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

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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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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 mCRC5 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 unarranged, and AKT1/PTEN/PIK3CA wild-type) and MSS/pMMR metastatic colorectal cancer (mCRC).

Knowledge Generated
Our data support the use of comprehensive genomic profiling in patients with negatively hyperselected (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.

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 unarranged, 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, ARAF/KRAS amplification, MAP2K1/MAP2K2 mutations, and MAP2K4 mutations without established inactivating phenotype [ie, S184L] given the cross-talk with the ERK-upstream branch of MAPK, PIK3CA (including AKT1/2 amplification and AKT2 mutations and PTEN loss), and EGFR-independent receptor tyrosine kinase (ie, IGF1R amplification, ERBB3 amplification/mutations, FGFR2 amplification, and NTRK tyrosine kinase [TK] domain mutations) signaling pathways and EGFR rearrangements involving the TK domain. Pathogenicity of single-nucleotide variants (SNVs) was determined taking advantage of FoundationOne CDx reports. 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. 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, χ², 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
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

| 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) |  
| Age, years                                    | Median 58 | 68 | 57 | .020 |
| Sex, No. (%)                                   | Female 65 (40) | 10 (42) | 55 (40) | > .999 |
| ECOG PS, No. (%)                               | 0 127 (78) | 19 (79) | 108 (78) | > .999 |
| Right colon                                    | 18 (11) | 5 (21) | 13 (9) | .149 |
| Left colon/rectum                              | 144 (89) | 19 (79) | 125 (91) | .538 |
| ECOG PS, No. (%)                               | Yes 137 (85) | 19 (79) | 118 (86) | .069 |
| Time to metastases, No. (%)                    | Synchronous 114 (70) | 12 (50) | 102 (74) | .033 |
| Metachronous                                   | 48 (30) | 12 (50) | 36 (26) | .098 |
| Metastatic sites, No. (%)                      | 1 85 (52) | 8 (33) | 77 (56) | .132 |
| Anti-EGFR line, No. (%)                        | 1 120 (74) | 14 (58) | 106 (77) | .438 |
| Anti-EGFR mAb, No. (%)                         | 14 (9) | 4 (17) | 10 (7) | .266 |
| Panitumab                                       | 96 (59) | 12 (50) | 84 (61) | .069 |
| Cetuximab                                       | 66 (41) | 12 (50) | 54 (39) | .056 |

Bold entries 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.
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-fourty 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.](image-url) 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.
patients with PRESSING2-positive status had significantly worse PFS compared with PRESSING2-negative ones (median PFS: 7.4 vs 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 vs 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)-high for 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

(Figs 2C and 2D), patients with PRESSING2-positive status had significantly worse PFS compared with PRESSING2-negative ones (median PFS: 7.4 vs 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 vs 48.8 months, HR 2.03, 95% CI, 0.90 to 4.61, \( P = .087 \)).

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| Characteristic                      | PFS Univariable Models |          | Multivariable Model |          | OS Univariable Models |          | Multivariable Model |          |
|-----------------------------------|------------------------|----------|---------------------|----------|-----------------------|----------|---------------------|----------|
|                                   | 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     | Ref                 |          |                       | .609     | Ref                 |          |
| Female                            |                        |          | Ref                 |          |                       |          | Ref                 |          |
| Male                              | 0.98 (0.70 to 1.37)    |          | 1.15 (0.67 to 1.97) |          |                       |          | Ref                 |          |
| ECOG PS                           |                        | .654     | Ref                 |          |                       | .001     | Ref                 |          |
| 0                                 | Ref                    |          | Ref                 |          |                       | .003     | Ref                 |          |
| 1-2                               | 0.91 (0.60 to 1.37)    | .063     | Ref                 |          | 2.44 (1.39 to 4.31)   | .132     | Ref                 |          |
|                                  |                        |          | Ref                 |          | 2.55 (1.35 to 4.83)   |          | Ref                 |          |
| Primary tumor location            |                        | .063     | <.001               |          | 1.60 (0.97 to 2.65)   | <.001    | 3.17 (1.67 to 6.04) | <.001    |
| Left colon/rectum                 | Ref                    | .132     | Ref                 |          | 1.49 (0.88 to 2.52)   |          | Ref                 |          |
| Right colon                       | 1.60 (0.97 to 2.65)    |          | 3.17 (1.67 to 6.04) |          | 3.51 (1.76 to 7.03)   |          | Ref                 |          |
| Primary tumor resection           | .414                   |          | Ref                 |          | <.001                 | .001     |                      |          |
| No                                | Ref                    |          | Ref                 |          | 0.82 (0.52 to 1.30)   |          | 0.36 (0.19 to 0.68) |          |
| Yes                               | 0.82 (0.52 to 1.30)    |          | 0.28 (0.15 to 0.51) |          | 0.36 (0.19 to 0.68)   |          | Ref                 |          |
| Time to metastases                | .487                   |          | Ref                 |          | 0.347                 |          | Ref                 |          |
| Metachronous                      | Ref                    |          | Ref                 |          | 1.33 (0.72 to 2.46)   |          | Ref                 |          |
| Synchronous                       | 0.87 (0.61 to 1.26)    |          | 1.33 (0.72 to 2.46) |          |                       | .003     | Ref                 |          |
| Metastatic sites                  | .667                   |          | Ref                 |          | 1.07 (0.77 to 1.48)   |          | 1.33 (0.72 to 2.46) |          |
| 1                                 | Ref                    | .487     | Ref                 |          | 2.22 (1.30 to 3.80)   | .347     | Ref                 |          |
| > 1                               | 1.07 (0.77 to 1.48)    |          | 2.22 (1.30 to 3.80) |          | 1.87 (1.08 to 3.26)   |          | Ref                 |          |
| Anti-EGFR line                    | .085                   | .097     | Ref                 |          | .819                  |          | Ref                 |          |
| 1                                 | Ref                    | .085     | Ref                 |          | 1.07 (0.57 to 2.00)   | .767     | Ref                 |          |
| > 1                               | 1.38 (0.95 to 2.01)    | .575     | 1.38 (0.94 to 2 to 2.04) |          | 1.07 (0.57 to 2.00)   |          | Ref                 |          |
| Anti-EGFR monotherapy             | .575                   |          | Ref                 |          | 1.07 (0.57 to 2.00)   | .767     | Ref                 |          |
| No                                | Ref                    | .575     | Ref                 |          | 1.07 (0.57 to 2.00)   | .767     | Ref                 |          |
| Yes                               | 0.82 (0.42 to 1.61)    |          | 1.16 (0.42 to 3.22) |          | Ref                 |          | Ref                 |          |
| PRESSING2                         | < .001                 | < .001   | < .001              |          | 1.07 (0.57 to 2.00)   | .767     | 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 entries 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

| 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)      | 3.99        | 0.09  | 28.0 (18.8 to NA)    | 2.68        | 0.56  |
| Right-sided/PRESSING2-negative          | 9.4 (7.0 to NA)       | 1.37        | 0.76  | 27.7 (22.2 to NA)    | 2.81        | 0.68  |
| Right-sided/PRESSING2-positive          | 6.3 (5.9 to NA)       | 9.14        | 3.47  | 18.1 (16.8 to NA)    | 9.90        | 3.33  |

Abbreviations: mPFS, median progression-free survival; mOS, median overall survival; HR, hazard ratio; 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, NFI mutations,11 KRAS amplification,26 ERBB3 mutations,18 MAP2K1 mutations,10 IGF1R amplification,10 EGFR fusions,22 and PTEN loss16) 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 amplification15). 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 PRESSING2 panel–positive status.27,28

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. 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% vs 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. These patients may be identified by combining different profiling data: genomics-based molecular ultra selection, high AREG/EREG expression, or CMS2/epithelial subtypes on the basis of transcriptomics. 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. In fact, several PRESSING2 alterations found in this cohort might be actionable (eg, bemarituzumab or pemigatinib for FGFR2 amplified, trametinib for MAP2K1 or NF1 mutated, pan-HER inhibitors for ERBB3 mutated, and EGFR TKIs for EGFR fusions) 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 ultra-selected 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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