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Original Research

Negative hyperselection of elderly patients with *RAS* and *BRAF* wild-type metastatic colorectal cancer receiving initial panitumumab plus FOLFOX or 5-FU/LV

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ABSTRACT

Background: Upfront anti-EGFR therapy represents the standard of care for patients with left-sided, MSS/pMMR, *RAS* and *BRAF* wild-type mCRC. Molecular 'hyperselection' may optimize EGFR inhibition by detecting additional resistance alterations.

Materials and methods: We used comprehensive genomic profiling on archival samples of elderly patients enrolled in the PANDA trial to detect: HER2 amplification/mutations; MET amplification; NTRK/ROS1/ALK/RET rearrangements; PIK3CA exon 20 mutations; PTEN alterations; AKT1 mutations; MAP2K1 mutations. We defined 'Gene Altered' (GA) patients whose tumour harboured at least one alteration, and 'Hyperselected' (HS) those without. Survival and tumour response outcomes were correlated to hyperselection status alone or combined with primary tumour sidedness or treatment arm.

Results: Genomic alterations were detected in 41/147 patients (27.9%). PFS, OS and ORR were inferior in GA versus HS (median PFS: 7.6 versus 12.8 months, HR = 2.08, 95% CI: 1.43–3.03, $p < 0.001$; median OS: 20.0 versus 29.5 months, HR = 1.82, 95% CI:1.23–2.69, $p = 0.002$; ORR: 51% versus 71%; OR = 0.43, 95% CI: 0.21–0.91, $p = 0.02$). In the multivariable models, the impact of hyperselection on PFS and OS was confirmed. Lower ORR was observed with 5-FU/LV/panitumumab in GA (40% versus 62%), but not in HS (70% versus 72%). GA was associated with worse survival and response regardless of primary tumour sidedness, whereas in the HS subgroup, right-and left sided tumours had similar outcomes.

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Conclusions: Molecular hyperselection and comprehensive genomic profiling have a potential usefulness in elderly patients with *RAS/BRAF* wild-type, pMMR/MSS mCRC, eligible for upfront EGFR inhibition.

1. Introduction

In patients with metastatic colorectal cancer (mCRC), the efficacy of anti-EGFR-based therapies relies on multiple crucial factors, such as molecular profile and primary tumour sidedness. It is well established that *RAS* and BRAF V600E mutations confer resistance to anti-EGFR agents, with right primary tumour sidedness and microsatellite instability-high (MSI-H) status being additional negative predictive factors [1–4]. However, primary resistance to EGFR inhibition still represents a relevant issue and patient selection may be improved by detecting other uncommon genomic drivers in *RAS* and *BRAF* wild-type tumours.

We previously developed the PRESSING panel, that groups together the following rare resistance alterations: *HER2* amplification/activating mutations; *MET* amplification; *NTRK/ROS1/ALK/RET* rearrangements; *PIK3CA* exon 20 mutations; *PTEN* inactivating mutations; *AKT1* mutations [5]. In patients with *RAS* and *BRAF* wild-type disease, the negative prognostic and potentially predictive role of the PRESSING panel alterations detected in tumour tissue has been firstly shown by a case-control prospective study in the chemorefractory setting, and subsequently confirmed by a pre-specified exploratory analysis of the Valentino trial evaluating upfront mFOLFOX/panitumumab followed by two different panitumumab-based maintenance strategies [5,6]. These studies led to the definition of the paradigm of 'negative hyper-selection' to further maximise the therapeutic index of anti-EGFR-based regimens, despite still requiring further validation in additional or larger datasets. More recently, the potentially predictive role of molecular hyper-selection has been evaluated in a translational analysis of patients with *RAS* wild-type mCRC enroled in the phase 3 PARADIGM trial and randomised to chemotherapy plus either panitumumab or bevacizumab [7,8]. In this study, the molecular profiling of baseline ctDNA confirmed the clinical usefulness of genomic-based hyperselection for upfront anti-EGFR-based doublets, regardless of primary tumour location [8].

We have previously reported the main results of the randomised, phase 2 PANDA trial, that randomised elderly patients with *RAS* and *BRAF* wild-type mCRC to upfront panitumumab plus mFOLFOX or 5-FU/LV [9]. In the primary study report, we showed that both treatment regimens are reasonable options based on the superimposable progression-free survival (PFS) and overall survival (OS), with panitumumab plus mFOLFOX being associated with higher overall response rate (ORR) and panitumumab plus 5-FU/LV being characterised by a more favourable safety profile.

With the aim of further investigating the external consistency of molecular hyperselection in the first-line setting, we conducted a translational analysis of the PANDA trial on the prognostic role of a slightly modified version of the PRESSING panel, both in the overall biomarker-evaluable population and according to sidedness or treatment arm.

2. Materials and methods

2.1. Patient population

The PANDA trial (NCT02904031) was an open-label, randomised, non-comparative phase II trial that included 183 previously untreated elderly patients with unresectable *RAS/BRAF* wild-type mCRC. Patients were randomised 1:1 to mFOLFOX/panitumumab (arm A, n = 91) or 5-FU/LV/panitumumab (arm B, n = 92) for up to 12 cycles, followed by maintenance with panitumumab alone in both arms [9].

Main eligibility criteria were: age of 70–75 years with an Eastern Cooperative Oncology Group (ECOG) performance status 1 or 2, OR age > 75 years with an ECOG PS of 0 or 1; measurable disease according to RECIST v1.1; no prior systemic chemotherapy for metastatic disease (previous adjuvant fluoropyrimidine monotherapy was allowed if at least 6 months had elapsed between the end of treatment and disease relapse); availability of tumour tissue sample (from primary and/or metastatic site on formalin-fixed paraffin-embedded [FFPE]) obtained prior to enrolment; *RAS* and *BRAF* wild-type status, centrally confirmed prior to enrolment and performed by MALDI-TOF MassArray (Sequenom) at Veneto Institute of Oncology IRCCS, Padua, and including the sequencing of codons 12, 13, 59, 61, 117, 146 of both *KRAS* and *NRAS*, as well as codon 600 of *BRAF*.

2.2. Molecular analyses

Molecular profiling was performed in baseline FFPE tumour samples obtained prior to the administration of the study treatment. Tissue samples were submitted to comprehensive genomic profiling by means of Foundation One CDx (F1CDx) assay. Therefore, *RAS* and *BRAF* mutational status was reassessed with deeper coverage and higher sensitivity compared to the initial assessment with Sequenom assay, and MSI status was re-evaluated by means of MSI sensor in addition to the locally performed standard IHC and/or multiplex PCR, if available.

The slightly modified version of the PRESSING panel adopted in this study implemented the following alterations: *MAP2K1* (MEK1) mutations, because of the increasing and strong preclinical and clinical evidence on their role as drivers of primary resistance; PTEN loss, added to the inactivating mutations with the overall definition of *PTEN* alterations. Patients with tumours bearing at least one 'modified PRESSING panel' alteration were included in the 'Gene Altered' subgroup, whereas those with no alterations were classified as 'Hyper-selected'.

2.3. Statistical analysis

The χ^2 test, the Fisher exact test, or the Mann-Whitney *U* test were used, as appropriate, to evaluate the association between patients baseline characteristics and modified PRESSING panel status. PFS was defined as the time interval from randomisation to progressive disease (PD) or death by any cause, whichever occurred first (censored at last follow-up for patients alive and without PD). Overall survival (OS) was the time interval from randomisation to death from any cause (censored at the last follow-up for patients alive). Overall response rate (ORR) was defined as the proportion of patients achieving a complete (CR) or partial response (PR) relative to the total of subjects. Survival analyses were performed using the Kaplan-Meier method and the Cox proportional-hazards model. Variables with a p value < 0.1 at univariate analysis were entered into the multivariate models. An interaction term was included in the statistical models when subgroup analyses were performed. Median follow-up was calculated by the reverse Kaplan-Meier approach. The χ^2 test or Fisher exact test was used, as appropriate, to assess the association between sidedness and/or hyper-selection status with ORR. All statistical tests were two-sided, and p-values of 0.05 or less were deemed significant. ORs with 95% CIs were estimated with a logistic regression model. The analyses were carried out using SAS (version 9.4).

3. Results

3.1. Study population

The patients' flow is depicted in Supplementary Fig. 1. Briefly, out of

183 patients who received at least one study treatment administration, 23 were excluded after either quality check failure or indeterminate results. Out of 160 cases with a valid Foundation One CDx result, 13 were excluded due to the detection of *RAS/BRAF* mutations (eight cases) or microsatellite instability (five cases). The final biomarker-evaluable population included 147 (80.3%) patients with *RAS/BRAF* wild-type, pMMR/MSS mCRC. Overall, 41 (27.9%) and 106 (72.1%) out of 147 patients were included in the 'Gene Altered' and 'Hyperselected' subgroups, respectively.

Table 1 shows the main patients and disease characteristics, overall and according to hyperselection status. The baseline variables including primary tumour sidedness and the treatment arm were not significantly different in the two subgroups, except for a significantly higher frequency of liver-limited disease and single metastatic site in the Hyperselected subgroup.

Table 1
Patients and disease baseline characteristics, in the overall biomarker-evaluable population and according to hyperselection status.

	Overall N = 147	GA n = 41	HS n = 106	p-value
Age				0.5484[a]
Median (IQR), years	77 (74–79)	77.0 (74–80)	77.0 (74–79)	
Age subgroups (%)				0.6856[b]
≤75 yrs	54 (36.7)	14 (34.1)	40 (37.7)	
>75 yrs	93 (63.3)	27 (65.9)	66 (62.3)	
Sex, n (%)				0.2178[b]
Female	53 (36.1)	18 (43.9)	35 (33.0)	
Male	94 (63.9)	23 (56.1)	71 (67.0)	
ECOG PS (%)				0.2164[b]
0	74 (50.3)	24 (58.5)	50 (47.2)	
1–2	73 (49.7)	17 (41.5)	56 (52.8)	
G8 Screening Score (%)				0.8485[b]
≤14	95 (64.6)	26 (63.4)	69 (65.1)	
>14	52 (35.4)	15 (36.6)	37 (34.9)	
Primary Tumour Sidedness (%)				0.4563[b]
Right colon	30 (20.4)	10 (24.4)	20 (18.9)	
Left colon	117 (79.6)	31 (75.6)	86 (81.1)	
Liver-limited Disease (%)				0.0034[c]
Yes	40 (27.2)	4 (9.8)	36 (34.0)	
No	107 (72.8)	37 (90.2)	70 (66.0)	
Time to Metastases (%)				0.4204[b]
Synchronous	104 (70.7)	31 (75.6)	73 (68.9)	
Metachronous	43 (29.3)	10 (24.4)	33 (31.1)	
Primary tumour resection (%)				0.6007[b]
No	42 (28.6)	13 (31.7)	29 (27.4)	
Yes	105 (71.4)	28 (68.3)	77 (72.6)	
Prior Adjuvant Chemotherapy (%)				0.9034[b]
No	121 (82.3)	34 (82.9)	87 (82.1)	
Yes	26 (17.7)	7 (17.1)	19 (17.9)	
Number of Metastatic Sites (%)				0.0191[b]
Single	62 (42.2)	11 (26.8)	51 (48.1)	
Multiple	85 (57.8)	30 (73.2)	55 (51.9)	
Treatment arm (%)				0.8945[b]
Arm A, mFOLFOX-pan	74 (50.3)	21 (51.2)	53 (50.0)	
Arm B, FU/LV-pan	73 (49.7)	20 (48.8)	53 (50.0)	
Source of sequencing (%)				0.8697[b]
Primary tumour	128 (87.1)	36 (87.8)	92 (86.8)	
Metastatic site	19 (12.9)	5 (12.2)	14 (13.2)	

ECOG PS, Eastern Cooperative Oncology Group Performance Status; GA, gene altered; HS, hyperselected; IQR, interquartile range; Pan, panitumumab.
[a] Kruskal-Wallis p-value.
[b] Chi-Square p-value.
[c] Fisher's exact test.

The specific molecular alterations detected in the Gene-Altered subgroup are illustrated in the heatmap in Fig. 1 and their association with individual key features is summarised in Supplementary Table 1. Overall, 13 samples (8.8%) had *HER2* amplification, three (2%) had *MET* amplification and one (0.7%) a gene rearrangement involving *RET*. *HER2* mutations were found in six (4%) samples, of which one had a concomitant *HER2* amplification. Overall, *PIK3CA* exon 20/*PTEN/AKT1* alterations were found in 16 (10.9%) samples and were associated with other alterations in four cases. Finally, *MAP2K1* (*MEK1*) alterations were found in four (2.7%) samples.

At the time of data cut-off (15th December 2022), in the biomarker-evaluable population the median follow-up was 51.7 months (IQR 46.1–56.4) and the median PFS and OS were 10.5 (95% CI: 9.1–11.7) and 24 months (95% CI: 21.8–30.1), thus superimposable with results in the intention-to-treat population (Supplementary Fig. 2).

3.2. Survival outcomes according to hyperselection and primary tumour sidedness

PFS was inferior in Gene Altered versus Hyperselected subgroup (median PFS: 7.6 versus 12.8 months; hazard ratio [HR], 2.08, 95% CI 1.43–3.03; log-rank $p < 0.001$; Fig. 2A). The same was observed with regard to OS (median OS: 20.0 versus 29.5 months; 2-year OS: 34% versus 57%; HR 1.82, 95% CI 1.23–2.69; $p = 0.002$; Fig. 2B). No significant interaction was observed between hyperselection status and treatment arm in terms of both PFS ($p = 0.29$; Fig. 2C) and OS ($p = 0.78$; Fig. 2D). In detail, in the Hyperselected subgroup the median PFS and OS were 13.2 and 30.0 months in arm A versus 12.4 and 29.5 months in arm B, while in the Gene Altered subgroup the median PFS and OS were 6.5 and 20.0 months in arm A versus 7.8 and 21.4 months in arm B, respectively. Table 2 shows the results of univariate and multivariable analyses for PFS and OS. In the multivariable model, molecular hyperselection was independently associated with PFS (HR = 2.1, 95% CI 1.4–3.1; $p < 0.001$) and OS (HR = 1.6, 95% CI 1.1–2.4; $p < 0.001$). Of note, the number of metastatic sites was also associated with both outcomes, whereas baseline G8 score was associated only with OS.

Regarding the combined assessment of hyperselection and primary tumour sidedness, the presence of Gene Altered status was associated with inferior PFS and OS outcomes regardless of primary tumour sidedness. Of note, patients with hyperselected, right-sided primary tumours had similar survival outcomes to those with hyperselected, left-sided disease (Figs. 2E–F).

3.3. Tumour response according to hyperselection and primary tumour sidedness

The ORR according to RECIST v.1.1 was significantly lower in Gene Altered versus Hyperselected subgroups (51% versus 71%; Odds Ratio [OR], 0.43, 95% CI 0.21–0.91; $p = 0.027$; Fig. 3A). Although the interaction between treatment arm and hyperselection status, in terms of tumour response, was not statistically significant (interaction test $p = 0.29$), a numerically lower ORR with FU/LV/panitumumab versus mFOLFOX/panitumumab was observed in the Gene Altered subgroup (40% versus 62%; OR 0.41, 95% CI 0.12–1.44), but not in the Hyperselected one (70% versus 72%; OR 0.91, 95% CI 0.40–2.11), as shown in Fig. 3B.

Regarding the combined assessment of hyperselection and primary tumour sidedness, the presence of Gene Altered status was associated with inferior tumour response regardless of primary tumour sidedness. Of note, patients with hyperselected, right-sided primary tumours had similar responses to those with hyperselected, left-sided disease (Fig. 3C).

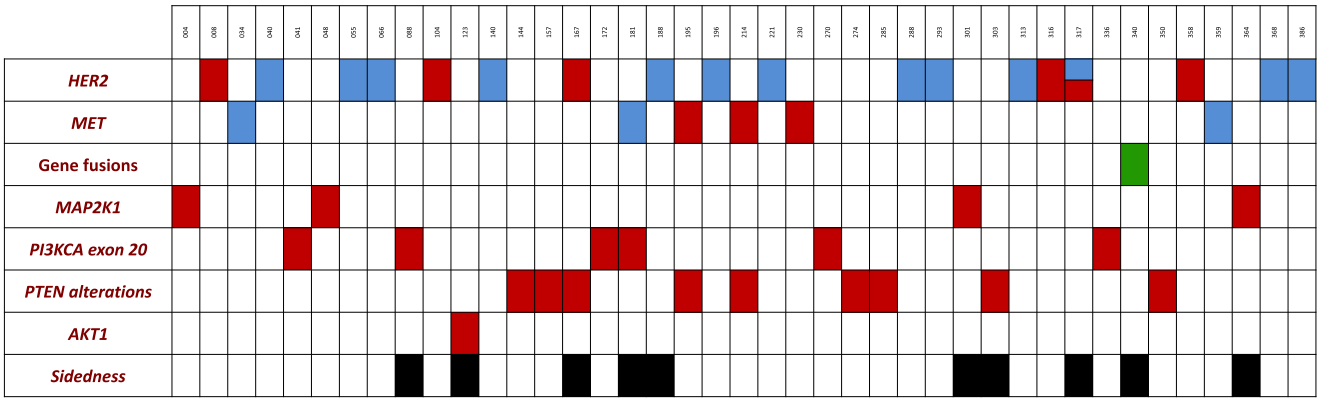

Fig. 1. Heatmap of the specific genomic alterations and primary tumour sidedness in the Gene Altered subgroup. The colour red identifies gene mutations, the colour blue identifies gene amplification and the colour green gene fusions. The colour black identifies right-sided primary tumours.

4. Discussion

In this translational analysis of the biomarker-evaluable subgroup (80.3%) of the PANDA trial, we validated the prognostic role of molecular hyperselection in elderly patients with *RAS* and *BRAF* wild-type mCRC treated with upfront anti-EGFR-based therapy. We confirmed that the presence of resistance alterations is associated with inferior activity and efficacy outcomes. Also, resistance alterations were significantly associated with multiple metastatic sites and thus with potentially more aggressive tumour biology. However, both genomic hyperselection and the number of metastatic sites were strongly associated with outcomes in the multivariable models, thus confirming the independent prognostic role of these molecular or clinical features.

As previously showed by our group, molecular hyperselection may be clinically useful for predicting the outcomes to anti-EGFR therapy, both in the chemorefractory and in the chemo-naïve settings [5,6]. Here we employed tissue-based comprehensive genomic profiling of primary tumours (87%) or metastases (13%) to parallelly detect several alterations associated with anti-EGFR primary resistance, including gene mutations, amplifications and fusions. In line with this research field, a recent translational analysis of the PARADIGM phase 3 trial employed an extensive NGS assay of 119 genes to detect some of these alterations in baseline ctDNA [8]. At date, no evidence favoring either tissue or liquid biopsies exists; in fact, both methods could have several advantages and limitations. Theoretically, liquid biopsy could overcome the limitation of tumour spatial heterogeneity, being able to detect alterations that would have otherwise been missed by tissue-based analyses. Nonetheless, subclonal alterations detected only in ctDNA may not have a role as oncogenic drivers and may not be associated with anti-EGFR resistance [10–14]. Additionally, despite the increased sensitivity of the most modern assays, the use of liquid biopsy may still be limited by false negative results, especially in non-shedding cancers and lymph nodal, lung and/or peritoneal disease. On the other side, given the potential discordance between the genomic profiling of primary tumours and metastases, liquid biopsy may overcome this intrinsic limitation of our analysis conducted mostly on primary tumour tissues.

Of note, the design of the PARADIGM trial allowed to explore the purely predictive role of molecular hyperselection thanks to the availability of an anti-EGFR-free, bevacizumab-based control arm. Similarly to our previous report of the Valentino trial [6], here we could not formally demonstrate the predictive role of hyperselection, since both arms of the PANDA trial were panitumumab-based; still, the strong biological rationale and the increasing amount of consistent evidences collected in this field may suggest a potentially predictive rather than just prognostic impact.

Consistently with data from PARADIGM, gene alterations were associated with worse survival outcomes and tumour response both in right- and left-sided tumours, with the poorest outcomes in the Gene Altered and right-sided subgroup.

Of note, the impact of identifying gene alterations of interest appears particularly relevant in the right-sided subgroup. In fact, patients with hyperselected and right-sided tumours may potentially still derive a clinically meaningful benefit from anti-EGFR-based initial therapy, since their outcomes may be similar to that of hyperselected and left-sided tumours. However, further refinement of their selection may be still needed by means of genomic ultra-selection or positive predictive biomarkers such as gene expression profiles linked to EGFR dependency (e.g. CMS2, AREG/EREG over-expression etc) [15–17].

A unique insight of this study is the potential added value of chemotherapy intensification in the Gene Altered subgroup, although this concept should be interpreted considering the main overall results of the PANDA study. In fact, the main results of the clinical trial showed that the mFOLFOX/panitumumab arm was associated with a higher ORR compared to 5-FU/LV/panitumumab, but this did not translate into an improvement in survival outcomes [9]. Similarly, in this analysis monochemotherapy plus panitumumab was associated with lower ORR in the Gene Altered subgroup, without significant differences in terms of PFS and OS compared to mFOLFOX/panitumumab arm. Of note, the lack of a statistically significant interaction test in terms of ORR may be at least partly related to the limited sample size.

Initial treatment of elderly patients with mCRC should be carefully chosen based on the risk of toxicities and the overall limited life expectancy, related to the limited feasibility of upfront aggressive combinations and post-progression sequential strategies. In line with this, 2-year OS was 34% in the Gene Altered versus 57% in the Hyperselected group, as compared to the 50% and 70% estimates previously reported in our Valentino first-line trial, that had enroled patients with *RAS* wild-type mCRC without a lower age limit [6]. In this context, genomic hyperselection of elderly patients with *RAS* and *BRAF* wild-type mCRC may be important, since patients with hyperselected disease may benefit from 5-FU/LV plus panitumumab, whereas those with Gene Alterations may be offered with alternative treatment options such as fluoropyrimidine monotherapy plus bevacizumab or oxaliplatin-based doublets at personalised dose/schedule.

In our opinion, pooled analyses of clinical trials are key to the delivery of the hyperselection paradigm into clinical practice. Furthermore, several molecular alterations of resistance are therapeutic targets and their routine testing is supported by clinical trials with matched therapeutic intervention. For instance, the most frequent alteration, *HER2* amplification, is now being investigated as the selection biomarker in the first-line setting by the MOUNTAINEER-03 phase III trial with tucatinib plus trastuzumab and chemotherapy experimental

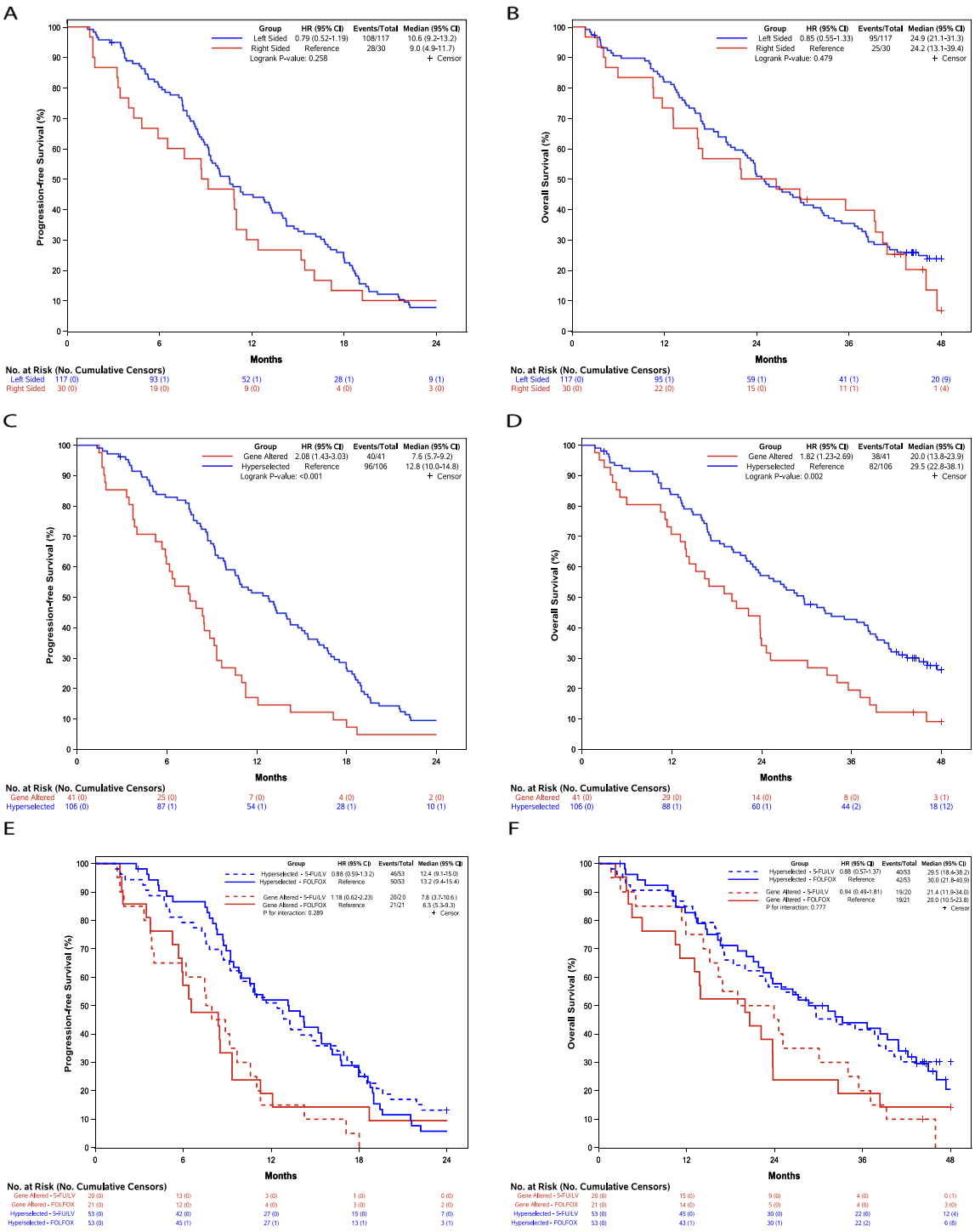

Fig. 2. Kaplan-Meier curves for progression-free survival and overall survival in Left-sided versus Right-sided subgroups (*panels A and B*), according to hyperselection status (*panels C and D*) and according to hyperselection status combined treatment arm (*panels E and F*).

strategy. The results of these trials may reinforce the clinical utility of upfront comprehensive genomic sequencing and, ultimately, may finally provide alternative, tailored first-line options for specific subgroups of patients with actionable drivers despite *RAS* and *BRAF* wild-type status.

This study has several limitations. First, we analysed a biomarker-evaluable population of the PANDA trial, though with baseline characteristics overlapping with those of the study population. Second, the specific alterations included in Gene Altered subgroup are rather uncommon, thus preventing the evaluation of their individual prognostic role and highlighting the need for larger studies or individual patient data meta-analyses to separately investigate each biomarker. Third, this study analysed the role of molecular hyperselection in elderly patients with lower life expectancy due to comorbidities and limited post-progression treatment options. Consequently, OS results should be carefully interpreted and applied to a younger population of patients.

Table 2
Univariate and multivariable analyses for progression-free survival and overall survival.

Characteristics	N (%)	*Progression-free survival*					*Overall survival*				
		Median (months)	Univariate analyses		Multivariable analysis		Median (months)	Univariate analyses		Multivariable analysis	
			HR (95% CI)	p	HR (95% CI)	p		HR (95% CI)	p	HR (95% CI)	p
Age											
≤75 years	54 (37)	9.9	1	0.88	-	-	23.8	1	-	-	-
>75 years	93 (63)	10.6	1.03 (0.72–1.46)		-	-	24.9	1.11 (0.76–1.62)	0.60	-	-
Sex											
Female	53 (36)	11.7	1	0.22	-	-	26.5	1	0.53	-	-
Male	94 (64)	9.3	1.24 (0.87–1.77)		-		23.8	1.13 (0.78–1.64)		-	
ECOG PS											
0	74 (50)	10.9	1	0.30	-	-	26.9	1	0.17	-	-
1–2	73 (50)	9.9	1.19 (0.85–1.67)		-		23.8	1.29 (0.90–1.84)		-	
G8 Screening Score											
≤14	95 (65)	10.0	1	0.361	-	-	23.2	1	**0.016**	1	**0.012**
>14	52 (35)	10.9	0.85 (0.60–1.21)		-		30.7	0.62 (0.42–0.92)		0.61 (0.41–0.90)	
Primary tumour sidedness											
Right	30 (20)	9.0	1	0.26	-	-	24.2	1	0.48	-	-
Left and rectum	117 (80)	10.6	0.79 (0.52–1.19)		-		24.9	0.85 (0.55–1.33)		-	
Liver-limited disease											
No	107 (73)	9.8	1	0.12	-	-	24.9	1	0.37	-	-
Yes	40 (27)	10.9	0.73 (0.50–1.08)		-		24.5	0.83 (0.55–1.25)		-	
Time to Metastases											
Metachronous	43 (29)	13.3	1	0.14	-	-	29.5	1	0.24	-	-
Synchronous	104 (71)	9.2	1.32 (0.91–1.92)		-		23.8	1.27 (0.85–1.90)		-	
Primary tumour resection											
No	42 (29)	10.2	1	0.72	-	-	23.3	1	0.42	-	-
Yes	105 (71)	10.6	0.93 (0.65–1.35)		-		27.2	0.85 (0.57–1.26)		-	
Previous Adjuvant chemotherapy											
No	121 (82)	9.4	1	**0.090**	1	**0.018**	23.8	1	0.34	-	-
Yes	26 (18)	13.8	0.68 (0.43–1.07)		0.57 (0.36–0.91)		32.3	0.79 (0.49–1.28)		-	
Treatment arm											
Arm A, FOLFOX + Pan	74 (50)	10.0	1	0.82	-	-	23.8	1	0.63	-	-
Arm B, 5-FU/LV + Pan	73 (50)	10.6	0.96 (0.69–1.35)		-		25.5	0.91 (0.64–1.31)		-	
Number of metastatic site (s)											
Multiple	85 (58)	8.9	1	**<0.001**	1	**<0.001**	21.8	1	**<0.001**	1	**<0.001**
Single	62 (42)	13.0	0.48 (0.33–0.68)		0.52 (0.36–0.75)		37.1	0.49 (0.33–0.72)		0.52 (0.35–0.77)	
Hyper-selection status											
Hyper-selected	106 (72)	12.8	1	**<0.001**	1	**<0.001**	29.5	1	**0.002**	1	**0.017**
Gene altered	41 (28)	7.6	2.08 (1.43–3.03)		2.10 (1.41–3.11)		20.0	1.82 (1.23–2.69)		1.62 (1.09–2.40)	

ECOG-PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ration; N, number; Pan, panitumumab.
Bold values represent statistically significant p values.

5. Conclusions

In conclusion, this study supports the potential clinical validity of molecular hyperselection on top of primary tumour location, although further validation is needed to establish its role as a driver of first-line treatment choices in patients with pMMR/MSS and *RAS/BRAF* wild-type mCRC. These data also strengthen the potential role of upfront comprehensive genomic profiling, which is still not recommended by the major guidelines for routine use in clinical practice, but may help define treatment choices, identify actionable drivers for targeted therapies and guide enrolment in clinical trials.

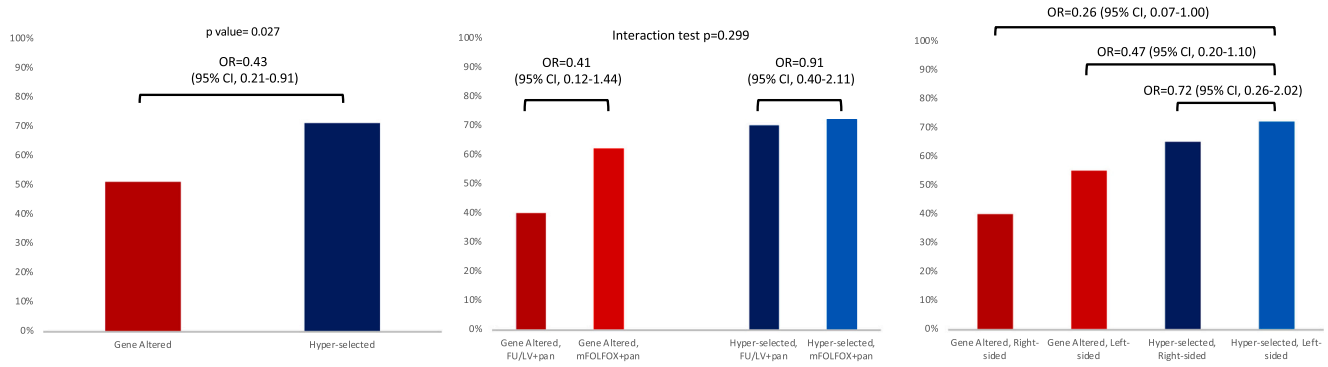

Fig. 3. Overall response rate according to RECISTv1.1 in Gene Altered versus Hyperselected subgroups (*panel A*), according to hyperselection status combined with treatment arm (*panel B*) and according to hyperselection status combined with primary tumour sidedness (*panel C*).

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Data availability statement

The data underlying this article will be made available by the authors upon reasonable request.

CRediT authorship contribution statement

Study concept and design: FP, FB, DR, MF, CC, SL. Acquisition, analysis or interpretation of data: All authors. Drafting of the manuscript: FP, FB, DR, FG, MF, CC, SL. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: DR. Study supervision: FP, FB, DR, MF, CC, SL.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: F. P. received honoraria from Amgen, Bayer, Servier, Merck-Serono, MSD, BMS, Takeda, Astellas, Pierre-Fabre and received research grants for academic studies from Bristol-Myers Squibb, AstraZeneca, Agenus, Incyte, Amgen. F.B. received personal honoraria as invited speaker from Eli-Lilly, MSD, EISAI, BMS; participation in advisory board for Servier, AAA Novartis. D.R. received honoraria from Amgen, MSD and Takeda. V.F. received personal honoraria as invited speaker from Merck Serono, Pierre Fabre, Amgen, MSD, Servier. G.L.F. received personal honoraria as invited speaker from: Servier, Merck Serono; participation in advisory board for Servier, Novartis, Merck Sharp & Dohme. S.D.D received personal honoraria as invited speaker from Roche, Servier, Merck Serono, Pierre Fabre, Amgen, Merck Sharp & Dohme, iNCYTE, Bayer; participation in advisory board for Amgen, Merck Serono, Servier, Bayer. L.A. received research funding (to institution) from Novartis, Merck Serono, Bristol Myers Squibb, Astra Zeneca; personal honoraria as invited speaker from Roche, Bristol Myers Squibb, Servier, AstraZeneca, Amgen, MSD; participation in advisory board for Amgen, Merk Serono, Ely Lilly, AstraZeneca, Incite, Bristol Myers Squibb, Servier, MSD. C.A. received speaker fees from Merck; Honoraria from Merck and Nordic Pharma. M.F. received research funding (to Institution) from QED, Macrophage pharma, Diaceutics, Astellas; personal honoraria as invited speaker from Roche, Eli Lilly, Bristol Myers Squibb, MSD, Pierre Fabre, GlaxoSmithKline, Amgen, Astellas, AstraZeneca. C.A. received speaker fees from Merck; Honoraria from Merck and Nordic Pharma. M.F. received research funding (to Institution) from QED, Macrophage pharma, Diaceutics, Astellas; personal honoraria as invited speaker from Roche, Eli Lilly, Bristol Myers Squibb, MSD, Pierre Fabre, GlaxoSmithKline, Amgen, Astellas, AstraZeneca. C.C. received research funding from Merck, Bayer, Servier; Honoraria from Amgen, Bayer, Merck, MSD, Pierre Fabre, Roche and Servier; Consulting or advisory role: Amgen, Bayer, MSD, Nordic Pharma, Roche. S.L. received research funding (to Institution) from Amgen,Merck Serono, Bayer, Roche, Eli Lilly, AstraZeneca, Bristol Myers Squibb; personal honoraria as invited speaker from Roche, Eli Lilly, Bristol Myers Squibb, Servier, Merck Serono, Pierre Fabre, GlaxoSmithKline, Amgen; participation in advisory board for Amgen, Merck Serono, Eli Lilly, AstraZeneca, Incyte, Daiichi-Sankyo, Bristol Myers Squibb, Servier, Merck Sharp & Dohme, Astellas, Takeda. FG, MCDG, MMG, GBa, GBo, SC, MA, VMP and FN declare no competing interests.

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Appendix A. Supporting material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.113396.

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