKA	0 (0.0)	0 (0.0)	1 (1.8%)	1 (1.8%)
APC	2 (3.6)	4 (7.1%)	0 (0.0)	0 (0.0)
BCL2L1	0 (0.0)	0 (0.0)	1 (1.8%)	2 (3.6%)
BRAF	1 (1.8%)	0 (0.0)	0 (0.0)	0 (0.0)
CCND2	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
CDKN2A	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
CDKN2B	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
DNMT3A	1 (1.8%)	0 (0.0)	0 (0.0)	0 (0.0)
EPHA6	1 (1.8%)	0 (0.0)	0 (0.0)	0 (0.0)
EPHB1	1 (1.8%)	0 (0.0)	0 (0.0)	0 (0.0)
ERBB2	1 (1.8%)	0 (0.0)	0 (0.0)	1 (1.8%)
FGF6	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
FGF23	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
FGFR1	0 (0.0)	1 (1.8%)	0 (0.0)	1 (1.8%)
FLT3	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8%)
GNAS	0 (0.0)	0 (0.0)	1 (1.8%)	1 (1.8%)
GRM3	1 (1.8%)	0 (0.0)	0 (0.0)	0 (0.0)
KRAS	1 (1.8%)	0 (0.0)	1 (1.8%)	0 (0.0)
LYN	0 (0.0)	0 (0.0)	1 (1.8%)	1 (1.8%)
MAP3K1	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
MYC	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8%)
MYST3	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.6%)
NF1	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
PARK2	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
PIK3CA	1 (1.8%)	0 (0.0)	0 (0.0)	0 (0.0)
PIK3CG	1 (1.8%)	1 (1.8%)	0 (0.0)	0 (0.0)
SMAD4	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8%)
SPTA1	1 (1.8%)	1 (1.8%)	0 (0.0)	0 (0.0)
SRC	0 (0.0)	0 (0.0)	1 (1.8%)	1 (1.8%)
TOP1	0 (0.0)	0 (0.0)	1 (1.8%)	1 (1.8%)
TOP2A	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8%)
TP53	0 (0.0)	1 (1.8%)	2 (3.6%)	0 (0.0)
TSC2	0 (0.0)	0 (0.0)	1 (1.8%)	0 (0.0)
ZNF217	0 (0.0)	0 (0.0)	1 (1.8%)	1 (1.8%)

Abbreviation: CNA, copy number alteration.

displayed in the Table S3.) Date of secondary resection was not recorded in one patient (Table 1).

3.2 | Outcome of patients undergoing secondary resection

No differences in PFS and DFS were observed with regard to treatment arms (Table S4). Compared to bevacizumab, nonsignificant trends towards a higher probability of response (ORR 92.0% vs 58.0%, odds ratio = 8.57 [1.09-182.88], $P = .07$), longer OS (60.4 vs 29.9 months, HR = 0.39 [95% CI 0.14-1.12], $P = .08$) and PSS (56.1 vs 32.2 months, HR = 0.42 [95% CI 0.14-1.23], $P = .11$) were observed for patients treated with cetuximab (Figure 1).

3.3 | Change of mutational profiles from baseline to post-baseline

(K)RAS mutations in colorectal primary tumors of the FIRE-3 intention-to-treat population were initially detected by pyrosequencing.

TABLE 3 Changes of alterations according to type of conversion treatment

Treatment	Baseline WT; post-baseline MUT, *n* (%)	Baseline MUT; post-baseline WT, *n* (%)	Total, *n* (%)
FOLFIRI + cetuximab	21 (50.0)	21 (50.0)	42 (100.0)
FOLFIRI + bevacizumab	10 (71.4)	4 (28.6)	14 (100.0)

Abbreviation: FOLFIRI, 5-fluorouracil, leucovorin and irinotecan; MUT, mutated; WT, wild-type.

This post-hoc analysis also evaluated patients included into FIRE-3 before the protocol amendment of 2008 (ie, inclusion of *KRAS* WT mCRC only), meaning that a small proportion of patients was initially treated despite presence of a *KRAS* mutation.

Prevalence of mutations was comparable in all patients at baseline (n = 373) and post-baseline (n = 57) except for *BRAF* (*V600E* and non-*V600E*, baseline: 12.1%; post-baseline: 5.3%), *NRAS* (baseline: 5.9%, post-baseline: 12.3%) and *PIK3CA* (baseline: 16.1%, post-baseline: 12.3%) mutations, respectively (Table S5).

Similar comparability was observed for patients with paired tumor samples (Figure 2A,B). Seven of 25 patients (5 with synchronous and 2 with metachronous disease) had no change of initial molecular status after conversion treatment. In a paired set of one patient, only variants classified as "likely" were detected.

In total, 168 genetic alterations in total (baseline and post-baseline) were detected in the remaining 18 patients (each 9 in the cetuximab and bevacizumab arm, respectively). Hundred and twelve alterations (66.7%) were observed in primary tumors and corresponding metastases after conversion treatment and resection. Conversely, 56 changes (cetuximab: n = 42; bevacizumab: n = 14), with a prevalence ranging from 1.8% to 7.1% (Table 2) were newly detected after conversion treatment and resection. Gains and losses of alterations were observed at a comparable frequency during cetuximab-based treatment, while mainly gains of alterations (n = 10, 71.4%) were detected during bevacizumab-based treatment (Table 3).

All changes are graphically displayed in Figure 2C. In summary, alterations associated with intrinsic resistance to cetuximab other than *RAS* mutations were observed in two patients after resection of metastatic tissue: patient 338 developed copy number alterations in *GNAS* and *SRC*, and *NF1* was inactivated by mutation in patient 494, respectively. Two patients treated with cetuximab lost previously detected markers of resistance (patients 709 and 889). Obvious biomarkers of resistance to bevacizumab were not observed. The complete mutational profile including mutated allele frequency (MAF) of mutations per patient is listed in Table S6.

3.4 | Change of RAS status from baseline to post baseline

In 25 patients providing paired samples, pyrosequencing initially detected *RAS* wild-type status in 19 and mutations in 6 patients, respectively.

RAS mutations were confirmed by NGS in 5 patients (3 in the cetuximab arm and 2 in the bevacizumab arm) at baseline. One *RAS* mutation initially detected by pyrosequencing was not confirmed by NGS. Subsequently, 20 patients were classified as *RAS* wild-type by NGS.

RAS mutations were maintained in the corresponding metastatic lesions after conversion treatment, albeit with different MAF (Table S4).

Nineteen of twenty patients (95.0%) with *RAS* WT tumor remained *RAS* WT after conversion therapy. Only one patient developed a new *KRAS* G12D mutation (MAF: 26.59) after treatment with cetuximab.

3.5 | Change of TMB status from baseline to post-baseline

Mean tumor mutational burden was lower post-baseline compared to baseline (5.56 vs 6.48 mutations per Mb). However, this difference was not significant ($P = .23$) (Figure 2D).

4 | DISCUSSION

In this retrospective exploratory analysis of the randomized phase III FIRE-3 trial, we evaluated the response and outcome of patients who underwent secondary resection, and compared the mutational profiles of paired samples before treatment start (primary tumor) and after secondary resection of metastases.

Secondary resection of metastases after conversion of initially irresectable disease during cytotoxic treatment is recommended by current treatment guidelines[3,4] and has been observed in clinical trials at rates ranging between 15% and 80%, depending on whether the trial was designed for the evaluation of resectability or not and whether disease was liver-limited or not.[6-8,16-18] Resectability was retrospectively assessed at baseline and at best response in FIRE-3 and only 29% of all patients actually underwent resection, while the proportion of potential resectability was significantly higher.[5] In this subgroup analysis, a strong, but nonsignificant trend towards higher ORR and longer OS and PSS was observed in patients treated with cetuximab compared to bevacizumab. The neoadjuvant addition of cetuximab to FOLFIRI was previously associated with numerically higher resection rates.[6,7] Nevertheless, the addition of cetuximab in a perioperative setting (ie, neoadjuvant and adjuvant treatment) in *KRAS* exon 2 WT mCRC patients with (suboptimal) resectable liver metastases was associated with unfavorable outcome compared to chemotherapy alone in the NewEPOC trial.[18] Platinum-containing cytotoxic treatment in combination with cetuximab, the quality of surgery and the presence of occult *RAS* mutations might have contributed to the adverse outcome, compared to FIRE-3.

The diagnostic approach to the detection of *RAS* mutations plays an important role for treatment selection, as anti-*EGFR* agents are notably not effective in the presence of *RAS* mutations.[1] Inclusion of patients with *KRAS* WT mCRC was required by amendment of FIRE-3 after 336 patients had been recruited, and therefore, a small proportion of patients received treatment despite of the presence of a *KRAS* mutation prior to this amendment. Nevertheless, these patients were included in this analysis, as the primary objective was the longitudinal comparison of mutational profiles, and all patients achieved secondary resectability. Comprehensive molecular profiling confirmed the presence of *RAS* mutations, providing a higher sensitivity compared to pyrosequencing.[13]

During cetuximab treatment, one patient with initial *RAS* WT mCRC developed a new *KRAS* G12D mutation, but eight patients did not. Additional *RAS* mutations with low mutated allele frequency were described as a mechanism of resistance by clonal selection.[19,20] Beyond *RAS*, inactivation of *NF1* and gain of CNA in *GNAS* and *SRC*, respectively, were observed after resection of metastases in two patients, for which intrinsic resistance towards cetuximab has been reported previously.[21-23] Conversely, 6 of 9 patients who underwent secondary resection did not display any known markers of resistance by NGS. It should be noted that other biomarkers of *EGFR* resistance, such as immunohistochemical testing of *HER2/neu* overexpression in *RAS* WT mCRC, were not assessed. Moreover, only known alterations with high evidence were included in this analysis, which might have excluded inactivating mutations of the *EGFR*.[24-26]

Interestingly, alterations associated with *EGFR* resistance or other newly detected alterations did not significantly affect overall survival of patients treated with cetuximab significantly. The former might be at least partially allocated to the better prognosis of secondary interventions compared to unresectable disease.[5,10] Disease burden is reduced or ideally not evident, and patients might not necessarily continue systemic treatment including cetuximab. Thus, secondarily evoked resistance mechanisms might disappear during the treatment break, with the option of anti-*EGFR* re-challenge in case of delayed disease progression.[20] Notably, these observations were limited to secondarily evoked *RAS* mutations, and this hypothesis would have to be confirmed in further investigations.

With respect to the limited sample size and the descriptive nature of these findings, our observations indicated that patients with secondarily resectable disease after cetuximab containing conversion treatment might represent an exceptionally susceptible subgroup with loss or without development of mechanisms of resistance towards anti-*EGFR* treatment. Conversely, alterations associated with anti-*EGFR* resistance presumably would have to be detected more frequently in nonresponding patients. Nonetheless, our post-hoc analysis did not investigate metastatic tissue of patients with irresectable metastases to confirm this hypothesis.

In contrast, less alterations were detected in metastatic tissue of patients treated with bevacizumab, which is known to be an inhibitor of angiogenesis by blocking the vascular endothelial growth factor (*VEGF*).[27,28] Few biomarkers have been reported for efficacy of bevacizumab treatment such as chromosomal instability or angiogenesis activity, which has, however, not been considered in our analysis.[29,30] We can therefore not conclude if patients of FIRE-3 who underwent resection are particularly susceptible to anti-*VEGF* treatment or not.

Clonal evolution of tumor lesions must be acknowledged when analyzing more than one lesion by comprehensive genomic profiling. We did not observe significant differences in terms of synchronous or metachronous, liver-limited or nonlimited metastatic disease in the mutational profiles of FIRE-3 patients. Some data suggest that mutational profiles of primary tumors remained consistent during systemic treatment, but that metastases are more heterogenous with a higher rate of private mutations.[31] Here, the timepoint of metastatic disease played a crucial role: while synchronous metastases showed a rate of concordance to primary tumors of 14%-84%, metachronous metastases might have a different mutational profile due to delay of treatment progression and subsequent evolution of tumor cell clones.[32-35] Although the comparison of mutational profiles in FIRE-3 before treatment start and after resection of metastases was considered longitudinal (ie, a change of the mutational profile during treatment), two aspects could have biased interpretation of these results. First, the alterations found after resection of metastases might have existed at baseline already in the metastatic tissue, while the primary tumor tissue was analyzed. Secondly, the origin of specimens could notably have biased these findings, as the post-baseline specimens originated from different metastatic sites, and the histology of these specimens was not documented. For precise results, comparisons would have to be performed within specimens of each individual site, even though the sample size would decrease further. Nevertheless, our approach provided at least partially insights of molecular tumor evolution during a common oncological procedure (primary tumor biopsy at diagnosis, biopsy of metastasis during treatment).

Moreover, genomic heterogeneity of multiple lesions of one site was described, but it would not be feasible to investigate all resected lesions, even if it would provide high resolution of the mutational profile. This approach would be limited to academic centers and clinical decision making would still be difficult owing to tumor heterogeneity. Our data rather support the option to biopsy a progressive lesion in case of rapid and unexpected disease progression or delayed metachronous disease. As a compromise, liquid biopsies could additionally assess the current status of known mutations in case of divergent results between primary tumor and metastases.

In this analysis, we were able to obtain paired DNA sequencing results of 25 well-described mCRC patients evaluated within a randomized controlled trial. Prior cohorts comparing DNA sequencing results between primary tumors and metastases were of comparable or lower sample size. However, the explanatory power remains limited, and the number of patients is too small to draw definite conclusions. The missing documentation for the underlying metastatic site for NGS testing, missing analyses of patients with irresectable disease and clonal heterogeneity within tumor lesions could have additionally biased our results.

5 | CONCLUSION

In conclusion, we observed largely comparable mutational profiles in patients with initially unresectable metastatic colorectal cancer before

treatment start and after conversion and secondary resection of the corresponding metastases. Three of nine patients treated with cetuximab and a documented change in their mutational profile developed alterations associated with intrinsic resistance towards anti-*EGFR* treatment, which conversely implies a high susceptibility. No specific mechanisms of resistance were observed in patients treated with bevacizumab, and TMB status remained unchanged.

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CONFLICT OF INTEREST

AS received honoraria for talks by Roche Pharma and Servier and reimbursement for travel, accommodation and expenses by Roche Pharma, Merck KGaA, MSD Sharp & Dohme, Pfizer and Amgen. VH received honoraria for talks by Merck, Roche Pharma, Celgene, Amgen, Sanofi, Lilly, SIRTEX, Boehringer-Ingelheim, Taiho, Servier, worked in advisory role for Merck KGaA, Roche Pharma, Amgen, Sanofi, SIRTEX, Servier, Celgene, Boehringer-Ingelheim, Halozyme, MSD Sharp & Dohme and BMS, received research funding by Merck KGaA, Roche Pharma, Amgen, SIRTEX, Servier, Celgene, Boehringer-Ingelheim and Shire and received reimbursement for travel, accommodation and expenses by Merck KGaA, Roche Pharma, Amgen, SIRTEX, Servier, Shire, MSD and BMS. JWH worked in advisory role for and received honoraria for talks from Roche Pharma, and received reimbursement for travel and accommodation from Novartis. JvE received honoraria for talks by Merck KGaA, Roche Pharma, Amgen, Sanofi, Pierre-Fabre, Servier, Taiho, BMS, Eisai and Novartis, worked in advisory role for Amgen, Pierre-Fabre, Bristol-Myers Squibb and Servier and received reimbursement for travel by AstraZeneca and apceth. CBW received personal and speakers' fees and scientific grants by Roche and worked in advisory boards for Roche.. KH received honoraria for talks by Roche, worked in advisory boards for Servier and received reimbursement for travel by Amgen, Celgene and Lilly. AHSA received honoraria for advisory role by Roche and MSD Sharp & Dohme and reimbursement for travel by Pfizer, Roche, Eli Lilly Oncology, Novartis and PharmaMar. LW received honoraria for talks from Roche Pharma. DPM received honoraria for talks by Merck KGaA, Amgen, BMS, MSD Sharp & Dohme, Servier, Pierre-Fabre, Lilly Oncology, Sanofi, Onkowissen and CORZED and scientific grants by Servier and Amgen. TD received honoraria for advisory role by Novartis and Lilly Oncology. MM reported honoraria from Merck KGaA, MSD Sharp & Dohme, BMS, Servier, Pierre-Fabre, Lilly Oncology and Dragonfly. FK worked in advisory role for Elsevier. TK worked in advisory role for Amgen, Astra Zeneca, Bayer Pharmaceuticals, BMS, Boehringer Ingelheim, Merck KGaA, MDS, Pfizer, Novartis, Qiagen, Roche Pharma and Takeda, received scientific grants by Merck KGaA and Roche Pharma and worked for the speaker's bureau of Merck KGaA and AstraZeneca. AJ worked in advisory role for and received honoraria for talks and reimbursement for travel, accommodation and expenses from Amgen, AstraZeneca, Bayer Pharmaceuticals, BMS, Biocartis, Boehringer Ingelheim, Merck KGaA, Lilly Oncology, MSD Sharp & Dohme, Novartis, QuIP GmbH, Roche Pharma, Takeda and Thermo Fisher. SS received honoraria for talks from and worked in consultancy role for Amgen, Bayer Pharmaceuticals, BMS, Eisai, Lilly Oncology, Merck KGaA, MSD Sharp & Dohme, Pierre-Fabre, Roche Pharma, Sanofi, Servier, Taiho Pharmaceuticals and Takeda and received scientific grants from Merck KGaA, Pierre Fabre, Servier and Roche Pharma.

All remaining authors have declared no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of our study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The trial was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the University of Munich (registry-no: 186-15). All patients provided written informed consent for treatment within this clinical trial. The trial was registered at clinicaltrials.gov (NCT00433927).

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REFERENCES

1. Van Cutsem E, Lenz HJ, Kohne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol*. 2015;33:692-700.
2. Stintzing S, Modest DP, Rossius L, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. *Lancet Oncol*. 2016;17:1426-1434.
3. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol*. 2016;27:1386-1422.
4. Benson AB, Venook A, Al-Hawary MM, et al. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Comprehensive Cancer Netw*. 2021;19:329-359.
5. Modest DP, Denecke T, Pratschke J, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. *Eur J Cancer*. 2018;88:77-86.
6. Kohne CH, Poston G, Folprecht G, et al. FOLFIRI plus cetuximab in patients with liver-limited or non-liver-limited RAS wild-type

metastatic colorectal cancer: a retrospective subgroup analysis of the CRYSTAL study. *Eur J Surg Oncol*. 2016;42:1540-1547.
7. Folprecht G, Gruenberger T, Bechstein W, et al. Survival of patients with initially unresectable colorectal liver metastases treated with FOLFOX/cetuximab or FOLFIRI/cetuximab in a multidisciplinary concept (CELIM study). *Ann Oncol*. 2014;25:1018-1025.
8. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. *Lancet Oncol*. 2010;11:38-47.
9. Choti MA, Thomas M, Wong SL, et al. Surgical resection preferences and perceptions among medical oncologists treating liver metastases from colorectal cancer. *Ann Surg Oncol*. 2016;23:375-381.
10. de Haas RJ, Wicherts DA, Flores E, Azoulay D, Castaing D, Adam R. R1 resection by necessity for colorectal liver metastases: is it still a contraindication to surgery? *Ann Surg*. 2008;248:626-637.
11. Modest DP, Heinemann V, Folprecht G, et al. Factors that influence conversion to resectability and survival after resection of metastases in RAS WT metastatic colorectal cancer (mCRC): analysis of FIRE-3-AIOKRK0306. *Ann Surg Oncol*. 2020;27:2389-2401.
12. Heinemann V, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2014;15:1065-1075.
13. Stahler A, Stintzing S, von Einem JC, et al. Single-nucleotide variants, tumour mutational burden and microsatellite instability in patients with metastatic colorectal cancer: next-generation sequencing results of the FIRE-3 trial. *Eur J Cancer*. 2020;137:250-259.
14. Stintzing S, Miller-Phillips L, Modest DP, et al. Impact of BRAF and RAS mutations on first-line efficacy of FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab: analysis of the FIRE-3 (AIO KRK-0306) study. *Eur J Cancer*. 2017;79:50-60.
15. Heinemann V, von Weikersthal LF, Decker T, 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;124:587-594.
16. Cremolini C, Loupakis F, Antoniotti C, 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;16:1306-1315.
17. Cremolini C, Antoniotti C, Rossini D, 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;21:497-507.
18. Bridgewater JA, Pugh SA, Maishman T, et al. Systemic chemotherapy with or without cetuximab in patients with resectable colorectal liver metastasis (new EPOC): long-term results of a multicentre, randomised, controlled, phase 3 trial. *Lancet Oncol*. 2020;21:398-411.
19. Morelli MP, Overman MJ, Dasari A, et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. *Ann Oncol*. 2015;26:731-736.
20. Parseghian CM, Loree JM, Morris VK, et al. Anti-EGFR-resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge. *Ann Oncol*. 2019;30:243-249.
21. Georgiou A, Stewart A, Cunningham D, Banerji U, Whittaker SR. Inactivation of NF1 promotes resistance to EGFR inhibition in KRAS/NRAS/BRAF(V600)-wild-type colorectal cancer. *Mol Cancer Res*. 2020;18:835-846.
22. Bray SM, Lee J, Kim ST, et al. Genomic characterization of intrinsic and acquired resistance to cetuximab in colorectal cancer patients. *Sci Rep*. 2019;9:15365.
23. Nozaki M, Yasui H, Ohnishi Y. Ligand-independent EGFR activation by anchorage-stimulated Src promotes cancer cell proliferation and cetuximab resistance via ErbB3 phosphorylation. *Cancers (Basel)*. 2019;11:1552.
24. Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2016;17:738-746.
25. Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol*. 2019;20:518-530.
26. Braig F, Marz M, Schieferdecker A, et al. Epidermal growth factor receptor mutation mediates cross-resistance to panitumumab and cetuximab in gastrointestinal cancer. *Oncotarget*. 2015;6:12035-12047.
27. Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature*. 1993;362:841-844.
28. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004;350:2335-2342.
29. Smeets D, Miller IS, O'Connor DP, et al. Copy number load predicts outcome of metastatic colorectal cancer patients receiving bevacizumab combination therapy. *Nat Commun*. 2018;9:4112.
30. van Dijk E, Biesma HD, Cordes M, et al. Loss of chromosome 18q11.2-q12.1 is predictive for survival in patients with metastatic colorectal cancer treated with bevacizumab. *J Clin Oncol*. 2018;36:2052-2060.
31. Adua D, Di Fabio F, Ercolani G, et al. Heterogeneity in the colorectal primary tumor and the synchronous resected liver metastases prior to and after treatment with an anti-EGFR monoclonal antibody. *Mol Clin Oncol*. 2017;7:113-120.
32. Kovaleva V, Geissler AL, Lutz L, et al. Spatio-temporal mutation profiles of case-matched colorectal carcinomas and their metastases reveal unique de novo mutations in metachronous lung metastases by targeted next generation sequencing. *Mol Cancer*. 2016;15:63.
33. Schweiger T, Liebmann-Reindl S, Glueck O, et al. Mutational profile of colorectal cancer lung metastases and paired primary tumors by targeted next generation sequencing: implications on clinical outcome after surgery. *J Thorac Dis*. 2018;10:6147-6157.
34. Sutton PA, Jithesh PV, Jones RP, et al. Exome sequencing of synchronously resected primary colorectal tumours and colorectal liver metastases to inform oncosurgical management. *Eur J Surg Oncol*. 2018;44:115-121.
35. Mogensen MB, Rossing M, Ostrup O, et al. Genomic alterations accompanying tumour evolution in colorectal cancer: tracking the differences between primary tumours and synchronous liver metastases by whole-exome sequencing. *BMC Cancer*. 2018;18:752.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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