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SHORT COMMUNICATION

Genomic profiling of high tumor mutational load in microsatellite-stable colorectal cancer uncovers MAPK signaling pathway alterations following anti-EGFR therapy

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Background: Translational studies have provided evidence that targeted therapies and chemotherapy might induce a status of adaptive mutability with an increase in the tumor mutational load.

Patients and methods: We conducted an analysis of pathogenic variants (PVs) detected by liquid biopsy (LBx)-based comprehensive genomic profiling in patients with chemo-refractory microsatellite-stable metastatic colorectal cancer (mCRC) pretreated with anti-epidermal growth factor receptor (EGFR) within the VELO and CAVE-2 GOIM studies compared with anti-EGFR naïve mCRC included in the CAPRI-2 GOIM trial.

Results: Overall, 559 patients with available samples for LBx analysis were included. EGFR pretreated tumors had significant enrichment for PVs in the MAPK signaling pathway with a median tumor mutational burden (TMB) [6 interquartile range (IQR 4-11) versus 4 (IQR 3-9), $P < 0.0001$]; 33.8% pretreated patients had TMB with $\geq$10 mutations per megabase compared with 9.7% patients before first-line anti-EGFR treatment. Higher mutational load correlated with *KRAS* (q = 0.07), *BRAF*^{V600} (q = 0.01), *ERBB2 AMP* (q = 0.06) and *EGFR ECD* (q = 0.07) PVs. Such association was not observed in patients naïve to anti-EGFR drugs. *MAPK* mutations were associated with

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higher TMB in anti-EGFR pretreated samples (beta = 4.0, $P < 0.0001$), but not in anti-EGFR-naïve samples (beta 1.2, $P = 0.4$).

Conclusion: These findings might support the investigation of immunotherapy in patients with mCRC pretreated with EGFR inhibitors with high mutational load.

Key words: TMB, liquid biopsy, anti-EGFR, comprehensive genomic profiling, MAPK

INTRODUCTION

Metastatic colorectal cancer (mCRC) is a malignancy characterized by several molecular alterations that promote cancer initiation, progression and invasiveness.[1] For patients with *RAS/BRAF*-wild type (wt), microsatellite-stable (MSS) mCRC the addition of anti-epidermal growth factor (EGFR) inhibitors to chemotherapy is a standard of care.[2] Despite initial antitumor activity, as for other target therapies, cancer cells will develop a mechanism of acquired resistance.[3]

It has been shown in preclinical models of CRC that blocking EGFR signaling might cause DNA damage, stimulating adaptive mutability in cancer cells with the accumulation of genomic alterations, which become responsible for loss of responsiveness to anti-EGFR therapy.[4] In this respect, increase in tumor mutational burden (TMB) could convert a low- into a high-immunogenic tumor microenvironment, supporting the rationale for the use of immunotherapy.[5] Despite this biologic rationale, available clinical evidence is limited; thus, further research is needed. Recently, liquid biopsy (LBx) has emerged as a noninvasive tool, which allows researchers to obtain a dynamic portrait of the tumor molecular landscape and to detect anti-EGFR drug resistance mutations.[6]

In this study, we carried out an exploratory analysis of pathogenic variants (PVs) identified through LBx-based comprehensive genomic profiling (CGP) in a homogeneous cohort of anti-EGFR pretreated patients with MSS mCRC enrolled in the VELO and CAVE-2 GOIM studies. This cohort was compared with a cohort of anti-EGFR-naïve patients from the CAPRI-2 GOIM trial.[7-9] We then investigated whether specific alterations in signaling pathways were associated with higher TMB. Finally, we explored the potential impact of prior exposure to anti-EGFR monoclonal antibodies on tumor heterogeneity.

METHODS

Study population

We conducted a pooled analysis of genomic data of patients enrolled in the VELO (NCT05468892), CAVE-2 GOIM (NCT05291156) and CAPRI-2 GOIM (NCT05312398).[7-9]

VELO was a randomized phase II trial evaluating panitumumab plus trifluridine-tipiracil versus trifluridine-tipiracil alone as third-line therapy for *RAS*-wt mCRC.[7] Patients should have obtained a partial response or complete response to first-line chemotherapy plus an anti-EGFR monoclonal antibody and an anti-EGFR free interval $\geq$4 months during second-line therapy. Plasma samples were collected at baseline for LBx analysis.

CAVE-2 GOIM is a randomized phase II study assessing the combination of cetuximab plus avelumab compared with cetuximab as anti-EGFR rechallenge strategy in pretreated patients with circulating tumor DNA (ctDNA) *RAS/BRAF*^{V600}-wt MSS/mismatch repair-proficient mCRC.[8]

CAPRI-2 GOIM trial investigates the efficacy and safety of a ctDNA biomarker-guided, cetuximab-based therapy over three treatment lines in mCRC patients with *RAS/BRAF*-wt tumors at start of first-line.[9] Here we evaluated results of baseline LBx analysis before the initiation of first-line treatment.

Cases with baseline pretreatment plasma samples adequate for LBx-based ctDNA CGP by FoundationOne Liquid (F1L) CDx assay (https://www.foundationmedicine.com/test/foundationone-liquid-cdx) assessing 324 genes were considered eligible. Main exclusion criteria were (i) nonavailability of baseline plasma sample; (ii) inadequate ctDNA for LBx analysis; (iii) F1L CDx assay evaluating 78 genes; (iv) microsatellite instable (MSI) tumors; (v) withdrawal of patient consent to participate.

Genomic analyses

Only pathogenic genomic alterations detected by F1L CDx assay were included for analyses. Tumor clonality was calculated as the ratio between the detected variant allele frequency and the estimated tumor fraction (TF). Considering the potential role as mechanism(s) of resistance to anti-EGFR therapies, the following genes were included in the hyper-selection panel included *RAS*, *BRAF*^{V600}, *NF1*, *MAP2K1*, *EGFR-extracellular domain (ECD)*, *PIK3CA* exon 20, *PTEN* mutations, *MET, FGFR1/2/3*, *ERBB2* amplifications (*ERBB2 AMP*), *NTRK1/2/3*, *ROS1*, and *ALK* rearrangements.

Statistical analyses

Continuous variables were reported as median and interquartile range (IQR) and compared between groups using the Mann—Whitney *U* test. Categorical variables were reported as frequencies and proportions and compared using Fisher's exact test. The linear association between gene alterations and an increase in TMB was calculated using ordinary least squares linear regression analysis. All tests were carried out assuming a two-sided statistical significance with an α level of <0.05. Family-wise error correction, where specified, was carried out using the Benjamini—Hochberg procedure. Statistical analyses were conducted with Python software version 3.9.21.

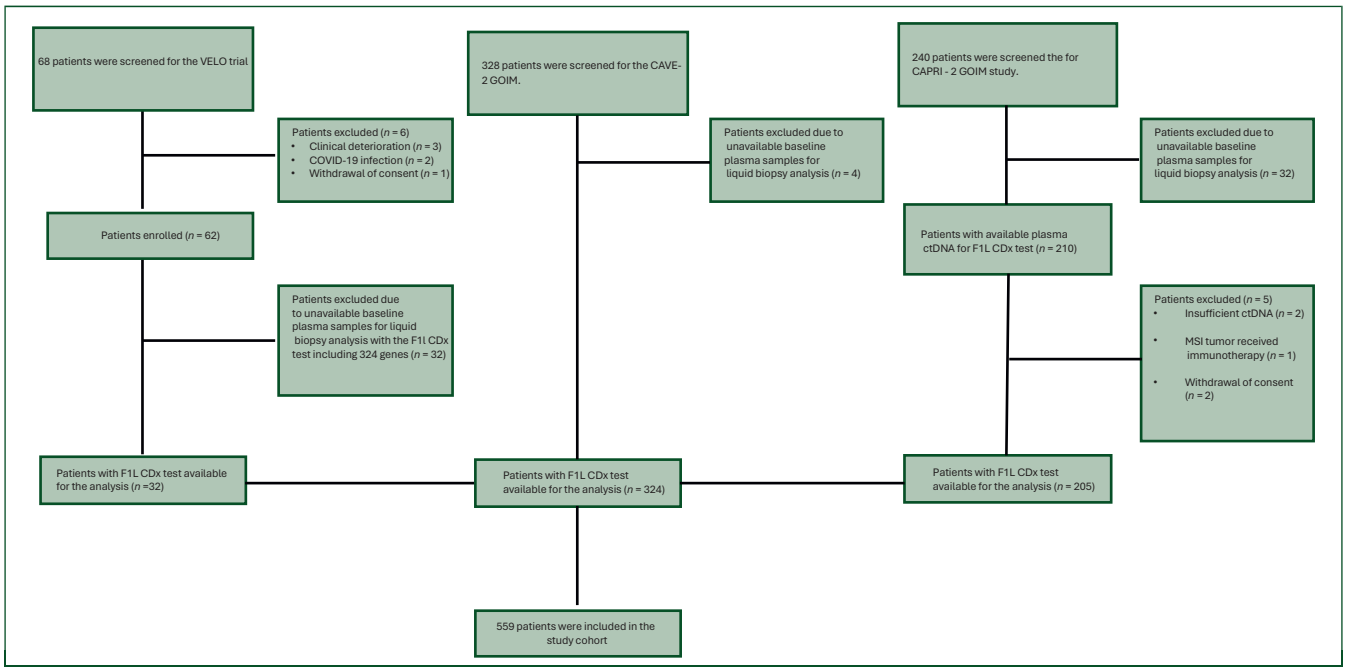

Figure 1. Study diagram. ctDNA, circulating tumor DNA; F1 CDx, FoundationOne CDx; F1L CDx, FoundationOne Liquid CDx; mCRC, metastatic colorectal cancer; MSI, microsatellite instable; MSS, microsatellite stable; pMMR, mismatch repair-proficient; wt, wild type.

Results

Overall, a total of 559 patients met the inclusion criteria and their data were incorporated in the current analysis (Figure 1). The study cohort includes 205 patients (36.6%) who were anti-EGFR naïve and enrolled in the CAPRI-2 trial, and 354 (63.3%) patients who were candidates for anti-EGFR rechallenge therapy in the CAVE-2 and VELO trials.

We first sought to investigate the different prevalence of gene alterations between the two cohorts. In the overall study population, we observed *APC* (481 mutations), *TP53* (394 mutations) and *KRAS* (141 mutations) to be the most common PVs. Previous studies have shown a potential clearance of anti-EGFR resistance PVs following exposure to chemotherapy during an anti-EGFR free interval.[10,11] Comparing the two clinical settings, anti-EGFR pretreated patients who were candidates for rechallenge therapy had significant enrichment for alterations included in the MAPK signaling pathway (Figure 2A): *KRAS* [33.9% versus 6.0%, odds ratio (OR) 8.3, q $<$ 0.001], *EGFR ECD*, 13.9% versus 1.0%, OR 15.9, q $<$ 0.0001), *MAP2K1* (13.9% versus 3.0%, OR 5.20, q $=$ 0.003), $BRAF^{nonV600}$ (7.9% versus 0.5%, q $=$ 0.0004, OR 17.1) and *NRAS* (13.0% versus 3.0%, OR 4.8, q $=$ 0.0005) (Figure 2B). This result was confirmed by conducting a pathway-level analysis. Of note, beside a slight enrichment of mutations in the TP53-pathway among pretreated tumors (77.9% versus 61.9%, OR 2.17, q $=$ 0.0002), only mutations involving MAPK signaling were statistically significantly enriched among patients previously exposed to anti-EGFR drugs (48.3% versus 17.0%, OR 4.53, q $<$ 0.0001) (Figure 2C). Emergence of anti-EGFR resistance PVs (RPVs) has been described in tumors that were transitioning in a genomic instability state, by favoring an increased rate of somatic mutations that are pathogenic hits in key resistance genes.[4] Patients in the advanced treatment lines had higher median TMB [6 (IQR 4-11) versus 4 (IQR 3-9), P $<$ 0.0001] (Figure 2D). More pretreated tumors (33.8%) than tumors that were candidates for first-line anti-EGFR treatment (9.7%) exhibited a TMB $\geq$10 mutations per megabase (mut/Mb) (Figure 2E). This TMB value represents the diagnostic cut-off for the approved clinical indication for tumor-agnostic use of immune checkpoint inhibitors.[12]

We next assessed whether augmented mutational load among anti-EGFR pretreated samples could be correlated with specific subgroups. A linear increase in TMB was associated with mutations in *KRAS* (beta $=$ 2.91, q $=$ 0.005), $BRAF^{V600}$ (beta $=$ 7.16, q $=$ 0.005), *BRCA2* (beta$=$ 7.26, q $=$ 0.005), and *EGFR ECD* (beta $=$ 4.00, q $=$ 0.058). Once stratified per treatment line, a higher TMB was reported in pretreated tumors carrying *KRAS* (q $=$ 0.07), $BRAF^{V600}$ (q $=$ 0.01), *ERBB2 AMP* (q $=$ 0.06) and *EGFR ECD* (q $=$ 0.07) molecular alterations (Figure 2F). Nevertheless, such association was not observed among samples from patients who were naïve to anti-EGFR therapies. Moreover, *MAPK* mutations were associated with higher TMB in samples from anti-EGFR pretreated patients (beta $=$ 4.0, P $<$ 0.0001), but not in samples from patients before first-line treatment of metastatic disease (beta 1.2, P $=$ 0.4) (Figure 2G).

We next assessed the correlation between molecular hyper-selection and TMB. Overall, a higher proportion of tumors from patients who were previously exposed to anti-EGFR therapies had PVs of anti-EGFR drug resistance (56.7% versus 26.3%, OR 3.67, P $<$ 0.0001) (Figure 2H). Tumors that exhibited PVs potentially correlated with anti-EGFR resistance (positively hyper-selected) that increased

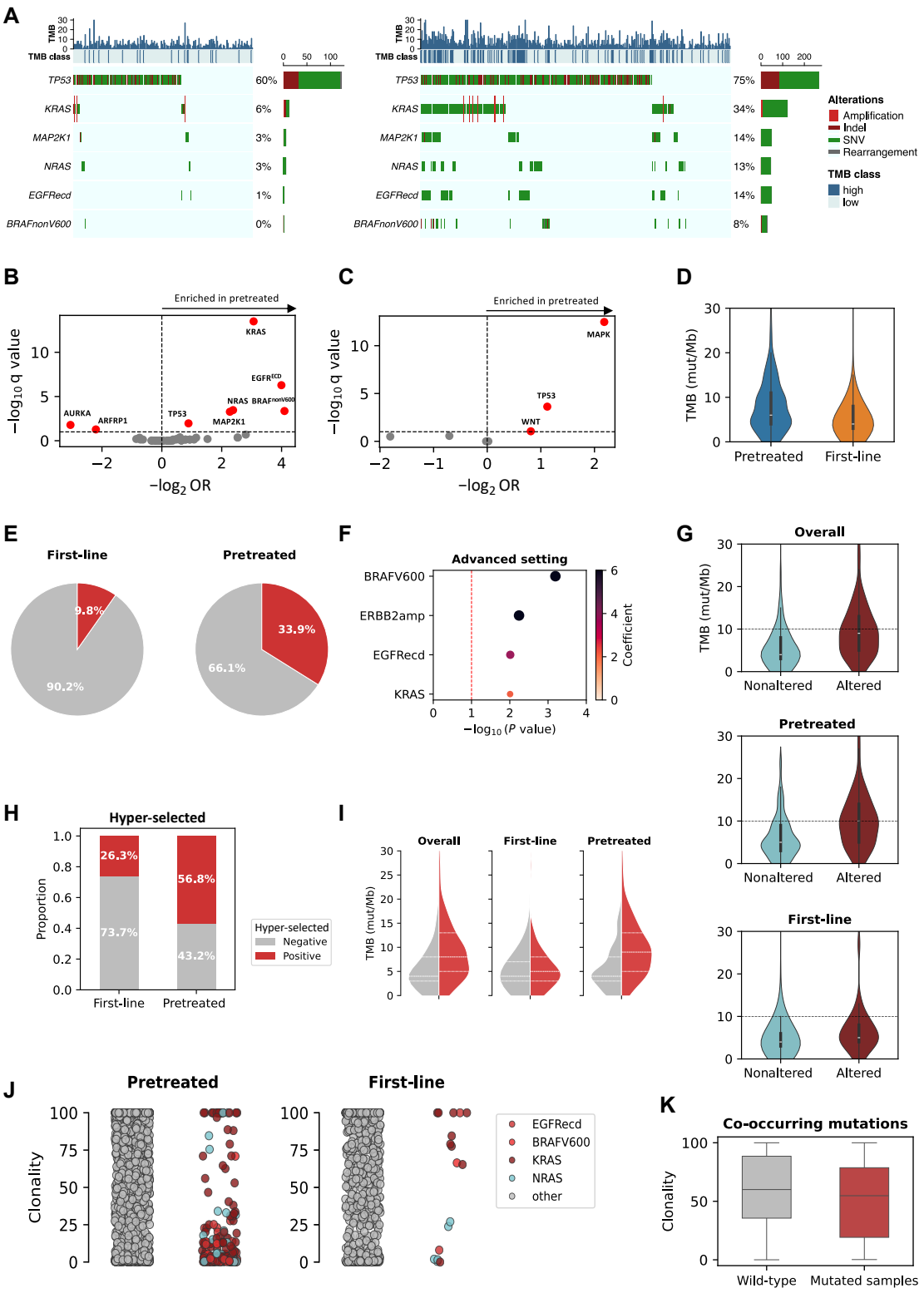

Figure 2. Analysis of genomic alteration by liquid biopsy based comprehensive genomic profiling in patient with untreated and pre-treated with anti-EGFR inhibitors and correlation with tumor mutational burden. (A) Oncoprint displaying gene alterations enriched in pretreated patients compared with those receiving first-line anti-EGFR based regimen. (B) Volcano plot of gene enrichment analysis according to the treatment setting. Pretreated samples from candidates for anti-EGFR rechallenge showed an enrichment of gene alterations involved in the MAPK signaling cascade. (C) Enrichment of pathway-level alterations according to the treatment setting. Again, pretreated samples demonstrated a particular enrichment of gene alterations involving the MAPK pathway (48.3% versus 17.0%, OR 4.53, q $<$ 0.0001). (D) TMB comparison between pretreated patients and those candidates for first-line treatment. Pretreated patients demonstrated a higher TMB compared with samples not previously exposed to anti-EGFR treatment [6 (IQR 4-11) versus 4 (IQR 3-9), $P < 0.0001$]. (E) Proportion of cases carrying a high TMB (TMB $\geq$10 mutations per megabase) according to the clinical setting. (F) Association between gene mutations and higher TMB among pretreated samples, with the linear regression coefficient (beta) denoting the median increase in TMB for mutated samples. (G) TMB between mutated and nonmutated tumors for *KRAS*, *EGFR ECD*, *ERBB* amplifications and *BRAF*^{V600}. Although mutated tumors showed a higher TMB in pretreated samples (beta = 4.0, $P < 0.0001$), this was not observed

TMB compared with tumors without resistance alterations (negatively hyper-selected tumors) [median TMB, 8 (IQR 5-13) versus 4 (IQR 3-8), $P < 0.0001$] (Figure 2I), which was consistent among tumors not carrying *RAS/BRAF*^{V600} PVs [median TMB, 8 (IQR 4-10) versus 4 [IQR (3-8), $P < 0.0001$]. Once considering genes included in the hyper-selection list of genes, but not in the MAPK signaling cascade, mutated tumors ($n = 47$) had higher TMB compared with wild-type tumors [median TMB, 6 (IQR 4-10) versus 4 (3-8), $P = 0.0006$], which was consistent in samples from pretreated patients [$n = 27$, median TMB 9, (IQR 6-15.5) versus 4 (IQR 3-8), $P < 0.0001$], but not in samples from patients who were not previously exposed to anti-EGFR drugs [$n = 20$, 4 (IQR 1-6.5) versus 4 (3-7), $P = 0.98$].

We finally evaluated if previous exposure to anti-EGFR therapies could account for a distinct tumor heterogeneity. Anti-EGFR drug pretreated tumors had lower cancer cell clonality of detected PVs [47.0% (IQR 8.2-72.5) versus 59% (IQR 35.3-87.0), $P < 0.0001$]. Mutations in *EGFR ECD* ($q < 0.0001$), *KRAS* ($q = 0.004$), *BRAF*^{V600} ($q = 0.006$) and *NRAS* ($q = 0.03$) were found in tumors with lower cancer cell clonality compared with nonmutated samples. Although mutations involving *EGFR ECD*, *KRAS*, *BRAF*^{V600} and *NRAS* were associated with lower cancer cell clonality compared with background mutations, this was not the case for samples from patients who were not previously exposed to anti-EGFR drugs (Figure 2L). Moreover, co-occurring gene mutations in tumors carrying *EGFR ECD*, *KRAS*, *BRAF*^{V600} and *NRAS* had lower cancer cell clonality compared with wild-type tumors [54.7% (IQR 19.9-78.6) versus 60.0% (IQR 35.5-88.5), $P = 0.01$] (Figure 2M).

DISCUSSION

Tumor heterogeneity represents a key determinant driving progression and resistance to targeted therapies.[13] Interestingly, cancer cell resistance and progression might occur through the emergence and expansion of sub-clones that carry private mutations not found in coexisting cancer cell clones, therefore being represented at lower clonality.[14]

In this study, we confirm that anti-EGFR drug exposure exerts a significant impact on the landscape of tumor genomic alterations in mCRC. In samples from anti-EGFR pretreated patients, we observed overall lower cancer cell clonality for *KRAS/NRAS/BRAF*^{V600} and *EGFR ECD* mutations compared with samples from patients who were candidates for first-line treatment (i.e. they were not previously exposed to anti-EGFR drugs). Moreover, coexisting PVs in the same sample displayed lower cancer cell clonality, underlining the potential presence of multiple clonal lineages emerging after exposure to anti-EGFR therapies and multiple lines of treatment. In a previous analysis of the CAVE-2 GOIM study we observed a correlation between the concomitant presence of sub-clonal *RAS/BRAF*^{V600} mutations and other sub-clonal PVs, suggesting that in case of a lower clonality multiple alterations were required to develop anti-EGFR resistance.[8]

Similarly, Topham and colleagues reported in a cohort of 66 patients with chemo-refractory MSS mCRC, that after progression to anti-EGFR drugs, cancer resistance occurred via a multiplicity of concurrent sub-clonal alterations, with 21% of tumors having more >10 putative RPVs.[15]

Although MSI or POLE/POLD-1 mCRC is characterized by a hyper-mutability state, most MSS tumors (90%-92%) display low TMB with <10 mut/Mb.[16] Notably, we report that 33.8% of MSS anti-EGFR/chemo-refractory tumors had high mutational load. In a previous report TMB was significantly increased in plasma samples of the anti-EGFR pretreated patients compared with patients who were not pretreated with anti-EGFR drugs.[17]

Consequently, we sought to investigate potential molecular mechanism(s) associated with increased mutational load. A correlation between *KRAS/BRAF*^{V600}*/EGFR ECD* and *BRCA2* PVs and increased TMB was reported. These findings were observed only for tumors of anti-EGFR drug treated patients, in which alterations of MAPK signaling cascade were also associated with an augmented TMB.

In this regard, a translational analysis of the IMblaze370 study showed that alterations in EGFR/MAPK signaling, but not in PI3K pathway genes, are selectively enriched in patients after anti-EGFR exposure but not following anti-angiogenic therapies in mCRC.[18] Taken together these results suggest that *MAPK* alterations correlate with increased TMB only in anti-EGFR pretreated tumors. Nevertheless, it is difficult to discern if this is a causal relationship (therapy-induced genomic instability) or a secondary correlation. Further translational studies are required to clarify this association.

Emerging evidence supports the concept that a deeper molecular stratification, beside *RAS/BRAF* evaluation, might better select patients who could benefit from anti-EGFR inhibition.[19] Given the overlap between MAPK signaling cascade genes and those promoting resistance to anti-EGFR therapies, we next assessed whether molecular hyper-selection could identify tumors carrying a higher mutational load. Positive hyper-selected tumors had higher TMB compared with negative hyper-selected tumors. The difference was also retained after excluding *RAS/BRAFV*^{600} PVs and MAPK alterations. Taken together, these findings underline the potential strict connection between hyper-mutability state and acquiring anti-EGFR RPVs in cancer cells treated with EGFR inhibitors.

among tumors not previously exposed to anti-EGFR treatment (beta 1.2, $P = 0.4$). (H) Proportion of tumors showing mutations in genes promoting anti-EGFR resistance (hyper-selected) according to the clinical setting. (I) TMB between positive and negative hyper-selected tumors according to the clinical setting. Positive hyper-selected tumors showcased a higher TMB among pretreated samples [9 (IQR 5/13) versus 4 (IQR 3-8), $P < 0.0001$] but not in patients not previously exposed to anti-EGFR treatment [5 (IQR 3-8) versus 4 (IQR 3-5.5), $P = 0.79$]. (J) Tumor clonality of *KRAS*, *NRAS*, *BRAF*^{V600} and *EGFR ECD* compared with background mutations, stratified according to the clinical setting. Notably, they consistently showed a lower tumor clonality only among pretreated samples. (K) Clonality of co-occurring mutations in tumors carrying *KRAS*, *NRAS*, *BRAF*^{V600} and *EGFR ECD* alterations. Notably, co-occurrent alterations showed a lower clonality compared with mutations detected in tumors not carrying *KRAS*, *NRAS*, *BRAF*^{V600} and *EGFR ECD* alterations [54.7% (IQR 19.9-78.6) versus 60.0 (IQR 35.5-88.5), $P = 0.01$]. IQR, interquartile range; OR, odds ratio; SNV, single nucleotide variant; TMB, tumor mutational burden.

Recently, pembrolizumab has been approved as an agnostic-therapy for the treatment of tumors with high TMB.[12] However, limited evidence is available for mCRC.[12] The CO.26 trial investigated the clinical activity of dual checkpoint blockade with durvalumab/tremelimumab in a cohort of pretreated patients with mCRC.[15,19] A plasma TMB threshold of 28 mut/Mb was useful to identify patients who could benefit from immunotherapy. It could be argued that only clonal and more immunogenic alterations might represent a potential predictive biomarker for immunotherapy efficacy. In the CO.26 study, the authors reported a threshold of 25.9 mut/Mb for separating patients based on overall survival for sub-clonal plasma TMB.[19] However, it should be considered that this threshold is significantly higher than the cut-off used in the KEYNOTE 158 study.[12] Thus, the optimal cut-off to select patients with MSS mCRC that might benefit from immunotherapy combinatory strategies deserves further investigation.

In this regard, there is a rationale for immunotherapy use in MSS tumors with a mutational load induced secondary to anti-EGFR therapies. The CAVE-GOIM trial demonstrated the safety and provided signal of clinical activity for combining cetuximab with the anti-programmed death-ligand one (PD-L1) avelumab, as a rechallenge strategy in patients with refractory *RAS*-wt mCRC.[20] The antitumor activity might be related to the capability of cetuximab to promote activation of natural killer cells and enhancement of antibody-dependent cell cytotoxicity. In this scenario, mature results of the CAVE-2 GOIM trial will provide further evidence.[8] The study has completed the enrollment and mature data are awaited.

Limitations

Several limitations should be considered when interpreting the results presented in this study. The CAVE-2 GOIM and VELO trials initially enrolled patients with *RAS*-wt mCRC, who experienced clinical benefit from front-line anti-EGFR-based chemotherapy. These patients subsequently experienced disease progression and were treated with another line of therapy, ultimately becoming candidates for an anti-EGFR rechallenge strategy.[9,10] However, serial plasma samples from the first to later lines of therapy were not available, and therefore, comparisons were made using a cohort of patients with *RAS/BRAF*^{V600}-wt MSS, prior to treatment for metastatic disease.[11] Moreover, matched tissue samples for patients who were candidates for anti-EGFR rechallenge were not available. However, the previous genomic analysis of CAPRI-2 GOIM trial showed a good concordance between tissue and LBx-based CGP especially in case of TF >10.[9] Thus, in these cases LBx evaluation might represent a valuable noninvasive option for monitoring tumor molecular profiling in patients treated with tailored treatment. However, in the case of low TF (<1), LBx could not allow to identify PVs with a lower clonality or complex genomic alterations such as deletion or rearrangement of copy number variation.

Finally, for patients in the CAVE-2 GOIM and VELO trials, detailed data regarding the duration of first-line therapy, the number of anti-EGFR therapy cycles, and the maintenance strategies employed were unavailable. As a result, the potential impact of these factors on the development and clearance of PVs could not be assessed. In this context, the ongoing CAPRI-2 GOIM trial presents a unique opportunity to investigate cancer cell clonal evolution across three consecutive lines of therapy for metastatic disease in *RAS/BRAF*^{V600}-wt MSS mCRC patients, through longitudinal LBx-based CGP evaluation.

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DISCLOSURE

GM reported receiving honoraria from Servier, Incyte and Pierre Fabre. AA reported receiving personal fees from Amgen, AstraZeneca, Merck, Sharp & Dohme (MSD), Eisai and Bristol Myers Squibb (BMS). FP reported receiving research funding (to Institution) from Lilly, BMS, Incyte, AstraZeneca, Amgen, Agenus and Rottapharm; personal honoraria as an invited speaker from BeiGene, Daiichi Sankyo, SeaGen, Astellas, Ipsen, AstraZeneca, Servier, Bayer, Takeda, Johnson and Johnson, BMS, MSD, Amgen, Merck-Serono and Pierre Fabre; advisory/consultancy from BMS, MSD, Amgen, Pierre Fabre, Johnson and Johnson, Servier, Bayer, Takeda, Astellas, GlaxoSmithKline (GSK), Daiichi Sankyo, Pfizer, BeiGene, Jazz Pharmaceuticals, Incyte, Rottapharm, Merck-Serono, Italfarmaco, Gilead, AstraZeneca and Agenus. RB reported receiving honoraria from Novartis, AstraZeneca, Sanofi, Amgen, Roche, Pfizer, Janssen-Cilag and BMS; consulting or advisory role from Novartis, Bayer, AstraZeneca, Sanofi, Amgen, Roche, Pfizer, Janssen-Cilag and BMS; speakers' bureau from AstraZeneca, Sanofi, Novartis, Bayer, Amgen, Roche, Pfizer,

Janssen-Cilag and BMS. EM reported serving as adviser and speaker for AstraZeneca, Eli Lilly, Servier, Sanofi Genzyme, Roche, Merck, Eisai and Pfizer. GT reported a consulting or advisory role for BMS, AstraZeneca, Merck, MSD and Servier. GS reported receiving research funds (to Institution) from BMS, Mirati and Nouscom; serving on an advisory board for Amgen and Bayer; travel grants from AstraZeneca, Merck and Servier. RB reported serving as a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Novartis, MSD, Otsuka, Eli Lilly and Roche. SL reported personal financial interests for serving on an advisory board for Amgen, Astellas, AstraZeneca, Bayer, BMS, Daiichi Sankyo, GSK, Incyte, Lilly, Merck-Serono, MSD, Servier, Takeda, Rottapharm, BeiGene, Fosun Pharma and Nimbus Therapeutics; for serving as an invited speaker for Amgen, AstraZeneca, BMS, Incyte, GSK, Lilly, Merck-Serono, MSD, Pierre Fabre, Roche and Servier; served as an unremunerated member of the board of directors for the Italian No-Profit Oncology Research Foundation supporting academic clinical trials: GONO. CC reported receiving grants and personal fees from Merck and Amgen outside the submitted work. AS-B reported a consulting or advisory role for Bayer, Novartis, Pierre Fabre, Servier and Takeda; personal honoraria as an invited speaker from Amgen, Guardant Health, Pierre Fabre and SeaGen. SS reported serving on an advisory board for Agenus, AstraZeneca, Bayer, BMS, CheckmAb, Daiichi Sankyo, GSK, MSD, Merck, Novartis, Pierre Fabre, SeaGen and T-One Therapeutics. NF received honoraria as a consultant and speaker from Novartis and ITM. MGZ reported receiving honoraria for serving on an advisory board from GSK. NN reported receiving honoraria from Thermo Fisher Scientific, Lilly, MSD, Illumina, Merck-Serono, Incyte, Biocartis, AstraZeneca and MSD; for a consulting or advisory role from Biocartis, AstraZeneca, Bayer, Incyte, Novartis and Roche; research funding (to Institution) from AstraZeneca, Biocartis, Illumina, Incyte, Merck-Serono, Qiagen, Roche, and Thermo Fisher Scientific; travel, accommodations, expenses from Merck-Serono. PP served as speaker/pathologist consultant for Amgen, Pierre Fabre, MSD, Servier, BeiGene, AstraZeneca, Bristol, Incyte, Pharmacogenetics, Daiichi Sankyo, Astellas and GSK. EM reported serving as adviser and speaker for AstraZeneca, Eli Lilly, Servier, Sanofi Genzyme, Roche, Merck, Eisai and Pfizer outside the submitted work. TT received travel grants from AstraZeneca and Pierre Fabre and is an advisory board member for AstraZeneca, Bayer, Amgen, Merck, Roche, Sanofi, Servier and Pierre Fabre. GC reported receiving honoraria for speaker's engagement from Roche, Seattle Genetics, Novartis, Lilly, Pfizer, Foundation Medicine, NanoString, Samsung, Celltrion, BMS and MSD; honoraria for providing consultancy from Roche, Seattle Genetics and NanoString; honoraria for participating on an advisory board from Roche, Lilly, Pfizer, Foundation Medicine, Samsung, Celltrion and Mylan; honoraria for writing engagement from Novartis and BMS; honoraria for participation in the Ellipsis Scientific Affairs Group; institutional research funding for conducting phase I and II clinical trials from Pfizer, Roche, Novartis, Sanofi, Celgene, Servier, Orion, AstraZeneca, Seattle Genetics, AbbVie, Tesaro, BMS, Merck-Serono, MSD, Janssen-Cilag, Philogen, Bayer, Medivation and Medimmune. FC received institutional research grants from Amgen, Merck KGaA, MSD, Pfizer, Pierre Fabre, Roche and Servier; and service on advisory boards for Bayer, Merck KGaA, MSD, Pierre Fabre, Roche, and Servier. SN reported receiving personal fees from Novartis and a travel grant from Amgen. DC declares advisory board/consultation for Bayer, Merck KgA, Foundation Medicine; and travel support from Sanofi, BMS and Merck KgA. All other authors declare no conflicts of interest.

DATA SHARING

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

ETHICS STATEMENT

The three studies were conducted in accordance with the principles of the Declaration of Helsinki and were approved by the ethical committees of all participating centers. Patients provided written informed consent for participation.

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