BIOMARKERS

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Negative Ultraselection of Patients With *RAS/BRAF* Wild-Type, Microsatellite-Stable Metastatic Colorectal Cancer Receiving Anti–EGFR-Based Therapy

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abstract

PURPOSE Several uncommon genomic alterations beyond *RAS* and BRAFV600E mutations drive primary resistance to anti–epidermal growth factor receptors (EGFRs) in metastatic colorectal cancer (mCRC). Our PRESSING panel (including *PIK3CA* exon 20/*AKT1*/*PTEN* mutations, *ERBB2*/*MET* amplifications, gene fusions, and microsatellite instability-high status) represented a paradigm of negative hyperselection with more precise tailoring of EGFR blockade. However, a modest proportion of hyperselected mCRC has intrinsic resistance potentially driven by even rarer genomic alterations.

MATERIALS AND METHODS A prospective data set at three Italian Academic Hospitals included 650 patients with mCRC with comprehensive genomic profiling by FoundationOne CDx and treated with anti-EGFRs. PRESSING2 panel alterations were selected on the basis of previous clinico-biologic studies and included *NTRKs*, *ERBB3*, *NF1*, *MAP2K1/2/4*, *AKT2* pathogenic mutations; *PTEN*/*NF1* loss; *ERBB3*, *FGFR2*, *IGF1R*, *KRAS*, *ARAF*, and *AKT1-2* amplification; and *EGFR* rearrangements. These were collectively associated with outcomes in patients with hyperselected disease, ie, *RAS*/*BRAF* wild-type, PRESSING-negative, and microsatellite stable.

RESULTS Among 162 hyperselected patients, 24 (15%) had PRESSING2 alterations, which were mutually exclusive except in two samples and were numerically higher in right-sided versus left-sided cancers (28% *v* 13%; $P = .149$). Independently of sidedness and other factors, patients with PRESSING2-positive status had significantly worse progression-free survival and overall survival compared with PRESSING2-negative ones (median progression-free survival 6.4 *v* 12.8 months, adjusted hazard ratio 4.19 [95% CI, 2.58 to 6.79]; median overall survival: 22.6 *v* 49.9 months, adjusted hazard ratio 2.98 [95% CI, 1.49 to 5.96]). The combined analysis of primary tumor sidedness and PRESSING2 status allowed us to better stratify outcomes.

CONCLUSION Negative ultraselection warrants further investigation with the aim of maximizing the benefit of EGFR blockade strategies in patients with *RAS* and *BRAF* wild-type, microsatellite stable mCRC.

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ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Anti–epidermal growth factor receptors (EGFRs) monoclonal antibodies, cetuximab and panitumumab, are guideline-recommended treatments for patients with *RAS* and *BRAF* wild-type metastatic colorectal cancer (mCRC).[1] Moreover, right sidedness of the primary tumor is a predictive factor of worse survival upon treatment with anti-EGFRs,[2] because of the enrichment for genomic mechanism and molecular profiles associated with primary resistance to EGFR inhibition.[3,4] However, EGFR blockade is effective only in a small subset (10%-15%) of patients with mCRC[5] and primary resistance still represents a relevant issue despite improved treatment personalization and exclusion of patients with *RAS* or *BRAF* class 1/2 mutations.[6] We and others contributed to the development of a new paradigm of negative hyperselection, which helps to further refine the proportion of patients eligible for anti-EGFRs. Our PRESSING panel included several uncommon genomic alterations of primary resistance (ie, *ERBB2* amplification/activating mutations, *MET* amplification, *NTRK*/*ROS1*/*ALK*/*RET* rearrangements, and *PIK3CA* exon 20/*PTEN*/*AKT1* mutations) and was associated with worse outcomes independently of primary tumor sidedness.[7,8] Finally, mismatch repair deficient (dMMR)/microsatellite instability (MSI)-

CONTEXT

Key Objective

To assess the prognostic impact of ultrarare alterations involving receptor tyrosine kinases, mitogen-activated protein kinase or PIK3CA pathways on epidermal growth factor receptors (EGFR)-targeted therapies in patients with negatively hyperselected (*RAS*/*BRAF* wild-type, *ERBB2*/*MET* nonamplified, *NTRKs*/*RET*/*ROS1*/*ALK* unrearranged, and *AKT1*/*PTEN*/*PIK3CA* wild-type) and MSS/pMMR metastatic colorectal cancer (mCRC).

Knowledge Generated

The use of comprehensive genomic profiling allowed us to identify sound drivers of primary resistance with very low frequency (negative ultraselection) that were collectively associated with poor outcomes in patients with molecularly hyperselected mCRC receiving anti–EGFR-based regimens, irrespective of primary tumor sidedness.

Relevance

Our data support the use of comprehensive genomic profiling in patients with *RAS* and *BRAF* wild-type mCRC. Rarer alterations in EGFR downstream/parallel pathways warrant further investigation as negative predictive biomarkers of EGFR inhibitors. Several of these alterations may be targetable with novel agents and combinations.

high tumors are hypermutated—thus highly enriched with several of the abovementioned primary resistance mechanisms—and more frequently right-sided, thus explaining inferior outcomes reported with cetuximab-based versus bevacizumab-based initial regimens.[9] On top of this, initial treatment with immune checkpoint inhibitors is recommended in patients with dMMR/MSI-high mCRC because of its superiority to doublet chemotherapy with or without targeted agents. Even if patients with *RAS*/*BRAF* wild-type, PRESSING-negative, and pMMR/MSS (negatively hyperselected) mCRC achieved unprecedented outcomes with an upfront anti–EGFR-based strategy,[7] there is still a modest proportion of patients with limited or absent benefit, which may be driven by even rarer genomic alterations. Therefore, we conducted this large and multicenter PRESSING2 study aimed at investigating in molecularly hyperselected patients with mCRC and treated with an anti–EGFR-based strategy, the clinical impact of negative ultraselection by adding a group of resistance mechanisms with extremely uncommon prevalence, but highly sound biologic rationale as resistance drivers.

MATERIALS AND METHODS

Patient Population

The study flowchart is depicted in the Data Supplement. Patients with *RAS*/*BRAF* wild-type, PRESSING panel–negative (hyperselected; ie, *ERBB2* nonamplified/wild-type, *MET* nonamplified, *NTRK*/*ROS1*/*ALK*/*RET* unrearranged, *PIK3CA* exon 20/*PTEN*/*AKT1* wild-type), MMR proficient (pMMR)/microsatellite stable (MSS), and *POLE* exonuclease domain wild-type mCRC treated with anti-EGFRs in any line were retrospectively retrieved from a common prospective data set established at three Academic Hospitals. Patients were included in two cohorts of PRESSING2-positive versus PRESSING2-negative (ie, ultraselected) tumors. Additional inclusion criteria were as follows: at least one measurable lesion according to RECIST 1.1, at least one postbaseline imaging scan, and written informed consent to study participation. The study was approved by the Fondazione IRCCS Istituto Nazionale dei Tumori di Milano Institutional Review Board (INT 117/15) and was conducted in accordance with the ethical principles for medical research involving human subjects adopted in the Declaration of Helsinki.

Molecular Analyses

PRESSING2 alterations were as follows: pathogenic alterations in genes involved in mitogen-activated protein kinase (MAPK) (ie, *NF1* mutations/loss,[10,11] *ARAF*[12]/*KRAS* amplification,[10] *MAP2K1*/*MAP2K2* mutations, and *MAP2K4* mutations without established inactivating phenotype [ie, S184L] given the cross-talk with the ERK-upstream branch of MAPK[13]), PIK3CA (including *AKT1*/*2* amplification and *AKT2* mutations[14,15] and *PTEN* loss[16]), and EGFR-independent receptor tyrosine kinase (ie, *IGF1R* amplification,[10] *ERBB3* amplification/mutations,[17,18] *FGFR2* amplification,[10,19] and *NTRK* tyrosine kinase [TK] domain mutations[20,21]) signaling pathways and *EGFR* rearrangements involving the TK domain.[22] Pathogenicity of single-nucleotide variants (SNVs) was determined taking advantage of FoundationOne CDx reports.[23] Variants of uncertain significance as assessed by FoundationOne CDx reports were excluded. *FGFR1* amplification and *PIK3CA* exon 9 mutations were not included in the PRESSING2 panel since the role of these alterations in mediating resistance to EGFR inhibition is unclear.[10] A heat map was used to depict genetic alterations.

Statistical Analyses

Association between PRESSING2 alterations and patients and/or disease characteristics was assessed by means of Kruskal-Wallis, χ^2, or Fisher exact tests, as appropriate. Progression-free survival (PFS) was defined as the time from the beginning of the EGFR inhibitor–based treatment to the radiologic evidence of disease progression or death from any cause. Overall

TABLE 1. Baseline Characteristics, Overall and According to the Presence of PRESSING2 Alterations

Characteristic	Study Population (N = 162)	PRESSING2-Positive (n = 24)	PRESSING2-Negative (n = 138)	*P*
Age, years				**.020**
Median	58	68	57	
IQR	50-66	49-71	51-65	
Sex, No. (%)				> .999
Female	65 (40)	10 (42)	55 (40)	
Male	97 (60)	14 (58)	83 (60)	
ECOG PS, No. (%)				> .999
0	127 (78)	19 (79)	108 (78)	
≥ 1	35 (27)	5 (21)	30 (22)	
Primary tumor location, No. (%)				.149
Right colon	18 (11)	5 (21)	13 (9)	
Left colon/rectum	144 (89)	19 (79)	125 (91)	
Primary tumor resection, No. (%)				.538
Yes	137 (85)	19 (79)	118 (86)	
No	25 (15)	5 (21)	20 (14)	
Time to metastases, No. (%)				**.033**
Synchronous	114 (70)	12 (50)	102 (74)	
Metachronous	48 (30)	12 (50)	36 (26)	
Metastatic sites, No. (%)				.069
1	85 (52)	8 (33)	77 (56)	
> 1	77 (48)	16 (67)	61 (44)	
Anti-EGFR line, No. (%)				.098
1	120 (74)	14 (58)	106 (77)	
> 1	42 (26)	10 (42)	32 (23)	
Anti-EGFR monotherapy, No. (%)				.132
No	148 (91)	20 (83)	128 (93)	
Yes	14 (9)	4 (17)	10 (7)	
Anti-EGFR mAb, No. (%)				.438
Panitumumab	96 (59)	12 (50)	84 (61)	
Cetuximab	66 (41)	12 (50)	54 (39)	

Bold entires indicate statistically significant *P* values.

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; IQR, interquartile range; mAb, monoclonal antibody.

survival (OS) was defined as the time from the beginning of the EGFR inhibitor–based treatment to death from any cause or last follow-up. PFS and OS analyses were determined according to the Kaplan-Meier method. The Kaplan-Meier estimator and Cox proportional hazards regression were used for survival analysis using the survival, survminer, and survMisc packages. Follow-up time was estimated using the reverse Kaplan-Meier method. In Cox proportional hazards regression models, all the covariates associated with PFS and OS in the univariable analyses with a *P* value < .10]were included in the multivariable model. *P* values < .05 were considered statistically significant.

RESULTS

Patient Population

A total of 650 samples were profiled by means of FoundationOne CDx. Among them, 291 (45%) samples were *RAS*/*BRAF* wild-type. Among these, PRESSING panel alterations were found in 103 (35%). Overall, 42 samples were MSI-high/*POLE*-mutated (6%); among these, 16 samples harbored *RAS*/BRAF V600E mutations or PRESSING alterations. The

final study population included 162 patients with *RAS*/BRAF V600E wild-type, PRESSING panel–negative, pMMR/MSS, and *POLE* wild-type mCRC. Patient and disease characteristics overall and according to PRESSING2 panel status are reported in Table 1. One hundred-twenty (74%) received anti–EGFR-based therapy as the first-line regimen, 22 (14%) as the second-line regimen, and 20 (12%) as the third-line or later-line treatment. PRESSING2 alterations were detected in 24 (15%) patients. Patients with PRESSING2-positive tumors were older (median age 68 *v* 57 years, $P = .020$) and had more frequently metachronous onset of metastases (50% *v* 26%, $P = .033$) compared with PRESSING2-negative ones. The frequency of PRESSING2 alterations was 28% versus 13% in right-sided versus left-sided mCRC, respectively ($P = .149$). Individual PRESSING2 alterations are specified in the Data Supplement.

Molecular Profiling

The alterations profiles of PRESSING2-negative and PRESSING2-positive tumors are depicted in the heat map in Figure 1. The schematic diagram of the signaling pathways of PRESSING2 alterations is shown in the Graphical Abstract in the Data Supplement. PRESSING2 alterations were mutually exclusive in 22 (92%) samples; one sample harbored both *KRAS* amplification and NF1 E2430* SNV, and one sample both *NF1* loss and MAP2K1 E203K SNV. One hundred-forty seven (91%) were evaluable for tumor mutational burden status. Median tumor mutational burden did not differ significantly according to PRESSING2 status (5.04 *v* 3.78 mutations/Mb for PRESSING2-positive and PRESSING2-negative tumors, respectively, $P = .326$).

Survival Analysis

The median follow-up was 34.1 (interquartile range 23.5-49.3) months. Overall, patients with PRESSING2-positive status had significantly worse PFS and OS compared with PRESSING2-negative ones (median PFS: 6.4 *v* 12.8 months, hazard ratio [HR] 4.25, 95% CI, 2.64 to 6.84, $P < .001$; median OS: 22.6 *v* 49.9 months, HR 2.98, 95% CI, 1.59 to 5.60, $P < .001$; Figs 2A and 2B). In the multivariate model (Table 2), the presence of PRESSING2 alterations had an adjusted HR of 4.19 for PFS and 2.98 for OS, whereas right sidedness had an adjusted HR of 1.41 and 3.51, respectively. One hundred twenty (74%) patients received an anti–EGFR-based therapy upfront. In this first-line cohort

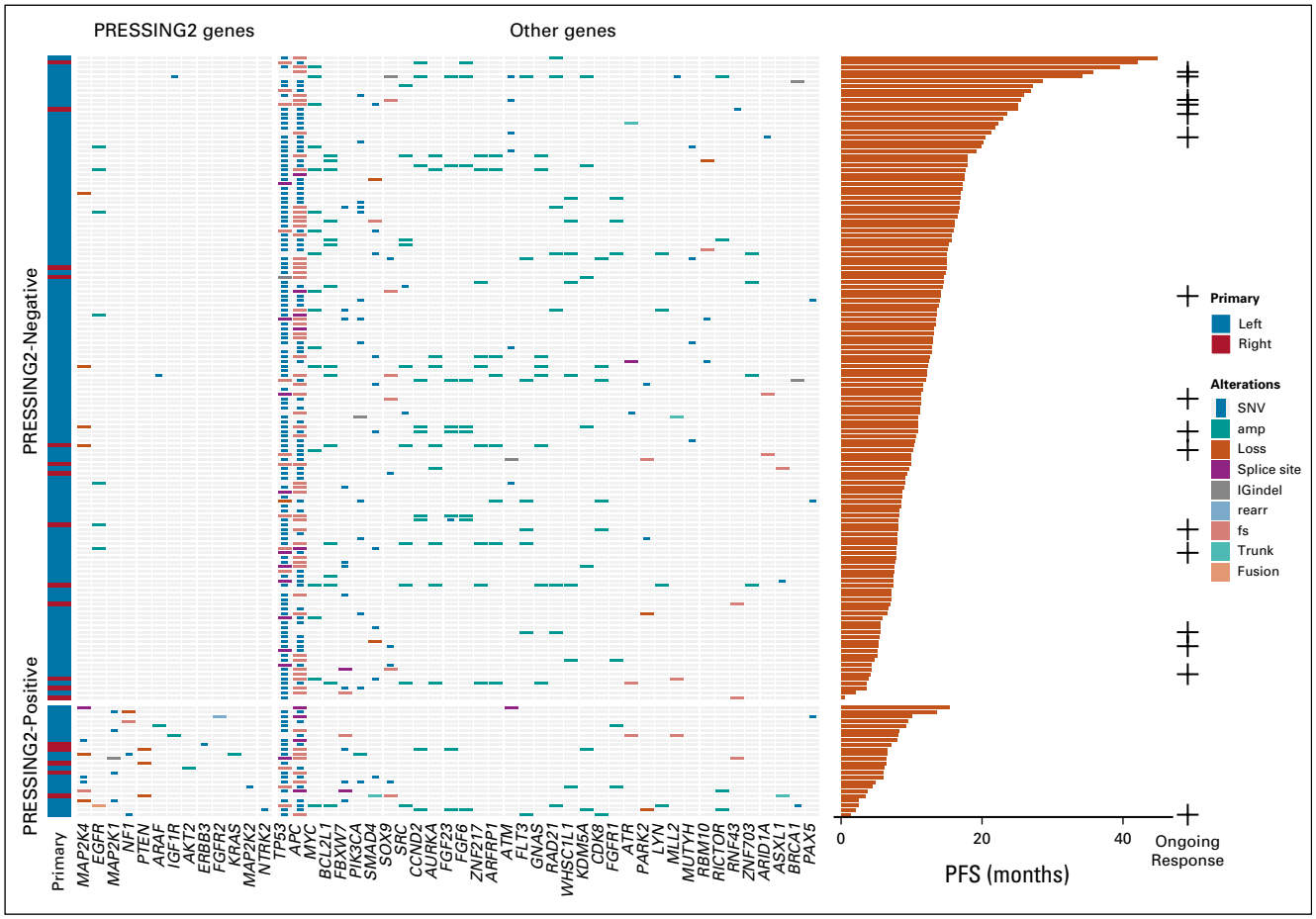

FIG 1. Heat map showing the genomic profiles according to the presence or absence of PRESSING2 alterations. Patients in the two groups were ordered according to PFS. amp, amplification; fs, frameshift; PFS, progression-free survival; rearr, rearrangements; SNV, single-nucleotide variant.

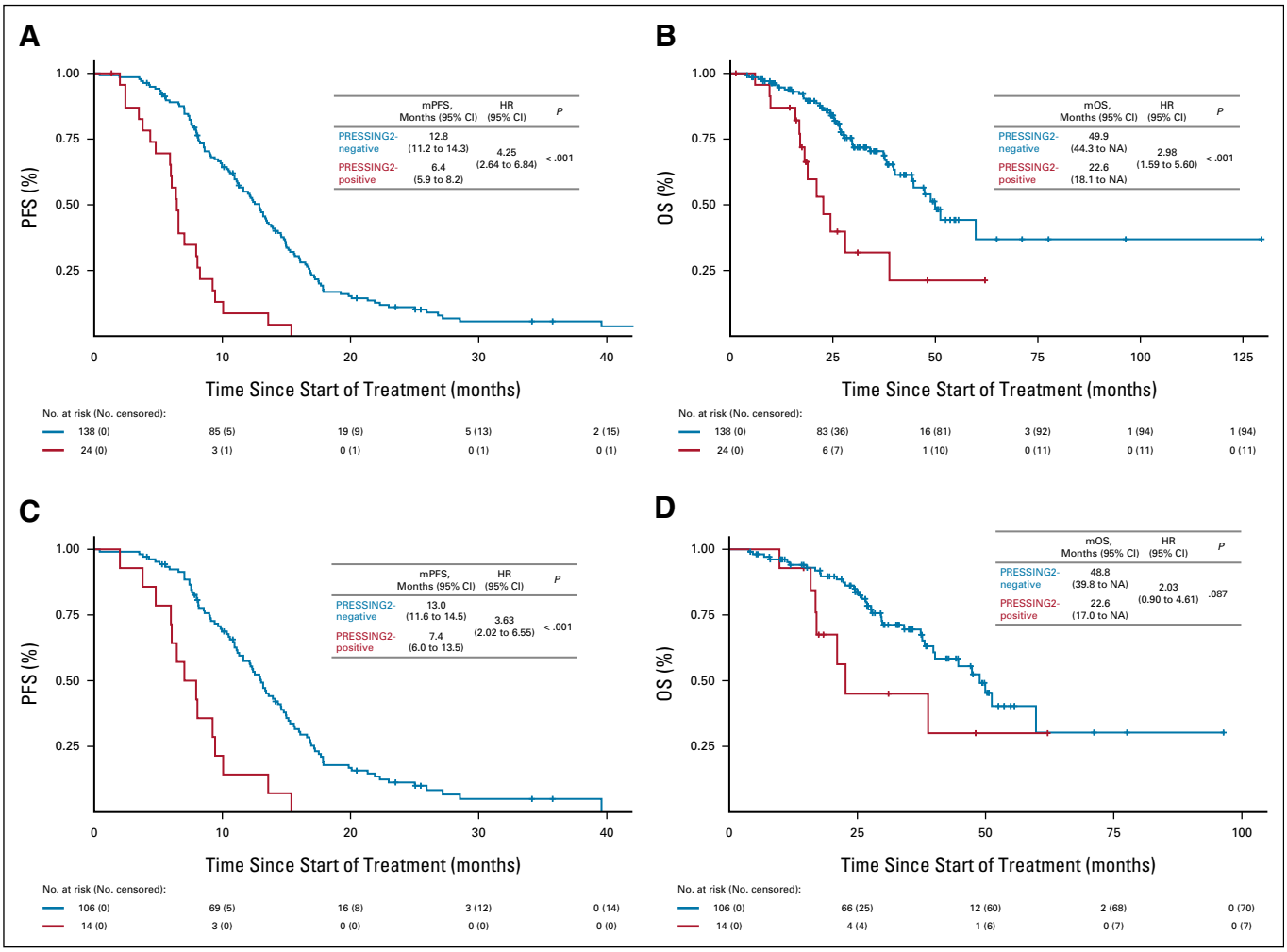

FIG 2. Kaplan-Meier curves of (A and C) PFS and (B and D) OS according to the presence or absence of PRESSING2 alterations in the entire study population and in patients receiving first-line anti–EGFR-based therapy. OS, overall survival; mOS, median overall survival; mPFS, median progression-free survival; NA, not assessable; PFS, progression-free survival.

(Figs 2C and 2D), patients with PRESSING2-positive status had significantly worse PFS compared with PRESSING2-negative ones (median PFS: 7.4 *v* 13.0 months, HR 3.63, 95% CI, 2.02 to 6.55, $P < .001$). Also, OS was nonsignificantly shorter in the PRESSING2-positive group (22.6 *v* 48.8 months, HR 2.03, 95% CI, 0.90 to 4.61, $P = .087$).

Prognostic Analyses According to Sidedness and the PRESSING2 Panel

Overall, the median PFS mismatch repair deficient (dMMR)/microsatellite instability (MSI)-highfor patients with PRESSING2-positive versus PRESSING2-negative tumors was 6.5 and 12.9 months in the left-sided subgroup and 6.3 versus 9.4 months in the right-sided one ($P < .001$; Table 3 and Fig 3A). Consistently, the median OS for patients with PRESSING2-positive versus PRESSING2-negative tumors was 28.0 versus 51.2 months in the left-sided subgroup and 18.1 versus 27.7 months in the right-sided one ($P < .001$; Table 3 and Fig 3B).

Activity of Anti-EGFRs According to the PRESSING2 Panel and Primary Tumor Location

The objective response rate according to RECIST v1.1 was 79% (including 10 [8%] complete responses) in patients with left-sided and PRESSING2-negative mCRC versus 56% (with no complete responses) in patients with PRESSING2-positive and/or right-sided mCRC (OR, 2.87; 95% CI, 1.22 to 6.76; $P = .009$; Data Supplement).

DISCUSSION

EGFR dependency may be defined by the reliance on the interaction between EGFR and its ligands (such as AREG/EREG) for sustaining colorectal cancer growth. It accounts for the clinically meaningful activity of anti-EGFRs in a relatively small subset—up to 15%—of patients with mCRC. Improved patient selection for this targeted treatment has been achieved through the paradigm of negative selection by excluding patients with *RAS*-mutated[24] or BRAF V600E–mutated[25] mCRC; more recently, negative hyperselection consisted of

TABLE 2. Cox Proportional Hazards Regression Models for PFS and OS in the Entire Study Population

	PFS				OS			
	Univariable Models		Multivariable Model		Univariable Models		Multivariable Model	
Characteristic	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (years) 1-year increase	1.01 (0.99 to 1.03)	**.080**	1.01 (0.99 to 1.03)	.063	1.02 (0.99 to 1.05)	**.051**	1.01 (0.98 to 1.04)	.216
Sex		.932				.609		
Female	Ref				Ref			
Male	0.98 (0.70 to 1.37)				1.15 (0.67 to 1.97)			
ECOG PS		.654				**.001**		**.003**
0	Ref				Ref		Ref	
1-2	0.91 (0.60 to 1.37)				2.44 (1.39 to 4.31)		2.55 (1.35 to 4.83)	
Primary tumor location		**.063**		**.132**		**< .001**		**< .001**
Left colon/rectum	Ref		Ref		Ref		Ref	
Right colon	1.60 (0.97 to 2.65)		1.49 (0.88 to 2.52)		3.17 (1.67 to 6.04)		3.51 (1.76 to 7.03)	
Primary tumor resection		.414				**< .001**		**.001**
No	Ref				Ref		Ref	
Yes	0.82 (0.52 to 1.30)				0.28 (0.15 to 0.51)		0.36 (0.19 to 0.68)	
Time to metastases		.487				.347		
Metachronous	Ref				Ref			
Synchronous	0.87 (0.61 to 1.26)				1.33 (0.72 to 2.46)			
Metastatic sites		.667				**.003**		**.025**
1	Ref				Ref		Ref	
> 1	1.07 (0.77 to 1.48)				2.22 (1.30 to 3.80)		1.87 (1.08 to 3.26)	
Anti-EGFR line		**.085**		**.097**		.819		
1	Ref		Ref		Ref			
> 1	1.38 (0.95 to 2.01)		1.38 (0.94 to 2 to 2.04)		1.07 (0.57 to 2.00)			
Anti-EGFR monotherapy		.575				.767		
No	Ref				Ref			
Yes	0.82 (0.42 to 1.61)				1.16 (0.42 to 3.22)			
PRESSING2		**< .001**		**< .001**		**< .001**		**.001**
Negative	Ref		Ref		Ref		Ref	
Positive	4.25 (2.64 to 6.84)		4.19 (2.58 to 6.79)		2.98 (1.59 to 5.60)		2.98 (1.49 to 5.96)	

Bold entires indicate statistically significant *P* values.

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; HR hazard ratio; OS, overall survival; PFS, progression-free survival; ref, reference.

TABLE 3. PFS and OS According to the Combined Evaluation of Primary Tumor Sidedness and PRESSING2 Panel Status

	PFS			OS		
Patient Subgroup	**mPFS, Months (95% CI)**	**HR (95%CI)**	***P***	**mOS, Months (95% CI)**	**HR (95%CI)**	***P***
Left-sided/PRESSING2-negative	12.9 (11.6 to 14.5)	Ref	< .001	51.2 (47.3 to NA)	Ref	< .001
Left-sided/PRESSING2-positive	6.5 (4.7 to 9.4)	3.89 (2.31 to 6.55)		28.0 (18.8 to NA)	2.68 (1.28 to 5.60)	
Right-sided/PRESSING2-negative	9.4 (7.0 to NA)	1.37 (0.76 to 2.46)		27.7 (22.2 to NA)	2.81 (1.30 to 6.08)	
Right-sided/PRESSING2-positive	6.3 (5.9 to NA)	9.14 (3.47 to 24.05)		18.1 (16.8 to NA)	9.90 (3.33 to 29.45)	

Abbreviations: HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; NA, not assessable; ref, reference.

the exclusion of patients with other uncommon oncogenic drivers such as *PIK3CA* exon 20 mutations, ERBB2 positivity, a variety of gene fusions, and dMMR/MSI status. Extended negative hyperselection beyond *RAS* and *BRAF*, using next-generation sequencing to detect these primary resistance alterations (PRESSING panel), coupled with primary tumor sidedness, allowed us to predict the EGFR dependency status. In fact, patients with left-sided and PRESSING-negative status reached unprecedented activity (objective response rate of 77.3%) and efficacy (median PFS of 13.2 months and 2-year OS of 69.7%) with FOLFOX/panitumumab upfront therapy.[7] On the contrary, *EGFR* amplification, albeit extremely rare (1%), is the only positive predictive biomarker and was associated with unprecedented outcomes in patients with *RAS/BRAF* wild-type mCRC receiving anti-EGFRs.[22]

For this work, we selected additional and even rarer alterations (PRESSING2 panel) with a putative role as drivers of primary resistance inferred from translational studies (eg, *NF1* mutations,[11] *KRAS* amplification,[26] *ERBB3* mutations,[18] MAP2K1 mutations,[10] *IGF1R* amplification,[10] *EGFR* fusions,[22] and *PTEN* loss[16]) and/or preclinical experiments (eg, *NTRK* mutations affecting the TK domain,[21] *FGFR2* amplification,[19] *NF1* mutations,[10] *ARAF* amplification,[12] *MAP2K1* mutations,[10] *MAP2K4* mutations,[13] and *AKT1/2* amplification[15]). As expected, patients with PRESSING2 alterations had significantly inferior outcomes after anti–EGFR-based therapy despite initial molecular hyperselection. It must be acknowledged that patients with PRESSING2 alterations were enriched for some poor prognostic indicators with respect to their PRESSING2-negative counterpart. However, the presence of PRESSING2 alterations retained independent negative association with both PFS and OS in the multivariable model. Moreover, we are aware that formal validation of the negative predictive impact of PRESSING2 alterations was not possible because of the lack of an anti–EGFR-free cohort. Such a level of evidence will not be achievable on the basis of the extreme rarity of PRESSING2 alterations and lack of pivotal randomized controlled trials with comprehensive genomic profiling data. Of note, our survival results in the resistant population (PRESSING2-positive) are superimposable to historical data in patients with *RAS* or *BRAF* mutations or with PRESSING panel–positive status.[7,27-29] Moreover, the clinical significance of our panel is further documented by the mutual exclusivity of PRESSING2

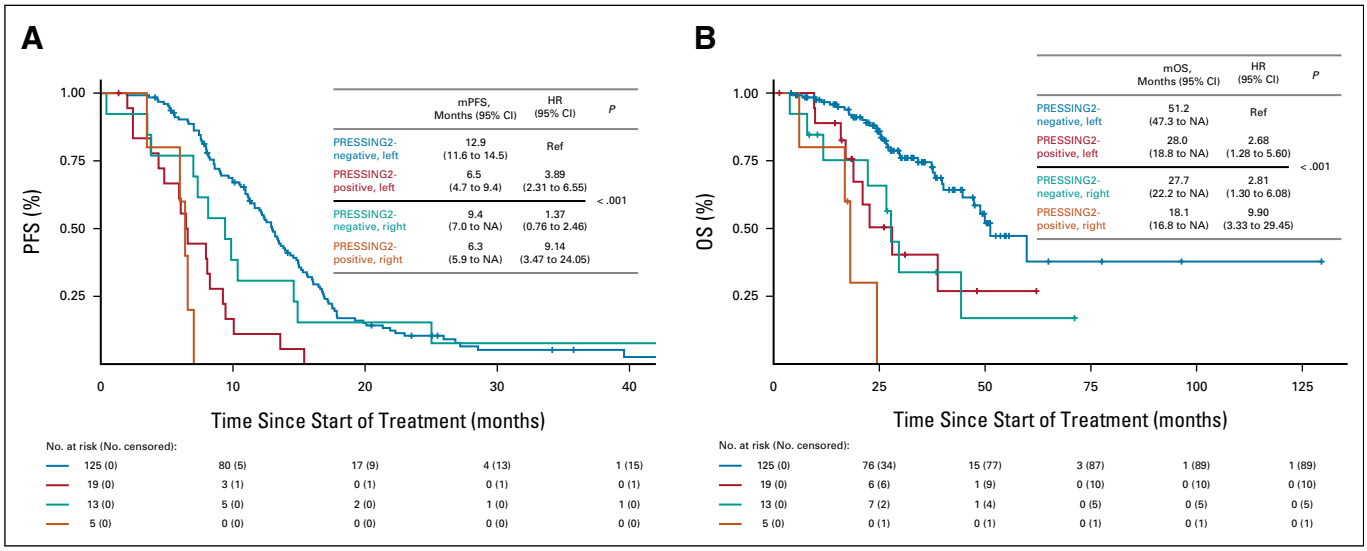

FIG 3. Kaplan-Meier estimates for (A) PFS and (B) OS in the four subgroups of patients identified by the combination of primary tumor sidedness and PRESSING2 status. OS, overall survival; mOS, median overall survival; mPFS, median progression-free survival; NA, not assessable; PFS, progression-free survival; ref, reference.

alterations, thus strengthening their potential role as oncogenic drivers in these tumors.

We believe that implementing the molecular hyperselection/ultraselection approach may be important for both patients with right-sided and left-sided cancers. Regarding patients with left-sided mCRC, the evaluation of PRESSING2 alterations may help to further refine the molecular selection of those eligible for anti-EGFR therapy, particularly considering the presence of alternative first-line options. Right-sidedness has a clear-cut negative predictive impact on EGFR-targeted therapy not only in all randomized controlled trials but also in independent series of hyperselected patients.[7,30,31] With the possible explanation of the sample size, the rare PRESSING2 alterations did not show a statistically significant association with right sidedness. As a matter of fact, their frequency was doubled vs left-sided subgroup (28% *v* 13%), in line with the enrichment of *BRAF* mutations, dMMR/MSI-high status, and PRESSING panel alterations in right-sided cancers. However, a small subset of patients with right-sided mCRC may show EGFR dependency and sensitivity to EGFR inhibition.[32] These patients may be identified by combining different profiling data: genomics-based molecular ultra selection, high AREG/EREG expression,[33] or CMS2/epithelial subtypes on the basis of transcriptomics.[34-36] Unfortunately, we could not investigate AREG/EREG expression in our cohort, but an observational UK study trial is evaluating the prognostic impact of AREG, EREG, and EGFR expression in patients with *RAS* wild-type mCRC receiving anti-EGFRs (ClinicalTrials.gov identifier: NCT03986541) and clinical trial validation is planned. Collectively, these data highlight the need of comprehensive molecular classification of CRC tumors to unveil the complexity of anti-EGFR resistance beyond the mutational status of key oncogenes and primary tumor location.

Comprehensive genomic profiling before initial treatment may allow the assessment of guideline-recommended biomarkers such as *RAS* and *BRAF*, with the concomitant detection of genomic alterations—such as those included in the PRESSING panels—that are increasingly recognized as resistance drivers of anti-EGFRs and, above all, as therapeutic targets. These considerations raise the question if extended genomic profiling should be obtained at baseline before any treatment to tailor the continuum of care and allow early access to innovative drugs and trials.[37] In fact, several PRESSING2 alterations found in this cohort might be actionable (eg, bemarituzumab or pemigatinib for *FGFR2* amplified,[38] trametinib for *MAP2K1* or *NF1* mutated,[39,40] pan-HER inhibitors for *ERBB3* mutated,[41] and EGFR TKIs for EGFR fusions[42]) and might be combined with EGFR inhibitors.

Our study has several limitations. First, we acknowledge that some patients with PRESSING2-positive tumors had relatively longer PFS to anti–EGFR-based therapy. All these patients (as well as the majority of included patients, ie, 91%) received chemotherapy in combination with anti-EGFRs; therefore chemosensitivity and an intrinsically favorable biology could have affected the PFS to anti-EGFRs at the individual patient level. Moreover, single PRESSING2 alterations might exert context-specific effects and dedicated preclinical works are needed for assessing the impact of specific molecular alterations on resistance to EGFR inhibition. Second, we cannot exclude that additional genomic alterations may aid to further refine molecular ultraselection of patients and will be identified in future works as drivers of primary resistance to anti-EGFRs. Third, this series is clinically heterogeneous and the results in the upfront setting were less robust because of the reduced sample size.

In conclusion, a relevant subset of molecularly hyperselected mCRCs harbor genomic alterations that are likely to impair sensitivity to EGFR-targeting therapies. Patients with ultraselected and left-sided mCRC achieve the best survival benefit on exposure to EGFR inhibitors, but analyses of big data on the issue of ultraselection are warranted.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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