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ORIGINAL ARTICLE

Comprehensive genomic profiling by liquid biopsy portrays metastatic colorectal cancer mutational landscape to predict antitumor efficacy of FOLFIRI plus cetuximab in the CAPRI-2 GOIM trial

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Background: Limited evidence is currently available on the role of liquid biopsy (LBx) in predicting the efficacy of anti-epidermal growth factor receptor (EGFR) therapies in metastatic colorectal cancer (mCRC).

Methods: The CAPRI-2 GOIM is a phase II trial investigating the use of LBx-comprehensive genomic profiling (CGP)-guided, cetuximab-based treatment through three subsequent lines of therapy in patients with *RAS/BRAF* wild-type (WT) mCRC. LBx-CGP is carried out at baseline and at progressive disease to first- and second-line therapies. In case of *RAS/BRAF* WT circulating tumor DNA at progressive disease, EGFR therapeutic blockade is continued by combining cetuximab with a different chemotherapy backbone. The primary endpoint is overall response rate (ORR) by RECIST 1.1 criteria. Tumor molecular characteristics by LBx-CGP are correlated with treatment efficacy.

Results: One hundred and ninety-two *RAS/BRAF* WT microsatellite stable mCRC patients treated with FOLFIRI plus cetuximab with baseline LBx-CGP and assessable for response were included in the analysis. One hundred and thirty-seven patients with WT tumors for potential anti-EGFR drug resistance genes *(RAS/BRAF/EGFR/PIK3CA/MAP2K1/MET/RET/ALK/ROS1/NTRK/NF1/FGFR*, and *HER2* amplification; 'negatively hyper-selected' cases) had 78.1% ORR compared with 54.5% ORR for patients with mutations [odds ratio 2.95, 95% confidence interval (CI) 1.44-6.10, $P = 0.001$]. 'Negatively hyper-selected' patients had median progression-free survival of 12.35 months (95% CI 10.58-15.4 months) compared with 8.68 months (95% CI 4.87-12.1 months) for patients with mutations (hazard ratio 0.64, 95% CI 0.44-0.92, $P = 0.017$). High cancer cell clonality of pathogenic variants (PVs) correlated with worse median progression-free survival (3.55 months, 95% CI 2.57 months to NE) compared with low cancer cell

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clonality of PV (9.63 months, 95% CI 7.16 months to NE, $P = 0.21$). After first-line therapy failure, approximately one out of five patients had acquired PVs of potential anti-EGFR drug resistance genes, whereas *RAS/BRAF* WT circulating tumor DNA was maintained in most patients (78.5%).

Conclusions: These results support the integration of LBx-CGP for implementing the efficacy and for optimizing the use of anti-EGFR therapies in *RAS/BRAF* WT mCRC.

Key words: colorectal cancer, liquid biopsy, cetuximab, comprehensive genomic profiling

INTRODUCTION

The choice of optimal first-line therapy for patients with metastatic colorectal cancer (mCRC) is based on clinicopathologic characteristics and on tumor molecular profile.[1-3] In case of *RAS/BRAF* wild-type (WT), microsatellite stable (MSS) mCRC, anti-epidermal growth factor receptor (EGFR) therapy (with the anti-EGFR monoclonal antibodies cetuximab or panitumumab) in combination with FOLFIRI or FOLFOX chemotherapy is a standard of care and represents the preferred option for left-sided primary tumors, although most patients experience cancer cell resistance and progress after initial response to treatments.

Growing evidence suggests that deeper molecular evaluation, especially by comprehensive genomic profiling (CGP), on tumor tissue may improve patient selection and efficacy of anti-EGFR therapies.[4,5] An intrinsic limitation of a single tumor tissue biopsy, however, is the inability to capture spatial and temporal heterogeneity of metastatic cancers.[6] Liquid biopsy (LBx)-based CGP could overcome these limitations by recapitulating in the circulating tumor DNA (ctDNA) the presence of genomic alterations from different metastatic sites and by providing dynamic molecular characterization if repeated over time during disease evolution. Exploratory biomarker analysis in the PARADIGM trial suggested that baseline LBx could help to better identify mCRC patients who benefit from anti-EGFR treatments.[7] Further evidence is required, however, and the use of LBx in clinical practice remains limited, mostly when tumor tissue is not available.[2]

Another potential advantage of ctDNA analysis by LBx is the possibility to better define tumor molecular heterogeneity in terms of cancer cell clones that may emerge during disease progression.[6] Moreover, a molecular pathogenic variant (PV) in most cancer cells (clonal mutation) rather than its presence in a subset of cancer cells (sub-clonal mutation) might affect efficacy of molecular targeted treatments.[8] Limited data, however, are currently available on the potential impact of cancer cell clonality on anti-EGFR drug efficacy in mCRC.

CAPRI-2 GOIM is a prospective single-arm phase II trial which evaluates the efficacy of a programmed sequence of three lines of therapy in *RAS/BRAF* WT MSS mCRC patients. Treatment is defined by LBx-CGP at baseline of each line of therapy.[9] We have recently shown high levels of concordance between LBx-CGP and tumor tissue-CGP by using the same validated next-generation sequencing platform before first-line treatment in the CAPRI-2 GOIM trial.[10] Here we report the value of baseline LBx-CGP in identifying tumor molecular heterogeneity as well as cancer cell clonality, which could better predict FOLFIRI plus cetuximab efficacy. Furthermore, LBx-CGP at progressive disease (PD) identifies patients without anti-EGFR drug resistance mutations, who may benefit from EGFR therapeutic blockade beyond first-line progression.

METHODS

Study design and treatment

The CAPRI-2 GOIM study (NCT05312398) is a non-profit, academic, phase II trial, investigating a biomarker-driven use of anti-EGFR therapy across three different lines of therapies in patients with mCRC. Patients with *RAS/BRAF* WT, MSS (defined by per practice evaluation on sample biopsy by local laboratories) mCRC received FOLFIRI plus cetuximab as first-line treatment. Pretreatment plasma samples were analyzed using the FoundationOne Liquid (F1L) CDx test.[11,12] After PD to first- and second-line therapies, treatment choices are defined by LBx using the F1L CDx assay: FOLFOX plus cetuximab (second line) and irinotecan plus cetuximab (third line) in case of *RAS/BRAF* WT ctDNA; whereas patients with *RAS/BRAF*^{V600E} mutant (MUT) ctDNA receive FOLFOX plus bevacizumab or investigator choice (trifluridine/tipiracil or regorafenib) as second- or third-line treatment, respectively. All therapies are administered according to standard schedules and doses until PD or unacceptable toxicity.

The study is conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation and Good Clinical Practice Guidelines and has been approved by the Ethics Committee of Università degli Studi della Campania Luigi Vanvitelli/Azienda Ospedaliera Universitaria Luigi Vanvitelli/AORN Ospedale dei Colli and by the ethics committees of the other centers involved in the study. All patients have provided written informed consent to participate.

Full study protocol has been already published and is available as Supplementary Tables S1-S12, available at https://doi.org/10.1016/j.esmoop.2025.104511.[9]

Statistical analysis

The primary endpoint of the trial is the overall response rate (ORR), defined as the sum of partial responses and complete responses for each line of treatment according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Key secondary endpoints are: progression-free survival (PFS), overall survival, safety (adverse events graded

according to NCI CTCAE v 5.0.), and evaluation of LBx-CGP on plasma samples at different time-points.[13] An exploratory pre-planned analysis has been done to evaluate the clinical significance of cancer resistance mutations on clinical outcome. Further, analysis of molecular hyper-selection based on potential anti-EGFR drug resistance genes (*RAS/BRAF/EGFR/PIK3CA/MAP2K1/MET/RET/ALK/ROS1/NTRK/NF1/FGFR* mutations and *HER2* amplification) and of cancer cell clonality in predicting anti-EGFR drug efficacy has been conducted.

Continuous variables are reported as medians, interquartile range (IQR), and range, as appropriate, and categorical variables were summarized as frequency counts and proportions. Clonality of PVs was calculated normalizing the variant allele frequency (VAF) using the estimated tumor fraction (TF) assessed on ctDNA (VAF divided by ctDNA fraction). Categorical and continuous variables between groups were compared using the Fisher's exact test and the Wilcoxon—Mann—Whitney test, respectively. Correlation tests were carried out using the Kendall's test assuming a non-bivariate normal distribution of ctDNA. The emergence and clearance of baseline compared with post-progression PVs were computed using the McNemar's chi-square test, with *P* values corrected using the Benjamini—Hochberg procedure. Time-to-event endpoints were calculated using the Kaplan—Meier (KM) method with confidence intervals (CIs) computed using the Brookmeyer and Crowley method and compared between groups using log-rank statistics. Hazard ratios (HRs) were calculated using the Cox-proportional model, once verifying the proportional hazard assumption using Schoenfeld residuals. Median follow-up was calculated using the reverse KM method. The optimal cut-off of tumor clonality was calculated using the maximally selected rank statistic. Bootstrap CIs were computed using 1000 resamples. Inferential statistical tests were carried out using a two-tailed alpha value of 0.05. Statistical analyses were conducted using R Software version 4.3.2.

RESULTS

Study patient population

Between July 2021 and November 2023, 240 patients were screened; 48 patients were excluded, and 192 patients treated with FOLFIRI plus cetuximab were included in the trial, having baseline LBx-CGP by F1L CDx and measurable disease (see Figure 1 for details). Patient characteristics are reported in Table 1.

Efficacy of FOLFIRI plus cetuximab according to baseline molecular genomic profiling

In the study cohort of 192 patients, who were considered *RAS/BRAF* WT by local laboratory assessment, ORR was 71.3% (95% CI 64.3% to 77.5%). Some 13 out of 192 patients (6.8%) had PD as best response (Figure 2A). Therefore, the disease control rate (DCR) (stable disease plus OR) was 93.2% (95% CI 88.4% to 96.1%). With a median follow-up of 20.1 months (IQR 15.4-27.7 months), 133 events of PD were recorded, with median PFS (mPFS) of 11.2 months (95% CI 9.67-13.5 months) (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.104511). Subgroup analysis including known prognostic factors did not show any difference in PFS in different subpopulations (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2025.104511).

Baseline F1L CDx analysis allowed to better define the molecular landscape of these tumors and to more precisely identify FOLFIRI plus cetuximab efficacy. In fact, in patients with *RAS/BRAF* WT ctDNA, ORR was 75.0% (95% CI 67.8% to 81.0%) compared with 31.2% (95% CI 12.1% to 58.5%) for *RAS/BRAF*^{V600E} MUT ctDNA patients [odds ratio (OR), 6.52, 95% CI 1.96-25.31, $P = 0.0006$] (Figure 1B); with DCR of 95.5% (95% CI 90.9% to 97.8%) versus 68.8% (95% CI 41.4% to 87.7%), respectively ($P < 0.0001$). Patients with ctDNA without any potential anti-EGFR drug resistance mutation ('negatively hyper-selected' cases: *RAS/BRAF/EGFR/PIK3CA/MAP2K1/MET/RET/ALK/ROS1/NTRK/NF1/FGFR* WT and *HER2* not amplified) had 78.1% ORR (95% CI 70.0% to 84.5%) compared with 54.5% (95% CI 40.6% to 67.8%) in patients with any of these mutations (OR, 2.95, 95% CI 1.44-6.10, $P = 0.001$) (Figure 2C), while DCR was 96.4% (95% CI 91.2% to 98.6%) versus 85.5% (95% CI 72.7% to 93.0%), respectively ($P = 0.01$).

Interestingly, if the 16 *RAS/BRAF*^{V600E} MUT ctDNA cases were excluded from the analysis, a numerical difference was still observed between WT and MUT ctDNA for the other anti-EGFR drug resistance genes (ORR, 78.1%, 95% CI 70.1% to 84.5%) versus 64.1% (95% CI 47.2% to 78.3%), OR 1.99 (95% CI 0.85-4.57), $P = 0.093$ (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2025.104511). In addition, patients with *RAS/BRAF* WT ctDNA had mPFS of 11.83 months (95% CI 9.67-13.5 months) versus 3.56 months (95% CI 2.13-12.3 months) in patients with *RAS/BRAF*^{V600E} MUT ctDNA, (HR 0.32, 95% CI 0.19-0.56, $P < 0.0001$) (Figure 2D).

The mPFS was 12.35 months (95% CI 10.58-15.4 months) in negatively hyper-selected WT ctDNA patients compared with 8.68 months (95% CI 4.87-12.1 months) in patients with at least one mutation (HR 0.64, 95% CI 0.44-0.92, $P = 0.017$) (Figure 2E). After removing the patients with *RAS/BRAF*^{V600E} MUT ctDNA, a numerically better mPFS was also observed for patients with WT ctDNA compared with mutated ctDNA (12.35 months, 95% CI 10.58-15.4 months versus 9.61 months, 95% CI 7.97-20 months, HR 0.8, 95% CI 0.53-1.23, $P = 0.32$) (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2025.104511).

Impact of cancer cell clonality of molecular pathogenic variants on the efficacy of FOLFIRI plus cetuximab

At baseline, 64 anti-EGFR drug resistance PVs were detected by F1L CDx (Figure 3A). A total of 9 out of 192 tumors (4.7%) had multiple co-occurring PVs of resistance to anti-EGFR drugs, with 6 of them carrying both *RAS* and *BRAF* PV.

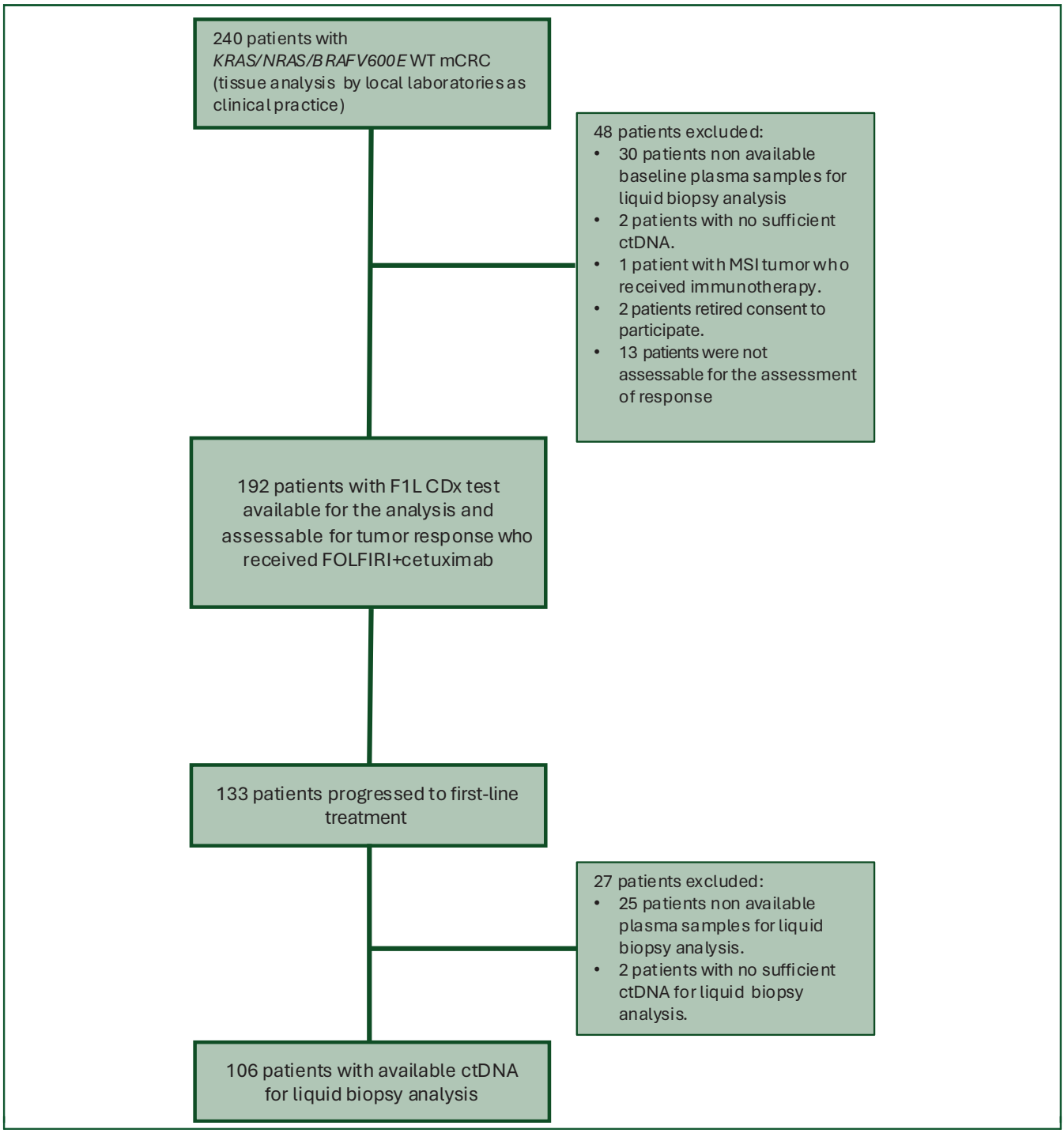

Figure 1. Study diagram of the CAPRI-2 GOIM study.
ctDNA, circulating tumor; mCRC, metastatic colorectal cancer; MSI, microsatellite instability; WT, wild-type.

Median VAF and median cancer cell clonality for 16 *RAS/BRAF*^{V600E} MUT ctDNA cases were 1.5% (IQR 0.28% to 24.0%) and 65.3% (IQR 1.9% to 84.4%), respectively. Of note, for 55 mutant cases for any of the extended anti-EGFR drug resistance genes, median VAF and median clonality were 1.6% (IQR 0.1% to 20.5%) and 17.0% (95% CI 1.1% to 87.3%), respectively (Figure 3B).

Patients experiencing PD as best response to FOLFIRI plus cetuximab had higher median clonality for both *RAS/BRAF*^{V600E} PVs (92.2%, 95% CI 63.8% to 100.0% versus 27.0%, 95% CI 1.5% to 66.4%, $P = 0.14$) and other anti-EGFR drug resistance PVs (61.4%, IQR 41.6% to 92.2% versus 17.0%, IQR 1.1% to 77.5%, $P = 0.10$). Moreover, only 1 out of 15 patients (6.7%) with cancer cell sub-clonal PVs (clonality below 10%) had PD as best response to FOLFIRI plus cetuximab compared with 7 of 40 patients (17.5%) with cancer cell clonal PVs who had PD (OR 0.34, 95% CI 0.01-3.09, $P = 0.4231$).

A direct correlation between increase in cancer cell clonal mutations and worse mPFS was observed for patients with

Table 1. Baseline patient characteristics. mets, metastases.

Patients' characteristic, *n* (%)	*N* = 192
Gender	
Female	74 (39)
Male	118 (61)
Age	65 (55-72)
Sidedness	
Left	140 (73)
Rectum	26 (14)
Right	20 (10)
Not specified	6 (3)
Synchronous	158 (82)
Metachronous	34 (18)
Number of metastatic sites	
≥3	40 (21)
1–2	152 (79)
Liver mets	
Yes	138 (72)
No	54 (28)
Lung mets	
Yes	55 (29)
No	137 (71)
Peritoneum mets	
Yes	36 (19)
No	156 (81)
Node mets	
Yes	77 (40)
No	115 (60)
Tumor fraction	
10%<	63 (33)
≥10%	129 (67)

RAS/BRAF^{V600E} MUT tumors (HR for each 10% increase in clonality 1.20, 95% CI 0.99-1.46). By using a minimal *P* value approach, an optimal cut-off of 27% for defining high or low cancer cell clonality for *RAS/BRAF*^{V600E} MUT cases was determined (bootstrap 95% CI 13.7% to 32.1%). In this respect, patients with high clonality *RAS/BRAF*^{V600E} MUT tumors had significantly worse mPFS compared with those with low clonality *RAS/BRAF*^{V600E} MUT tumors (3.43 months, 95% CI 2.10 months-NE versus 7.02 months, 95% CI 4.81 months-NE, HR 0.08, 95% CI 0.01-0.76, $P = 0.02$) (Figure 3C).

Similarly, for the group of patients with PVs for the other anti-EGFR drug resistance genes, an increase in cancer cell clonality of PVs was associated with a trend for worse mPFS (HR for each 10% increase in clonality 1.04, 95% CI 0.94-1.14, $P = 0.451$). Using a minimal *P* value approach, an optimal cut-off of 39.4% clonality was determined (95% bootstrap CI 9.7% to 34.2%). In fact, patients with high clonality mutations had worse mPFS (3.55 months, 95% CI 2.57 months-NE) compared with those with low clonality mutations (9.63 months, 95% CI 7.16 months-NE, $P = 0.21$) (Figure 3D).

Emergence and clearance of anti-EGFR drugs resistance molecular pathogenic variants at progressive disease

At PD after first-line therapy with FOLFIRI plus cetuximab, 108/133 (81.2%) patients had LBx-CGP test on post-treatment plasma samples. Among them, for two cases the F1L CDx test was not informative (failure rate 1.8%). For the other 106 cases, 157 PVs were detected. *KRAS* ($n = 52$), *EGFR extracellular domain* (*ECD*) ($n = 30$), *NRAS* ($n = 16$), and *BRAF* ($n = 15$) were the most common altered genes (Figure 4A). Considering multiple co-occurring gene mutations in the same tumor, *KRAS* ($n = 24$, 22.6%), *BRAF-nonV600* ($n = 12$, 11.3%), *NF1* ($n = 9$, 8.4%), and *EGFR ECD* ($n = 8$, 7.5%) represented the most common PVs that were detected at PD. Within the *RAS/BRAF*^{V600E} MUT group, *KRAS* was the most common acquired alteration at PD ($n = 16$, 17.7%), while *NRAS* and *BRAF*^{V600E} were found in four (4.4%) and two (2.2%) patients, respectively. Interestingly, they were found in cases that had also acquired co-occurring *KRAS* alteration.

A positive correlation between ctDNA TF and the number of anti-EGFR drug resistance PVs was observed (Kendall's tau 0.25, $P = 0.02$) (Figure 4B). Higher ctDNA TF was detected in cases with anti-EGFR drug resistance PVs compared with those without (29.0%, IQR 13.0% to 62.5% versus 8.65%, IQR 2.93% to 17.0%, $P < 0.0001$) and in patients with multiple acquired PVs compared with patients with a single acquired PV (50.5%, IQR 19.2% to 63.7% versus 21.0%, IQR 10.3% to 44.0%, $P = 0.03$).

Finally, if we evaluated the emergence of anti-EGFR drug resistance genes for each individual patient, *KRAS* ($n = 6$ pre-, $n = 22$ post-, $P < 0.001$, $q = 0.002$) and *EGFR ECD* ($n = 1$ pre-, $n = 9$ post-, $P = 0.01$, $q = 0.22$) were found as the most frequent acquired mutations in post-treatment samples at PD (Figure 4C).

We further determined the impact of single versus multiple acquired gene mutations at PD. A trend for better mPFS was observed for cases exhibiting multiple acquired *RAS/BRAF*^{V600E} PVs at PD (acquired single PV: 8.7 months, 95% CI 7.33 months-NE versus acquired co-occurring PVs: 11.84 months, 95% CI 8.45 months-NE, $P = 0.15$) (Figure 4D). These findings could be due to lower cancer cell clonality for cases with multiple *RAS/BRAF*^{V600E} PVs compared with cases with a single *RAS/BRAF*^{V600E} mutation [clonality of acquired co-occurring PVs: 0.5% (IQR 0.3% to 1.4%) versus clonality of acquired single PV: 3.9% (IQR 1.7% to 47.7%) versus clonality of baseline single PV 63.4% (IQR 35.5% to 70.5%), $P < 0.0001$] (Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2025.104511).

It has been previously hypothesized that baseline *RAS/BRAF* alteration could be cleared by chemotherapy, thus potentially restoring anti-EGFR drug sensitivity.[14] For 13 cases with baseline *RAS/BRAF*^{V600E} PVs, 11 (84.6%) patients maintained *RAS/BRAF*^{V600E} MUT ctDNA at LBx-CGP after PD. For one patient with sub-clonal *KRAS G12R* MUT ctDNA, and for one patient with *KRAS* copy number gain at baseline LBx-CGP, these PVs were not detected on post-progression tests (Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2025.104511). In addition, PV clearance was found at PD in eight patients with mutated tumors

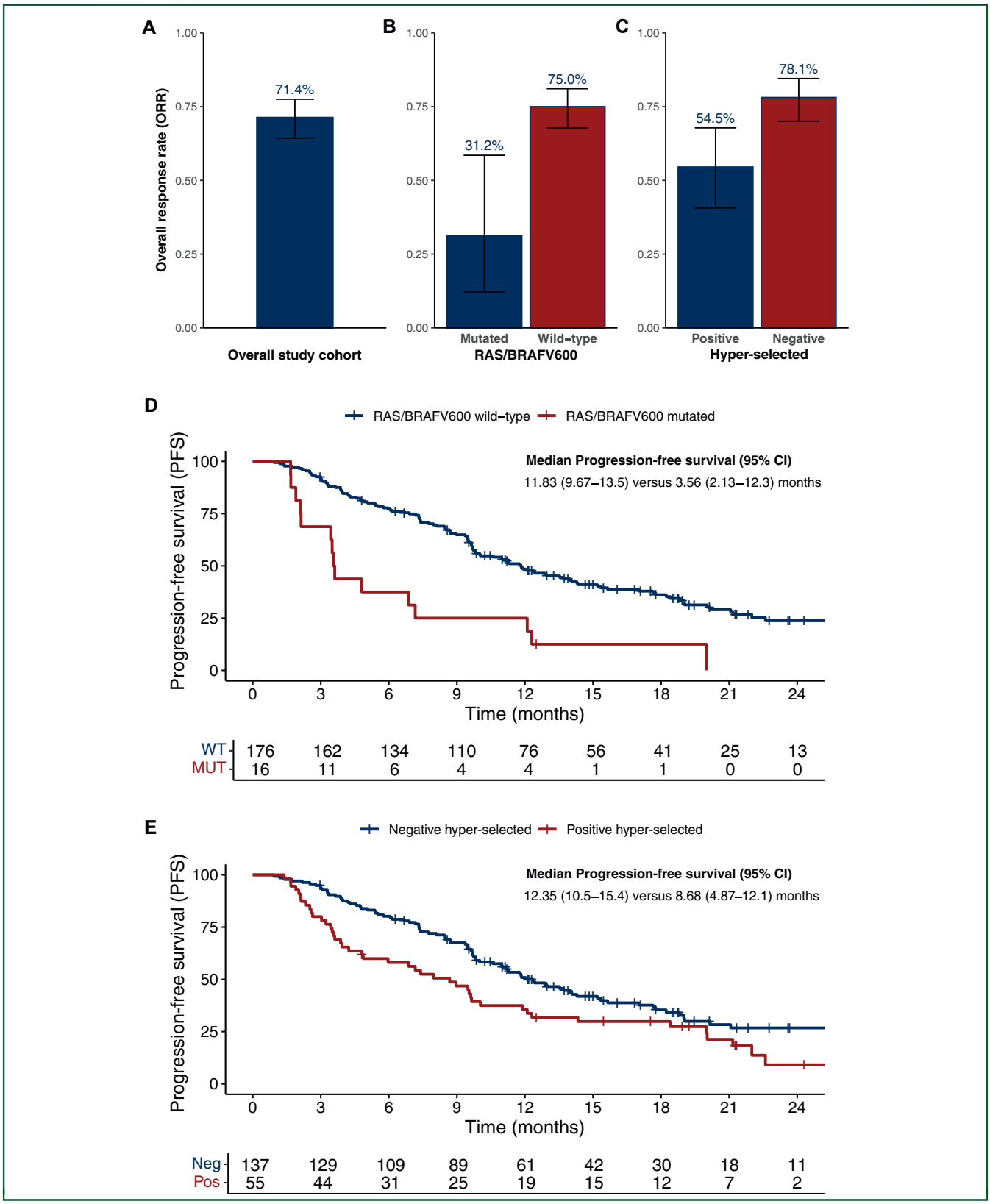

Figure 2. Overall response rate and progression-free survival according to baseline anti-EGFR resistance molecular pathogenic variants. Overall response rate in the overall study cohort (A) and according to *RAS/BRAF* (B) and to 'negatively hyper-selected' tumors (C). A higher response rate was observed for WT tumors compared with *RAS/BRAF*^{V600E} mutated [OR 6.52 (95% CI 1.96-25.31), $P = 0.0006$] and 'positive hyper-selected' mutated tumors [OR 2.95 (95% CI 1.44-6.10), $P = 0.001$]. Kaplan—Meier curves of PFS according to *RAS/BRAF* (D) and to 'negatively hyper-selected' tumors (E), with *RAS/BRAF*^{V600E} [HR 3.03 (95% CI 1.79-5.22), $P < 0.0001$] and 'positive hyper-selected' mutated tumors [HR 1.55 (95% CI 1.08-2.23), $P = 0.017$] have lower PFS compared with WT tumors.
CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; MUT, mutant; OR, odds ratio; PFS, progression-free survival; WT, wild-type.

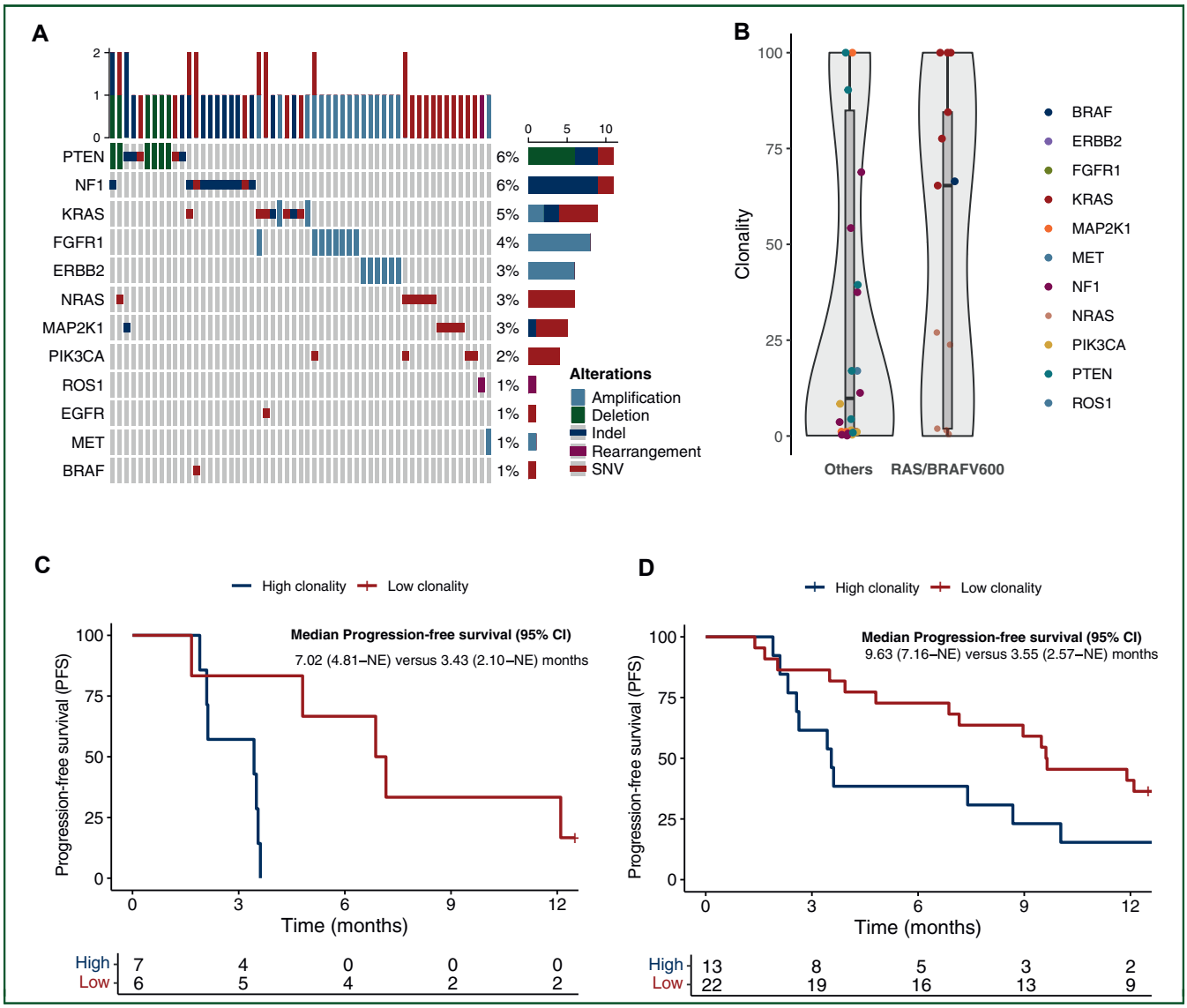

Figure 3. Prevalence and impact of baseline anti-EGFR resistance molecular pathogenic variants clonality. Oncoprint of baseline anti-EGFR resistance molecular PVs in 64/192 (33.3%) patients (A). Cancer cell clonality of *RAS/BRAF*^{V600E} and other anti-EGFR resistance PVs, with *RAS/BRAF*^{V600E} having higher clonality compared with other anti-EGFR resistance PVs (B). Association between PFS and cancer cell clonality of PV (C and D). Using an optimal cut-off of 27% for *RAS/BRAF*^{V600E} PVs, tumors with high-clonal *RAS/BRAF*^{V600E} PVs had lower PFS compared with tumors with low-clonal *RAS/BRAF*^{V600E} PVs ($P = 0.02$) (C). Using an optimal cut-off of 39% for all anti-EGFR drug resistance PVs, a similar negative impact of high-clonal PVs on PFS was observed ($P = 0.21$) (D).
CI, confidence interval; EGFR, epidermal growth factor receptor; NE, not evaluable; PFS, progression-free survival; PV, pathogenic variant; SNV, single nucleotide variant.

within the group of anti-EGFR drug resistance genes at baseline LBx-CGP. Notably, these patients had lower cancer cell clonal mutations compared with patients with tumors in which PVs were also maintained at PD LBx-CGP (0.65%, IQR 0.3% to 4.4% versus 65.3%, IQR 6.0% to 100%, $P = 0.018$) (Figure 4E).

Finally, among the 106 patients with F1L CDx test at PD, 93 had *RAS/BRAF* WT ctDNA at baseline. Of note, in 73/93 (78.5%) cases *RAS/BRAF* WT ctDNA was also found at PD, suggesting that the majority of patients after progression to FOLFIRI plus cetuximab could be potentially sensitive to EGFR therapeutic blockade and, therefore, treated with alternative chemotherapy (FOLFOX) plus cetuximab in second line (Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2025.104511). This approach is currently being investigated as second-line treatment in the CAPRI-2 GOIM trial.

DISCUSSION

Metastatic CRC is a multifaceted disease, which is characterized by a plethora of gene alterations.[1] They could be clonal PVs, which are present in most cancer cells and are generally 'driver' mutations, and/or sub-clonal PVs, which are present in a sub-group of cancer cells and are generally 'passenger' mutations. Further, cancer cells may accumulate

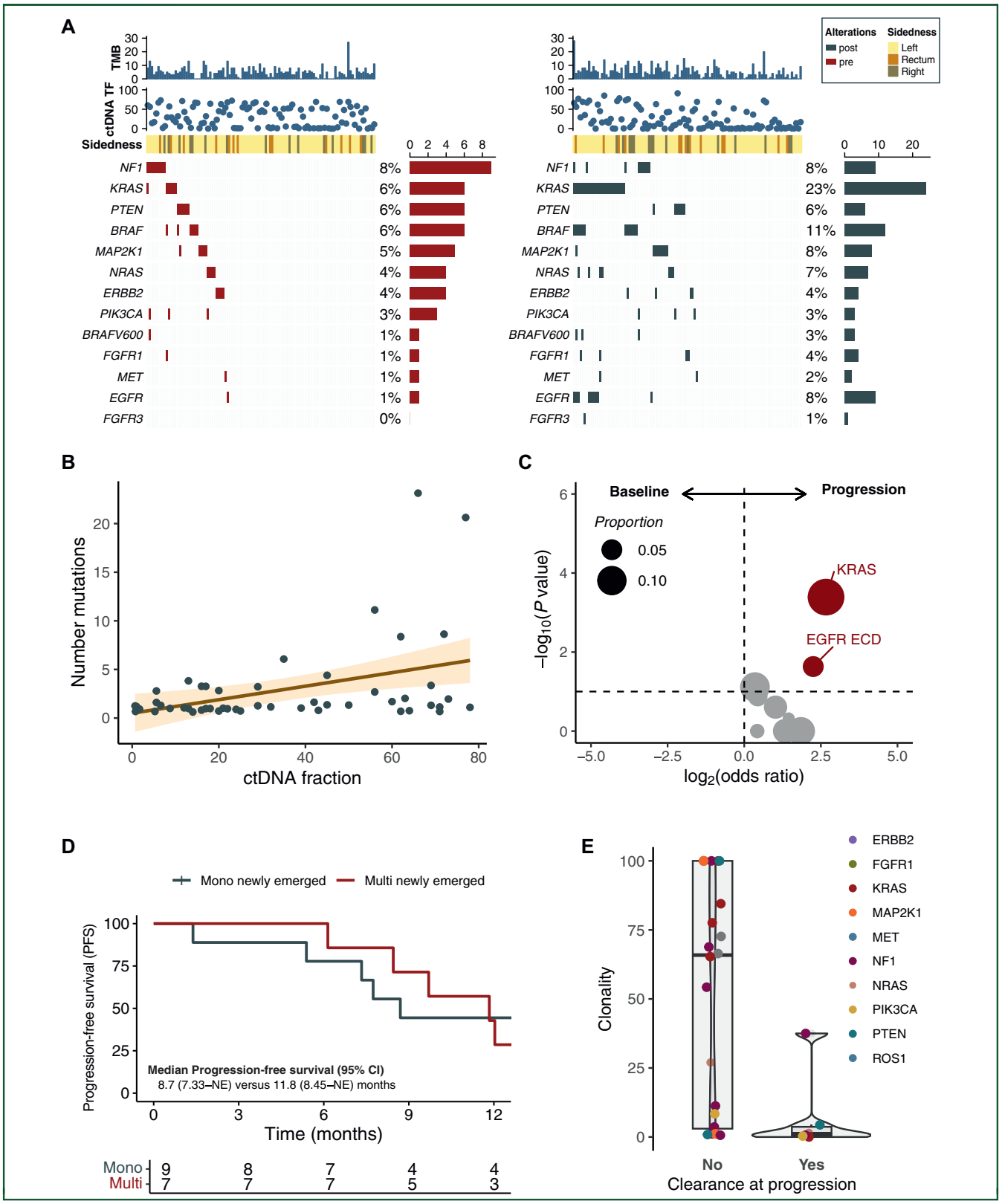

Figure 4. Emergence of anti-EGFR drug resistance molecular pathogenic variant at disease progression to FOLFIRI plus cetuximab. Oncoprints comparing the frequency of anti-EGFR drug resistance PV at baseline (left) and at disease progression (right) among 106 cases with matched baseline and post-progression F1L CDx test, with tumor sidedness, TMB, and ctDNA TF annotations on top of the oncoprints matrix (A). Correlation between ctDNA TF and number of anti-EGFR drug resistance PVs: higher numbers of PVs were observed in case with higher ctDNA (Kendall's tau 0.25, $P = 0.02$) (B). Prevalence of anti-EGFR drug resistance PV between baseline and post-progression samples. A statistically significant enrichment in post-progression compared with baseline samples was observed for *KRAS* ($P < 0.001$, $q = 0.002$) and *EGFR ECD* ($P = 0.01$, $q = 0.22$). Horizontal dotted line represents an alpha value of 5% (C). Kaplan–Meier plot of PFS for cases with acquired *RAS/BRAF*^{V600E} PVs: multiple RAS/BRAF^{V600E} PVs had trend for worse PFS ($P = 0.15$). Cases with baseline *RAS/BRAF*^{V600E} PVs were excluded from the analysis (D). Association between cancer cell clonality and clearance or persistence of PVs between baseline and disease progression time-points. For cases with cleared anti-EGFR

additional mutations under the selective pressure of anticancer therapies. This spatial and temporal tumor molecular heterogeneity might impair the efficacy of targeted therapies, including anti-EGFR drugs.[15,16] LBx-based CGP may portray the complex tumor molecular landscape, by identifying the cancer clones with PVs that are responsible for therapy resistance and for disease progression. In fact, an intrinsic advantage of LBx-CGP is the possibility to evaluate a broader range of PVs, to estimate the ctDNA TF and to estimate the clonality of cancer cells with a specific gene alteration.

The results of the first-line therapy with FOLFIRI plus cetuximab within the CAPRI-2 GOIM trial support the clinical utility of baseline LBx-CGP for implementing efficacy of anticancer treatment in *RAS/BRAF* WT MSS mCRC. In agreement with the results of previous retrospective studies,[7,17,18] the prospective CAPRI-2 GOIM trial demonstrates that extended molecular selection of genes potentially involved in EGFR drug resistance before first-line treatment could identify patients who highly benefit from EGFR therapeutic blockade. The ORR, DCR, and mPFS for these appropriately selected mCRC patients were 78.1%, 96.4%, and 12.35 months, respectively.

Interestingly, we observed that not all mutations had the same impact on the efficacy of FOLFIRI plus cetuximab. The sub-group of patients with *RAS/BRAF*^{V600E} MUT ctDNA had poor outcome with mPFS $<$4 months and ORR of $\sim$30%, which are lower than expected for FOLFIRI alone in mCRC.[19,20] This may be since the majority of patients with *RAS/BRAF*^{V600E} MUT ctDNA had higher clonality that could be correlated with a more aggressive disease. Therefore, for these patients with poor prognosis a tailored treatment with selective KRAS or BRAF^{V600E} inhibitors (if available) plus chemotherapy or the use of triplet chemotherapy might be a potential therapeutic option.

Moreover, we observed that, after excluding patients with *RAS/BRAF*^{V600E} PVs, patients with any mutation in the other genes which could determine anti-EGFR drug resistance achieved 64% ORR with 9 months mPFS. These findings support the concept that there is a hierarchy in the role of potential anti-EGFR drug resistance mutations. In this respect, the CAPRI-2 GOIM trial suggests that patients with PVs at low cancer cell clonality for genes other than *RAS/BRAF* might still benefit from anti-EGFR therapeutic blockade. Taken together, these findings support the concept that cancer cell clonality of anti-EGFR drug resistance mutations is the most relevant clinical determinant for appropriate molecular targeted therapy selection in these patients.

Of course, it should be noted that around one-third of the patients displayed a TF $<$10 and that could have an impact when trying to estimate the clonality. Moreover, the limited sample sizes might have represented a potential bias when calculating the optimal cut-off.

Nevertheless, in the analysis by Nakamura and colleagues,[8] a cut-off for clonality of 40% predicted the activity of target therapies. Within the obvious limitation of this kind of indirect comparison, our data suggest that a cut-off of 39% might help to predict the unresponsiveness to anti-EGFR therapies. Of course, this hypothesis should be considered with caution and requires further validation.

Emerging evidence indicates that after failure of first-line treatments, there is a subset of mCRC patients who maintain *RAS/BRAF* WT ctDNA and, therefore, could benefit from EGFR inhibition beyond progression.[21-25] LBx-CGP at PD may allow to identify this population.[6,9,12] Here we report that anti-EGFR drug resistance mutations are found only in a minority of patients at FOLFIRI plus cetuximab therapy failure. In fact, *RAS/BRAF* WT ctDNA was detected in approximately four out of five patients at PD. These data are in line with the retrospective and exploratory analysis by Parseghian and colleagues.[26] The authors reported that only 9% of the patients progressing to chemotherapy plus anti-EGFR will develop *RAS/BRAF* alterations. According to the CAPRI-2 GOIM trial design, these patients switch the chemotherapy backbone from FOLFIRI to FOLFOX and continue to receive cetuximab as second-line therapy.[9] This part of the study is currently on-going. Furthermore, we are planning to conduct a phase III randomized study (CAPRI-III GOIM-TTD) in LBx molecularly hyper-selected tumors to validate this treatment strategy.

Finally, we investigated the cancer cell clonality of anti-EGFR drug resistance PVs, that emerged as acquired mutations at PD. Patients with multiple, but sub-clonal PVs had longer PFS compared with patients who develop a single clonal PV. Therefore, that acquired sub-clonal PV may be a 'passenger' and transient mutations could have minor impact on anti-EGFR drug resistance. Moreover, these gene alterations may be cleared during second-line therapies with non-cross-resistant chemotherapy and without anti-EGFR inhibitors.[27,28] As result, *RAS/BRAF* WT ctDNA could be found at second-line or further-line/s disease progression in these patients, who could benefit from subsequent anti-EGFR drug rechallenge therapy.[29]

The present study has some limitations. In fact, despite the biological and clinical rationale, the internal validity of the results and the consistency with previous reports, the relatively small sample size of a prospective single-arm phase II trial, and the exploratory nature of the analysis of cancer cell clonality on treatment efficacy, render these findings, although of clinical relevance, as hypothesis generating. In this respect, further studies are warranted to define the optimal threshold of clonality to predict the efficacy of anti-EGFR therapies and implement LBX-CGP use in practice.

drug resistance PVs, low cancer cell clonality was observed compared with high cancer cell clonality of PVs which were maintained at disease progression [0.65% (interquartile range 0.3% to 4.4%) versus 65.3% (interquartile range 6.0% to 100%), $P = 0.018$] (E).

CI, confidence interval; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; NE, not evaluable; PFS, progression-free survival; PV, pathogenic variant; TF, tumor fraction; TMB, tumor mutational burden.

CONCLUSION

The CAPRI-2 GOIM trial provides novel evidence on the role of LBx-CGP in the management of *RAS/BRAF* WT mCRC. LBx captures the heterogenous molecular profile of mCRC and allows to predict FOLFIRI plus cetuximab therapeutic efficacy. Furthermore, longitudinal ctDNA evaluation may guide the choice of tailored therapies, including the identification of patients who could benefit from maintaining anti-EGFR drugs while switching chemotherapy backbone across subsequent lines of treatment.

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DC: reported receiving travel support from Merck KGaA, Sanofi, and Bristol Myers Squibb (BMS); advisory board Bayer outside the submitted work.

SN: reported receiving personal fees from Novartis and a travel grant from Amgen outside the submitted work.

RB: reported receiving honoraria: Novartis, AstraZeneca, Sanofi, Amgen, Roche, Pfizer, Janssen-Cilag, and BMS; consulting or advisory role: Novartis, Bayer, AstraZeneca, Sanofi, Amgen, Roche, Pfizer, Janssen-Cilag, and BMS; speakers' bureau: AstraZeneca, Sanofi, Novartis, Bayer, Amgen, Roche, Pfizer, Janssen-Cilag, and BMS.

SL: reported financial interests, personal, advisory board: Amgen, Merck Serono, Lilly, Servier, AstraZeneca, Merck Sharp & Dohme (MSD), Incyte, Daiichi Sankyo, BMS, Astellas, GlaxoSmithKline (GSK), Takeda, Bayer; financial interests, personal, invited speaker: Pierre Fabre, GSK, Roche, Servier, Amgen, BMS, Incyte, Lilly, Merck Serono, MSD; financial interests, institutional, invited speaker: Amgen, Merck Serono, Bayer, Roche, Lilly, AstraZeneca, BMS; non-financial interests, member of board of directors, 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.

GT: reported consulting or advisory role for BMS, AstraZeneca, Merck, MSD, Servier.

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EM: reported serving as advisor and speaker for AstraZeneca, Eli Lilly, Servier, Sanofi Genzyme, Roche, Merck, Eisai, and Pfizer outside the submitted work.

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All other authors have declared no conflicts of interest.

DATA SHARING

Researchers can request access to de-identified individual patient-level data from the corresponding author on a reasonable request.

REFERENCES

1. Ciardiello F, Ciardiello D, Martini G, Napolitano S, Tabernero J, Cervantes A. Clinical management of metastatic colorectal cancer in the era of precision medicine. *CA Cancer J Clin*. 2022;72(4):372-401.
2. Cervantes A, Adam R, Roselló S, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34(1):10-32.
3. Morris VK, Kennedy EB, Baxter NN, et al. Treatment of metastatic colorectal cancer: ASCO Guideline. *J Clin Oncol*. 2023;41(3):678-700.
4. Ciardiello F, Normanno N, Maiello E, et al. Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRI-GOIM trial. *Ann Oncol*. 2014;25(9):1756-1761.
5. Randon G, Maddalena G, Germani MM, et al. Negative ultraselection of patients with RAS/BRAF wild-type, microsatellite-stable metastatic colorectal cancer receiving anti-EGFR-based therapy. *JCO Precis Oncol*. 2022;6:e2200037 [Erratum in: *JCO Precis Oncol.* 2022 Jul;6:e2200356. https://doi.org/10.1200/PO.22.00356.
6. Patelli G, Mauri G, Tosi F, et al. Circulating tumor DNA to drive treatment in metastatic colorectal cancer. *Clin Cancer Res*. 2023;29(22):4530-4539.
7. Shitara K, Muro K, Watanabe J, et al. Baseline ctDNA gene alterations as a biomarker of survival after panitumumab and chemotherapy in metastatic colorectal cancer. *Nat Med*. 2024;30(3):730-739.
8. Nakamura Y, Ozaki H, Ueno M, et al. Targeted therapy guided by circulating tumor DNA analysis in advanced gastrointestinal tumors. *Nat Med*. 2025;31:165-175.
9. Martini G, Ciardiello D, Napolitano S, et al. Efficacy and safety of a biomarker-driven cetuximab-based treatment regimen over 3 treatment lines in mCRC patients with RAS/BRAF wild type tumors at start of first line: the CAPRI 2 GOIM trial. *Front Oncol*. 2023;13:1069370.
10. Ciardiello D, Boscolo Bielo L, Napolitano S, et al. Comprehensive genomic profiling by liquid biopsy captures tumor heterogeneity and identifies cancer vulnerabilities in patients with RAS/BRAFV600E wild-type metastatic colorectal cancer in the CAPRI 2-GOIM trial. *Ann Oncol*. 2024;35:1105-1115.
11. Woodhouse R, Li M, Hughes J, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLoS One*. 2020;15(9):e0237802.
12. Caputo V, De Falco V, Ventriglia A, et al. Comprehensive genome profiling by next generation sequencing of circulating tumor DNA in solid tumors: a single academic institution experience. *Ther Adv Med Oncol*. 2022;14:17588359221096878.
13. Division of Cancer Treatment & Diagnosis. Cancer Therapy Evaluation Program (CTEP). Adverse Events/CTCAE. Available at https://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm. Accessed December 1, 2024.
14. Osumi H, Shinozaki E, Nakamura Y, et al. Clinical features associated with NeoRAS wild-type metastatic colorectal cancer A SCRUM-Japan GOZILA substudy. *Nat Commun*. 2024;15(1):5885.
15. Normanno N, Rachiglio AM, Lambiase M, et al. Heterogeneity of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer and potential effects on therapy in the CAPRI GOIM trial. *Ann Oncol*. 2015;26(8):1710-1714.
16. Morano F, Corallo S, Lonardi S, et al. Negative hyperselection of patients with RAS and BRAF wild-type metastatic colorectal cancer who received panitumumab-based maintenance therapy. *J Clin Oncol*. 2019;37(33):3099-3110.
17. Manca P, Corallo S, Busico A, et al. The added value of baseline circulating tumor DNA profiling in patients with molecularly hyper-selected, left-sided metastatic colorectal cancer. *Clin Cancer Res*. 2021;27(9):2505-2514.
18. Stintzing S, Heinemann V, Fischer Von Weikersthal L, et al. Phase III FIRE-4 study (AIO KRK-0114): influence of baseline liquid biopsy results in first-line treatment efficacy of FOLFIRI/cetuximab in patients with tissue RAS-WT mCRC. *J Clin Oncol*. 2023;41(suppl 16):3507. 3507.
19. Van Cutsem E, Lenz HJ, Köhne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol*. 2015;33(7):692-700.
20. Bokemeyer C, Köhne CH, Ciardiello F, et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. *Eur J Cancer*. 2015;51(10):1243-1252.
21. Ciardiello F, Normanno N, Martinelli E, et al. Cetuximab continuation after first progression in metastatic colorectal cancer (CAPRI-GOIM): a randomized phase II trial of FOLFOX plus cetuximab versus FOLFOX. *Ann Oncol*. 2016;27(6):1055-1061.
22. Aparicio J, Virgili Manrique AC, Capdevila J, et al. Randomized phase II trial of FOLFIRI-panitumumab compared with FOLFIRI alone in patients with RAS wild-type circulating tumor DNA metastatic colorectal cancer beyond progression to first-line FOLFOX-panitumumab: the BEYOND study (GEMCAD 17-01). *Clin Transl Oncol*. 2022;24(11):2155-2165.
23. Martinelli E, Ciardiello D, Martini G, et al. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: challenges and future perspectives. *Ann Oncol*. 2020;31(1):30-40.
24. Sartore-Bianchi A, Pietrantonio F, Lonardi S, et al. Circulating tumor DNA to guide rechallenge with panitumumab in metastatic colorectal cancer: the phase 2 CHRONOS trial. *Nat Med*. 2022;28(8):1612-1618.
25. Ciardiello D, Martinelli E, Troiani T, et al. Anti-EGFR rechallenge in patients with refractory ctDNA RAS/BRAF wt metastatic colorectal cancer: a nonrandomized controlled trial. *JAMA Netw Open*. 2024;7(4):e245635 [Erratum in: *JAMA Netw Open.* 2024 May 1;7(5):e2418437. https://doi.org/10.1001/jamanetworkopen.2024.18437.
26. Parseghian CM, Sun R, Woods M, et al. Resistance mechanisms to anti-epidermal growth factor receptor therapy in RAS/RAF wild-type colorectal cancer vary by regimen and line of therapy. *J Clin Oncol*. 2023;41(3):460-471.
27. Morelli MP, Overman MJ, Dasari A, et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. *Ann Oncol*. 2015;26(4):731-736.
28. Parseghian CM, Loree JM, Morris VK, et al. Anti-EGFR-resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge. *Ann Oncol*. 2019;30(2):243-249.
29. Ciardiello D, Mauri G, Sartore-Bianchi A, et al. The role of anti-EGFR rechallenge in metastatic colorectal cancer, from available data to future developments: a systematic review. *Cancer Treat Rev*. 2024;124:102683.