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Molecular hyperselection for optimal choice of first-line targeted therapy independent of primary tumor sidedness: An exploratory analysis of the randomized FIRE-3 study performed in RAS wild-type metastatic colorectal cancer

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ABSTRACT

Introduction: Molecular diagnostics play a pivotal role in guiding therapy for metastatic colorectal cancer (mCRC). Current guidelines recommend stratification based on biomarkers such as RAS, BRAF, and DNA mismatch-repair (MMR) status to select between anti-EGFR (epidermal growth factor receptor) and anti-VEGF (vascular endothelial growth factor) therapies.
Materials and methods: This retrospective analysis evaluated the randomized FIRE-3 study that compared first-line treatment with FOLFIRI plus cetuximab to FOLFIRI plus bevacizumab in RAS wild-type patients. The present analysis included 199 patients with RAS/BRAF wild-type MMR proficient tumors. Next-generation sequencing (NGS) was successfully performed in all patients and allowed stratification into hyperselected (no predefined genetic alterations) or gene altered subgroups using the previously published approach of the PRESSING-studies.
Results: Hyperselection according to PRESSING-3 was associated with a survival benefit from anti-EGFR-based therapy compared to bevacizumab (38.5 months vs. 27.5 months; HR 0.68; 95 % CI, 0.44–1.05; P = 0.08). This benefit was observed in both, right- and left-sided tumors, (HR 0.58 and HR 0.70). Patients with gene alterations showed inferior survival compared to hyperselected patients across all subgroups. In this unfavorable

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subgroup, application of cetuximab and bevacizumab were associated with comparable OS (total cohort: HR 1.04; 95 % CI, 0.61–1.79). Again, this finding was independent of primary tumor sidedness (left-sided tumors: HR 1.10; 95 % CI, 0.59–2.07; right-sided tumors: HR 1.05; 95 % CI, 0.31–3.55).
Conclusion: Molecular hyperselection facilitated by next generation sequencing could replace primary tumor sidedness as a tool of decision making for optimal choice of targeted therapy in first-line treatment of RAS wild-type mCRC

Trial identification number: NCT00433927 [clinicaltrials.gov]

1. Introduction

The present standard of treatment for metastatic or recurrent, unresectable colorectal cancer involves treatment stratification based on biomarkers such as RAS (KRAS and NRAS), BRAF, and microsatellite status [2,10]. These predictive biomarkers guide the decision to initiate either an anti-EGFR-based or anti-VEGF-based therapy. According to current guidelines, anti-EGFR therapies such as cetuximab or panitumumab are indicated only for patients with RAS and BRAF wild-type tumors located in the left-sided colon [6,20]. However, primary resistance to EGFR-inhibitors remains a significant challenge despite the exclusion of patients based on these biomarkers.

In addition to the established biomarkers RAS and BRAF, other molecular alterations are associated with primary resistance to anti-EGFR therapies [19,22]. Incorporating these alterations into the current standard selection process could improve the identification of patients who are likely to benefit from targeted therapies, while sparing others from potentially toxic treatments.

The concept of molecular hyperselection, which enables more precise identification of patients likely to benefit from anti-EGFR-based therapy, was first described by Cremolini and coworkers in a cohort of RAS and BRAF wild-type patients treated with anti-EGFR directed therapies [5]. In this context, the PRESSING panel was developed to highlight the negative predictive role of specific alterations such as mutations of PIK3CA, PTEN, or AKT1 mutations, amplification of HER2- or MET, or rearrangements of NTRK, ROS1, ALK, and RET. These alterations are considered predictive markers of resistance to anti-EGFR therapies. In 2023, the PRESSING panel was expanded to also include MAP2K1 mutations and PTEN loss [12].

Despite the precise selection provided by the PRESSING panel, a small group of patients continues to derive limited benefit from anti-EGFR-based therapy. To better characterize this group, Randon et al. conducted the PRESSING-2 study, which investigated mCRC patients molecularly hyperselected by PRESSING-1 to exclude even rarer mechanisms of resistance (ultraselection)[13]. The PRESSING-2 study thus classified additional alterations in the MAPK, PIK3CA, and EGFR-independent receptor tyrosine kinase pathways as “gene altered”.

In the PARADIGM study, the concept of molecular hyperselection was demonstrated for the first time in a large, randomized study [20]. Hyperselected patients with ctDNA that lacked gene alterations showed a clear survival benefit when panitumumab versus bevacizumab was added to FOLFOX6. However, similar or inferior outcome with panitumumab was observed in patients with ctDNA that contained any gene alteration [14].

The present analysis examines the relevance of molecular hyperselection in patients treated in the FIRE-3 study. This randomized phase III trial evaluated patients with unresectable RAS wild-type mCRC, who underwent first-line chemotherapy with FOLFIRI (5-FU, leucovorin, irinotecan) combined with either the anti-EGFR antibody cetuximab or the anti-VEGF antibody bevacizumab [6].

Application of PRESSING-1 and PRESSING-2 in FIRE-3, thus allowed for the first time to evaluate the concept of molecular hyperselection in a cohort treated with an irinotecan-based chemotherapy backbone.

2. Methods and material

2.1. Patients

For this exploratory, retrospective analysis, 752 patients from the FIRE-3 study were included. Successful next-generation sequencing (NGS) analysis was conducted for 373 patients, as reported earlier [16]. Details regarding the study design, patient characteristics, treatment, statistical assumptions, and results have been reported previously [6].

2.2. Next-generation sequencing of DNA

For NGS, tumor tissue samples from formalin-fixed, paraffin-embedded (FFPE) specimens were used for DNA extraction and analyzed with the Foundation One® assay (Foundation Medicine, Penzberg, Germany). This assay enabled hybrid-capture NGS of 315 genes. Sequencing was performed on FFPE tissue samples obtained before the administration of study treatment. Patients were classified as gene altered if at least one of the predefined alterations (Table 2) were identified. Patients without any of the predefined alterations were assigned to the hyperselection group.

2.3. Primary tumor sidedness and exact primary tumor location

Information on exact primary tumor location was extracted from the respective study report forms. Right-sided primary tumors (RSPT) included caecum, ascending colon, hepatic flexure, and the transverse colon, while left-sided primary tumors (LSPT) included the splenic flexure, descending colon, sigmoid, and rectum. Patients for whom the primary tumor location could not be exactly determined, for example, when the tumor spanned multiple segments, were excluded from the analysis.

2.4. Standard selection

The starting point of the present analysis is the cohort of patients selected by Standard Selection. This reflects the current standard of treatment stratification and excludes all patients with a known dMMR/MSI-h status or mutations in RAS- or BRAF-genes as identified by PCR-based analysis (Standard Selection^{PCR}; see Table 2).

2.5. Selection by next generation sequencing

Due to its greater sensitivity, next-generation sequencing (NGS) allowed the identification of further RAS mutations. No additional BRAF mutations were detected. These NGS-detected RAS mutations were excluded to define a purely RAS/BRAF WT (wild-type) population (Standard Selection^{NGS} Cohort).

2.6. Hyperselection according to PRESSING-1

According to the PRESSING-1 concept, NGS-based hyperselection was performed in the Standard Selection^{NGS} Cohort (n = 171). For this analysis, we used the extended PRESSING-1 panel that also includes MAP2K1 mutations and PTEN loss [5,12]. The PRESSING-1 gene altered cohort was thus defined by detection of at least one alteration including point mutations (KRAS, NRAS, BRAFV600E, HER2/ERBB2, PIK3CA

exon 20, PTEN, AKT1, MAP2K1), amplifications (Her2/ERBB2, MET), loss of PTEN, or rearrangements (NTRK, ROS1, ALK, RET) in the analyzed tumors (Table 2). Conversely, the PRESSING-1 hyperselected subgroup consisted of tumors without any of these alterations.

2.7. Selection according to PRESSING-2

The PRESSING-2 analysis was performed in PRESSING-1-negative patients [13]. In this cohort, additional rare alterations associated with primary EGFR-resistance were analyzed including point mutations (NF1, MAP2K2, MAP2K4 (non S184L), AKT2, ERBB3, NTRK), amplifications (ARAF, KRAS, AKT1/2, IGF1R, ERBB3, FGFR2), loss of NF1 or rearrangement of EGFR (see Table 2). The PRESSING-2 gene altered cohort was thus defined by detection of at least one mutation of the following genes detected in the tumor.

2.8. Selection according to PRESSING-3

The PRESSING-3 analysis was performed in the Standard Selection^{NGS} Cohort. PRESSING-3 is a combined evaluation of all alterations tested in the PRESSING-1 plus PRESSING-2. It thus represents the most comprehensive panel in this analysis, comprising 26 different alterations.

2.9. Statistical analysis

All statistical analyses were performed using SPSS Version 29 for Windows (SPSS Inc, Chicago, IL). In univariate analyses, categorical variables were compared using the chi-square test to identify patient-, tumor-, and treatment-related characteristics associated with prolonged survival. Significant factors, including the presence of gene alterations according to PRESSING-1 and PRESSING-3, were further analyzed in multivariate analyses using linear and binary logistic regression models. The prognostic impact of gene alterations was specifically assessed by comparing survival outcomes in hyperselected versus gene-altered patients, as presented in Table 4 and the Kaplan-Meier survival curves (Supplement 1–3).

Survival estimates were calculated using the Kaplan-Meier method and described by median values. Survival data comparisons were conducted using log-rank tests and Cox regression analyses, presented as hazard ratios (HR) with 95 % confidence intervals (95 % CI). Associations and differences between variables were analyzed using chi-square statistics. The probability of response (ORR) was estimated using a logistic regression model, which included the odds ratio (OR) for cetuximab versus bevacizumab. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Patients

For this exploratory, retrospective analysis, 752 patients from the FIRE-3 study were included. Sufficient tumor material for a successful next-generation sequencing analysis was obtained in 373 patients, as previously reported. Ten patients were excluded from the analysis due to deficient mismatch repair (microsatellite instability). Within the FIRE-3 study, RAS and BRAF status had been centrally determined using PCR, resulting in the exclusion of an additional 164 patients with BRAF^{V600E} or RAS alterations.

After exclusion of dMMR/MSI-h and PCR-detected RAS/BRAF mutant tumors according to the Standard Selection^{PCR}, a cohort of 199 tumors remained. Central NGS identified additional 28 patients with KRAS or NRAS mutations. Exclusion of these RAS mutant patients resulted in the Standard Selection^{NGS} Cohort (n = 171) in which subsequently PRESSING-1 and PRESSING-3 analyses were performed. Details are provided in Fig. 1.

3.2. Patient characteristics

Patient characteristics are summarized in Table 1. The Standard Selection^{PCR} Cohort of 199 patients included 142 male and 57 female patients. Median age was 65.0 years (range: 31–76 years). In the majority of patients, primary tumors were located in the left-side of the colon (79.9 %), while location in the right-sided colon was observed in only 19.1 % of patients. Patient characteristics were comparable between the different cohorts of molecular selection (Table 1).

3.3. Effect of hyperselection on overall survival

The present analysis was performed as a retrospective evaluation of the randomized FIRE-3 study comparing anti-EGFR- versus anti-VEGF-based first-line therapy. The primary focus of this investigation was laid on the effect of hyperselection on overall survival.

3.4. Selection according to the PRESSING-1 panel

The PRESSING-1 panel was evaluated in the RAS/BRAF WT Standard Selection^{NGS} Cohort of 171 patients and led to the identification of a hyperselected subgroup (N = 119) without genetic alterations and a gene altered subgroup of 52 patients (30.4 %). Gene mutations were observed at the highest frequency in PIK3CA (11.7 %), PTEN (4.7 %) and ERBB2 (3.5 %). The highest rate of gene amplification was observed with regard to ERBB2 (7.6 %) (Fig. 3).

Table 1
Patient characteristics of the Standard Selection^{NGS} with and without PRESSING-1 alterations.

	Standard Selection^{NGS} Cohort N = 171	PRESSING-1 Gene AlteredN= 52	PRESSING-1hyperselectedN= 119
Median Age, Range [years]	64, 31–76	64, 44–74	64, 31–76
Sex [%]	126 [73.7]45 [22.6]	39 [75.0]13 [25.0]	87 [73.1]32 [26.9]
• **Male**			
• **Female**			
Primary tumor location [%]	140 [81.9]31 [18.1]	40 [76.9]12 [23.1]	100 [84.0]19 [16.0]
• **Left-sided**			
• **Right-sided**			
Treatment [%]	77 [45.0]94 [55.0]	27 [51.9]25 [48.1]	50 [42.0]69 [58.0]
• **FOLFIRI+cetuximab**			
• **FOLFIRI+bevacizumab**			
ECOG [%]	99 [57.9]70 [40.9]2 [1.2]	28 [53.8]22 [42.3]2 [3.8]	71 [59.7]22 [40.3]-
• **0**			
• **1**			
• **2**			

In the PRESSING-1 hyperselected subgroup, anti-EGFR-based therapy induced a median overall survival of 38.5 months compared to 27.5 months in the bevacizumab arm (p = 0.09; HR 0.70). Conversely, patients in the gene altered group showed comparable benefit from either therapy (HR 1.04). Among PRESSING-1 hyperselected patients, anti-EGFR therapy improved survival irrespective of tumor localization (left-sided: HR 0.74; right-sided: HR 0.55). In the gene altered subgroup, no survival advantage was observed (left-sided: HR 1.07; right-sided: HR 1.20). The results are shown in Table 3 and Supplement 1.

Table 2
Overview of different panels within this analysis. Blue: included in the panel, orange: excluded from the panel. Gene Altered Rate defines the percentage of alterations defined as gene altered according to the panel used.

	Standard Selection^{PCR} Cohort	**PRESSING-1**	**PRESSING-2**	**PRESSING-3**
Cohort of Selection		Standard Selection^{NGS} Cohort	PRESSING-1 hyperselected	Standard Selection^{NGS} Cohort
Evaluated pts	199	171	119	171
MSS-Status				
MSS, pMMR [%]	199 [100]			
Mutation				
KRAS [%]	23 [11.6]			
NRAS [%]	5 [2.5]			
BRAF^{V600E} [%]	0			
PIK3CA exon 20 [%]		20 [11.7]		20 [11.7]
PTEN [%]		8 [4.7]		8 [4.7]
Her2/ERBB2 [%]		6 [3.5]		6 [3.5]
AKT1 [%]		0		0
MAP2K1 [%]		0		0
MAP2K4 (non S184L) [%]			2 [1.7]	3 [1.8]
ERBB3 [%]			2 [1.7]	2 [1.2]
NF1 [%]			1 [0.8]	2 [1.2]
NTRK 1 [%]			0	2 [1.2]
MAP2K2 [%]			0	0
AKT2 [%]			0	0
NTRK 2 [%]			0	0
Amplification				
Her2/ERBB2 [%]		13 [7.6]		13 [7.6]
MET [%]		2 [1.2]		2 [1.2]
KRAS [%]			2 [1.7]	2 [1.2]
AKT 2 [%]			2 [1.7]	2 [1.2]
ARAF [%]			0	0
AKT1 [%]			0	0
IGF1R [%]			0	0
ERBB3 [%]			0	0
FGFR2 [%]			0	0
Loss				
PTEN [%]		5 [2.9]		5 [2.9]
NF1 [%]			0	0
Rearrangement				
NTRK 1 [%]		0		0
NTRK 2 [%]		0		0
ROS1 [%]		0		0
ALK [%]		0		0
RET [%]		0		0
EGFR [%]			0	0
Gene Altered Rate,		31.6	7.6	39.2

Table 3
Survival-analysis according to PRESSING-1 based on Standard Selection^{NGS} Cohort (N = 171).

	Hyperselection			Gene Altered		
	Treatment	**N**	**HR [95 % CI]**	**Treatment**	**N**	**HR [95 % CI]**
Total cohort	Cetuximab	50	0.70 [0.46–1.06]	Cetuximab	27	1.04 [0.58–1.87]
	Bevacizumab	69		Bevacizumab	25	
Left-Sided	Cetuximab	43	0.74 [0.47–1.16]	Cetuximab	22	1.07 [0.53–2.14]
	Bevacizumab	57		Bevacizumab	18	
Right-Sided	Cetuximab	7	0.55 [0.20–1.49]	Cetuximab	5	1.20 [0.34–4.17]
	Bevacizumab	12		Bevacizumab	7	

3.5. Selection according to the PRESSING-2 panel

For PRESSING-1 hyperselected patients (N = 119), an additional analysis was conducted, focusing on rare genetic alterations associated with EGFR resistance (PRESSING-2 panel). This resulted in 110 patients being assigned to the PRESSING-2 hyperselection group and nine patients to the gene altered group. Thus, the additional application of PRESSING-2 in the PRESSING-1 hyperselected subgroup resulted in a further exclusion of 7.6 % (9/119) of patients. The results are shown in Supplement 2.

3.6. Combined evaluation of PRESSING-1 and PRESSING-2 (PRESSING-3)

PRESSING-3 is the combined evaluation of PRESSING-1 and PRESSING-2. This evaluation was again performed in the Standard Selection^{NGS} Cohort and resulted in 110 patients found in the hyperselection group compared to 61 (35.7 %) patients in the gene altered group. Here, the previously observed trends were confirmed: anti-EGFR therapy conferred a survival benefit across the entire cohort, regardless of tumor localization (overall cohort: HR 0.68; left-sided: HR 0.70; right-sided: HR 0.58). For genetically altered tumors, neither therapy showed a survival advantage (overall cohort: HR 1.04; left-sided: HR 1.10; right-sided: HR 1.05). Detailed results are provided in Fig. 2, Table 4 and Supplement 3.

3.7. Effect of hyperselection on ORR and PFS

In the Standard Selection^{NGS} Cohort, objective response rate (ORR) was significantly greater with cetuximab compared to bevacizumab (83 % vs. 69 %; P = 0.049). Numerical superiority of cetuximab compared to bevacizumab was noted in PRESSING-1/3 hyperselected as well as in gene altered patients. This effect was independent of tumor sidedness. PFS was comparable in the total cohort for cetuximab compared to bevacizumab (HR 1.06). Detailed results on PFS and ORR can be found in Table 4.

4. Discussion

The present analysis investigates the molecular diagnostics in metastatic colorectal cancer and aims to assess their effect on clinical decision-making. Current guidelines recommend treatment stratification based on biomarkers such as RAS, BRAF, and microsatellite status (MSS/MMR) [2]. More recently, also HER-2 status has been introduced into the selection process [14]. These biomarkers are central to the initial choice between anti-EGFR and anti-VEGF therapies. However, questions remain regarding primary resistance to anti-EGFR therapies and the role of rare genetic alterations, which can be identified through advanced molecular diagnostics such as NGS.

The published analyses on hyperselection using the PRESSING- and PARADIGM-panel consistently show that hyperselected patients significantly benefit from anti-EGFR therapy. These analyses are based on patient cohorts that received platinum-based systemic therapy as a backbone [20,5,9]. Whether these findings are transferable to irinotecan-based chemotherapy was unclear prior to this analysis.

In the FIRE-3 study, a large cohort was retrospectively analyzed, and the results confirm that hyperselected patients also benefit from anti-EGFR therapy when treated with irinotecan-based regimens. At the same time, gene-altered tumors, regardless of localization, showed similar survival outcomes under both anti-EGFR and anti-VEGF therapies. However, it should be noted that on data on right-sided mCRC in this analysis are limited due to sample size constraints. Furthermore, the benefit observed in hyperselected right-sided patients did not reach statistical significance, as indicated by the wide confidence interval of the hazard ratio. Additional biomarkers, such as the expression degress of EGFR ligands AREG and EREG, may bei required to better identify EGFR-addicted right-sided tumors[7,8].

Methodologically, the PRESSING and PARADIGM analyses differ significantly. The PARADIGM analysis is based on NGS from liquid biopsies and classified tumors with NGS-detected RAS- and BRAF-mutations as gene altered, allowing for a broader inclusion strategy but leading to a more heterogeneous cohort. The same approach was used in the analysis by Stahler and coworkers analyzing negative hyperselection of resistance mutations for panitumumab maintenance within the PANAMA trial [15]. It should be acknowledged that the PANAMA analysis utilized a panel that did not include MMR, which may have influenced the findings. In the present investigation, we followed the PRESSING approach, where, on the basis of NGS, additionally identified RAS mutations were excluded to create a homogeneous all-wild-type cohort, enabling more precise therapeutic decisions. Nevertheless, the limitations of the PRESSING panels must be acknowledged. The negative predictive role of several alterations remains unclear, as illustrated by the case of PIK3CA mutations [21]. The question of whether grouping rare alterations is a sound method for assessing primary resistance remains open, necessitating continuous research efforts for the correct characterization of rare alterations as potential bypass mechanisms upon EGFR inhibition.

To apply the advantages of treatment stratification based on hyperselection as outlined in this manuscript, expanded molecular analyses are required at the time of initial diagnosis. According to current guidelines, such diagnostics can be recommended at an early stage of treatment [11]. Highly-sensitive RAS diagnostics before treatment start are supported by data from the FIRE-4 study, where liquid biopsies detected additional RAS alterations in 20 % of tumors classified as RAS wild-type based on tissue biopsies [17]. Accordingly, there is a clear necessity to discuss early access to advanced molecular diagnostics for mCRC patients.

The optimal panel size to address the question of hyperselection remains a subject of debate. Thus, it remains to be clarified whether a comprehensive approach with a panel size of over 500 genes is necessary to capture hyperselection analogous to PRESSING-1/-2/-3, or whether a smaller selection of alterations could also suffice.

Smaller panels evaluating gene alterations with a prevalence of at least 2 % (PIK3CA mutation, PTEN mutation, PTEN loss, ERBB2 mutation, ERBB2 amplification), are presented in Supplements 4 and 5. Even with these smaller panels, the previously described benefit of anti-EGFR therapy, can be observed in hyperselected patients independent of tumor sidedness.

Table 4
Outcome analysis within the PRESSING-3 panel based on the Standard Selection^{NGS} Cohort (N = 171).

		N	Overall survival					Progression-free survival						Best response			
			Cet*		Bev**		HR	P	Cet*		Bev**		HR	P	Cet*	Bev**	OR	P
			N	Median (months)	N	Median (months)			N	Median (months)	N	Median (months)			ORR (%)			
1	A	171	77	36.5	94	27.6	0.79 [0.57–1.11]	0.17	77	10.7	94	11.3	1.06 [0.78–1.45]	0.72	64 (83)	65 (69)	2.20 [0.05–4.60]	0.049
	B	110	46	38.5	64	27.5	0.68 [0.44–1.05]	0.08	46	11.8	64	11.3	0.99 [0.67–1.46]	0.95	39 (85)	47 (73)	2.02 [0.76–5.36]	0.17
	C	61	31	31.0	30	28.0	1.04 [0.61–1.79]	0.89	31	10.6	30	10.2	1.19 [0.70–2.04]	0.53	25 (81)	18 (60)	2.78 [0.88–8.79]	0.10
2	A	140	65	38.6	75	29.9	0.82 [0.56–1.19]	0.29	65	11.7	75	11.7	1.09 [0.77–1.54]	0.63	54 (83)	53 (71)	2.04 [0.90–4.61]	0.11
	B	92	39	42.7	53	29.9	0.70 [0.43–1.12]	0.14	39	10.7	53	11.7	1.02 [0.67–1.57]	0.93	33 (85)	39 (74)	1.97 [0.68–5.72]	0.31
	C	48	26	38.1	22	28.0	1.10 [0.59–2.07]	0.76	26	11.7	22	11.5	1.22 [0.66–2.26]	0.52	21 (81)	14 (64)	2.40 [0.65–8.86]	0.21
3	A	31	12	18.8	19	23.6	0.76 [0.35–1.64]	0.48	12	7.9	19	9.0	1.08 [0.51–2.29]	0.84	10 (83)	12 (63)	2.92 [.49–17.32]	0.42
	B	18	7	27.1	11	21.1	0.58 [0.21–1.62]	0.30	7	12.8	11	9.9	0.82 [0.31–2.17]	0.68	6 (86)	8 (73)	2.25 [.19–27.37]	1.00
	C	13	5	18.3	8	25.8	1.05 [0.31–3.55]	0.93	5	7.2	8	7.1	1.34 [0.41–4.44]	0.63	4 (80)	1 (13)	4.00 [.30–53.47]	0.57

1 = Total cohort, 2 = Left-sided mCRC,
A Total Cohort, B Hyperselection, C Gene Altered;
*FOLFIRI+cetuximab; **FOLFIRI+bevacizumab

At present time, combination of first-line chemotherapy with anti-EGFR directed agents is only recommended in patients with left-sided primary tumors [1,18,2–4]. The present analyses support the concept that molecular hyperselection may refine treatment selection beyond primary sidedness. They, however, suggest that molecular hyperselection could replace sidedness-based stratification. In our analysis, hyperselected patients had a significant benefit from anti-EGFR therapy, regardless of tumor sidedness. By contrast, patients with gene-altered tumors showed no survival advantage with either anti-EGFR or anti-VEGF therapies, irrespective of sidedness. The present data therefore infers that molecular characterization may play a pivotal role as a decision tool and may provide a more precise and mechanistic basis for treatment decisions in first-line therapy. However, the sample size for right-sided tumors was limited, leading to wide confidence intervals and statistical unvertainty in this subgroup. These limitations lightlight the need for cautious interpretation of the results and underline the necessity for further validations in prospective studies.

The main limitation of the present investigation is its retrospective nature. Specifically, the small sample size in some subgroup analyses may preclude final conclusions. However, it needs to be pointed out that the results presented in this analysis are in accordance with the published evidence [12,13,5] and specifically are supported by findings of the PARADIGM trial [14]. In fact, the latter trial is the only other study that compared an anti-EGFR and anti-VEGF-based first-line therapy and evaluated the effect of hyperselection. In this context, pooled analyses integrating data from multiple studies will be of key importance to further clarify the role of the molecular hyperselection in treatment decision-making for mCRC patients.

5. Conclusion

The results support the development of NGS-based hyperselection as an effective tool that outperforms standard selection in the prediction of treatment efficacy.

The data demonstrate that hyperselected patients significantly benefit from anti-EGFR therapies regardless of tumor sidedness. In genetically altered tumors, however, survival is inferior and targeted therapy with anti-VEGF or anti-EGFR agents induced comparable outcome.

Molecular hyperselection enables a more precise patient stratification by upfront elimination of resistance mechanisms. While our finding suggest that hyperselection may refine treatment decision-making beyond primary tumor sidedness, limitations such as small sample sizes in right-sided tumors associated with wide confidence intervals necessitate cautious interpretation. Prospective studies are certainly necessary to validate these findings and establish hyperselection as the new standard.

Data/Supplement

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Declarations

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

Written informed consent for the FIRE-3 trial participation and for publication of trial results was obtained from each patient before any study specific procedure. The protocol and informed consent forms were approved by the ethic committee of the Medical Faculty of the LudwigMaximilians-University (reference number: 370–06). Informed consent was obtained from all subjects prior to participating in the study. The study was conducted in accordance with the Declaration of Helsinki.

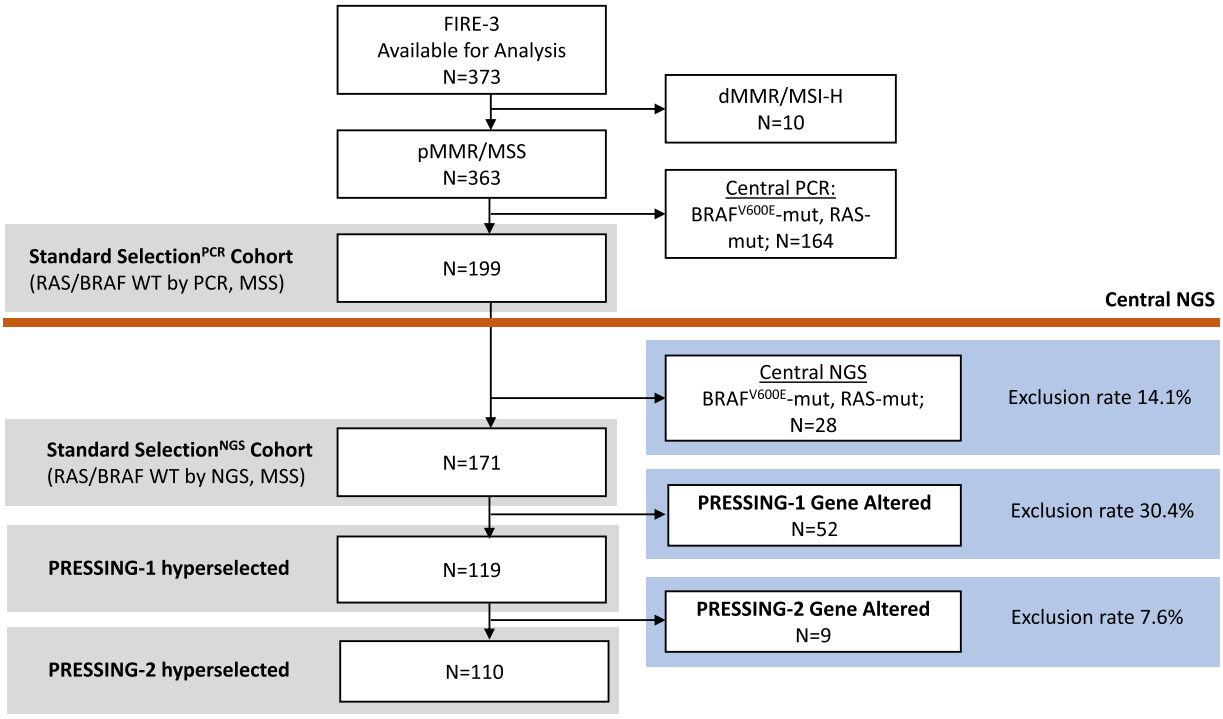

Fig. 1. Consort diagram of analyzed cohort.

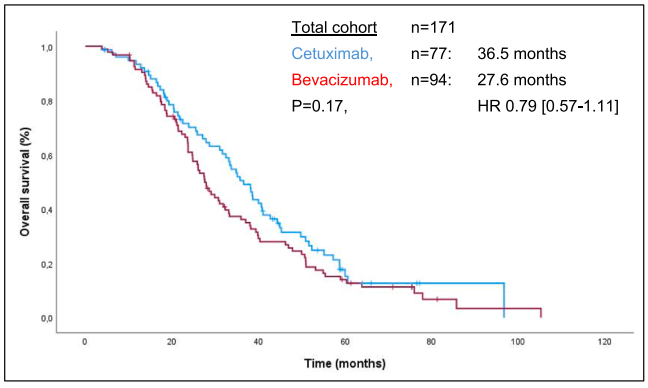

Fig. 2. Kaplan-Meier curve of PRESSING-3 based on Standard Selection^{NGS} Cohort (N = 171).

Data availability

Available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Seipelt Gernot: Writing – review & editing. **Stintzing Sebastian:** Writing – review & editing. **Kahl Christoph:** Writing – review & editing. **Weiss Lena:** Writing – original draft, Visualization, Formal analysis. **Stahler Arndt:** Writing – review & editing. **Kullmann Frank:** Writing – review & editing. **Holch Julian Walter:** Writing – review & editing. **Fischer von Weikersthal Ludwig:** Writing – review & editing. **Heinrich Kathrin:** Writing – review & editing. **Westphalen C. Benedikt:** Writing – review & editing. **Kiani Alexander:** Writing – review & editing. **Jung Andreas:** Writing – review & editing. **Alig Annabel:** Writing – review & editing. **Decker Thomas:** Writing – review & editing. **Heinemann Volker:** Writing – original draft, Supervision, Methodology, Conceptualization. **Al-Batran Salah-Edin:** Writing – review & editing. **Modest Dominik Paul:** Writing – review & editing. **Vehling-Kaiser Ursula:** Writing – review & editing. **Lerchenmüller Christian A.:** Writing – review & editing. **Heintges Tobias:** Writing – review & editing.

Declaration of Competing Interest

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

Lena Weiss: Honoraria: Roche, Servier; Travel Expenses: Merck KGaA, Amgen; Advisory Role: Roche.

Sebastian Stintzing: Consulting or Advisory Role: Merck KGaA, Roche, Amgen, Pierre Fabre, MSD, AstraZeneca, Servier, GalaxoSmithKline, Termuo, Nordic Bioscience, Seagen, Daiichi Sankyo Europe gmbH, CV6 Therapeutics, Isofol Medical; Travel Expenses: Merck KGaA, Roche, Sanofi, Bayer, Sirtex Medical, Amgen, Lilly, Takeda, Pierre Fabre, AstraZeneca Honoraria: Merck KGaA, Roche, Amgen, ervier, MSD, Pfizer, Pierre Fabre, BMS, Nordic Bioscience, AstraZeneca, Daiichi Sankyo Europe GmbH.

Arndt Stahler: Honoraria: Takeda; Merck KGaA, Amgen, Daiichi Sankyo Europe GmbH, MSD; Travel expenses: Amgen, Roche, Lilly, Pfizer, Merck KGaA; Consulting or Advisory Role: Takeda.

C. Benedikt Westphalen: has received honoraria from Amgen, Bayer, BMS, Chugai, Celgene, Falk, GSK, Janssen, Ipsen, MSD, Merck, Roche, Servier, SIRTeX, Taiho. Served on advisory boards for Bayer, BMS, Celgene, Incyte, Janssen, Lily, MSD, Servier, Shire/Baxalta, Rafael Pharmaceuticals, RedHill, Roche. Has received travel support by Bayer, Celgene, Janssen, MSD, RedHill, Roche, Servier, Taiho and research grants (institutional) by Roche and Deutsche Krebshilfe (institutional). Serves as faculty for European Society of Medical Oncology (ESMO), Deutsche Krebshilfe (DKH) and Arbeitsgemeinschaft internistische Onkologie (AIO). Is a member of the EU Commission expert group:

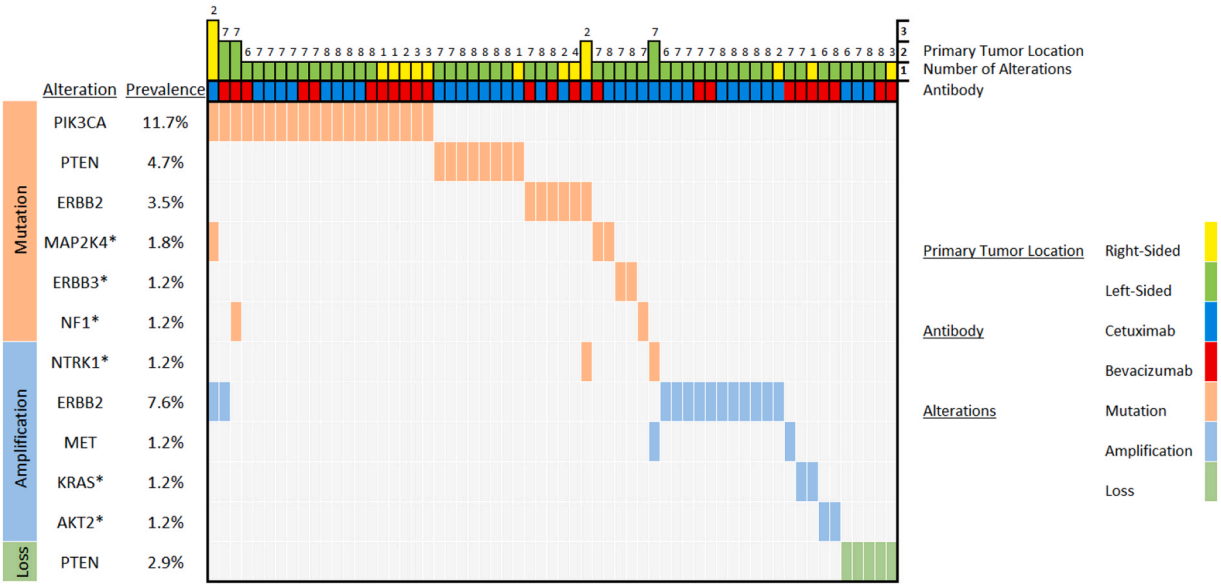

Fig. 3. Oncoprint diagram of detected alterations within the PRESSING panel performed in 171 patients (Standard Selection^{NGS} Cohort). Each row represents a genetic alteration. Alterations with * are included in PRESSING 2 and 3. Orange: mutations; Blue: amplifications; Green: loss; Yellow: Right-sided mCRC; Green: Right-sided mCRC. Antibody: Blue: Cetuximab, Red: Bevacizumab. Each box corresponds to one patient. Primary Tumor Location: 1:Caecum, 2:C. ascendens, 3:Right Flexure, 4:C. transversum, 5: Left Flexure, 6: C. descendens, 7: S. sigmoideum, 8: Rectum.

Mission Board for cancer. Is a member of the BMBF expert group: Forum Zukunftsstrategie. Is a member of the BMBF steering committee: Strategiekreis Dekade gegen Krebs

Thomas Decker: reports advisory role for Novartis, Lilly, Astra Zeneca.

Kathrin Heinrich: Consulting or Advisory Role: Amgen, Servier, MSD (Institutional), Merck, Janssen; Travel Expenses: Amgen, Servier, Merck KGaA; Honoraria: Amgen, BMS, Merck, MSD, Roche, Taiho, Servier, streamedup!

Julian Holch: served on advisory board for Merck, Roche and Servier, has received travel support from Novartis, Merck and Servier.

Annabel Alig: Honoraria: MSD, Servier, Merck KgaA, MSD, Pfizer, Pierre-Fabre, Roche, AMGEN, BMS; Travel Expenses: Nordic, Servier, Merck KgaA, MSD, Pfizer, Pierre-Fabre, Roche, AMGEN, Daiichi Sankyo; Advisory Role: Beigene.

Dominik Paul Modest: Consulting or Advisor Role: Merck, Amgen, Servier, Pierre Fabre, Lilly, Cor2Ed, Onkowissen, Regeneron, GalaxoSmithKline, Takeda, Incyte; Travel Expenses: Amgen, Merck, Servier; Honoraria: Merck, Amgen, Servier, BMS, Taiho, Onkowissen, Pierre Fabre, AstraZeneca, Lilly, Takeda, GalaxoSmithKline, Seagen, Cor2Ed, Boehringer Ingelheim, Regeneron, Bicara Therapeutics, Rottapharm Biotech, IKF Klinische Krebsforschung, 21up

Volker Heinemann: reports receiving fees for talks and advisory board roles from Merck, Amgen, Roche, Sanofi, Servier, Pfizer, Pierre-Fabre, AstraZeneca, BMS, MSD, Novartis, Terumo, Oncosil, NORDIC, Seagen, GSK and for receiving research funding from Merck, Amgen, Roche, Sanofi, Boehringer-Ingelheim, SIRTEX, Servier.

All remaining authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2025.115399.

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