The added value of baseline circulating tumor DNA profiling in patients with molecularly hyperselected, left-sided metastatic colorectal cancer

Running title: ctDNA profiling in Valentino trial

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Conflict of Interest statement

Dr Lonardi reports grants and personal fees from Amgen, grants and personal fees from Merck Serono, grants and personal fees from Astra-Zeneca, grants and personal fees from BMS, grants and personal fees from Lilly, grants from Bayer, grants and personal fees from Roche, personal fees from Incyte, personal fees from Daiichi-Sankyo, personal fees from Servier, personal fees from Pierre-Fabre, and personal fees from GSK outside the submitted work. Dr Manca reports grants from amgen during the conduct of the study. Dr Morano reports personal fees from SERVIER outside the submitted work. Dr Racca reports grants from Merck and grants from Amgen outside the submitted work. Dr Pietrantonio reports personal fees from Roche, personal fees from Sanofi, personal fees from Merck-Serono, grants from BMS, personal fees from Servier, personal fees from Bayer, personal fees from Lilly, and personal fees from Amgen outside the submitted work. Dr Sartore-Bianchi reports personal fees from Amgen, personal fees from Bayer, personal fees from Sanofi, and personal fees from Servier outside the submitted work. All other authors declared no conflicts of interests.
Abstract

Background: The routine use of liquid biopsy is not recommended for the choice of initial treatment of patients with metastatic colorectal cancer (mCRC).

Experimental design: We included patients with left-sided, RAS/BRAF wild-type, HER2-negative and microsatellite stable mCRC, treated with upfront FOLFOX-panitumumab in the Valentino study. We performed amplicon-based genomic profiling of 14 genes in baseline plasma samples and compared these data with tumor tissue ultra-deep sequencing results. Specific gene mutations in ctDNA and their clonality were associated with PFS, OS and radiological dynamics.

Results: Ten and 15 out of 120 patients had a mutation of RAS and PIK3CA in ctDNA, with a positive concordance with tissue deep-sequencing of only 31.3% and 47.1%, respectively. Presence of RAS or PIK3CA mutations in baseline ctDNA was associated with worse median PFS (8.0 vs. 12.8 months; HR=2.49, 95%CI: 1.28-4.81, p=0.007; 8.5 vs 12.9 months; HR=2.86, 95%CI: 1.63-5.04, p<0.001) and median OS (17.1 vs. 36.5 months; HR=2.26, 95%CI: 1.03-4.96, p=0.042; 21.1 vs 38.9 months; HR=2.18, 95%CI: 1.16-4.07, p=0.015). RAS mutations in ctDNA were associated with worse RECIST response, early tumor shrinkage and depth of response, while PIK3CA mutations were not. Patients with higher levels of RAS/PIK3CA variant allele fraction (VAF) in ctDNA had the worst outcomes (VAF ≥5% vs all-wild-type: median PFS: 7.7 vs 13.1 months, HR: 4.02, 95%CI: 2.03-7.95, p<0.001; median OS: 18.8 vs 38.9 months, HR: 4.07, 95%CI: 2.04-8.12, p<0.001).

Conclusion: Baseline ctDNA profiling may add value to tumor tissue testing to refine the molecular hyperselection of mCRC patients for upfront anti-EGFR-based strategies.
Statement of Translational Relevance

In this pre-specified exploratory analysis of the Valentino trial, next-generation sequencing of ctDNA obtained from baseline liquid biopsies allowed to detect new RAS or PIK3CA mutations in patients treated with upfront anti-EGFR therapy for metastatic colorectal cancer. Patients with left-sided, hyperselected mCRC receiving FOLFOX/panitumumab and with RAS/PIK3CA mutations in ctDNA had worse outcomes, particularly those with variant allele fraction $\geq 5\%$. Liquid biopsy may allow to refine the molecular selection of patients with highest likelihood of benefit from EGFR blockade as compared to tumor tissue profiling alone and it may guide decisions on upfront therapy in patients with mCRC.
Introduction

Anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab are guideline-recommended treatments for patients with RAS/BRAF wild-type metastatic colorectal cancer (mCRC).\(^1\) Both primary tumor sidedness and molecular hyperselection beyond RAS and BRAF status allow to select patients with the highest likelihood of benefit from EGFR inhibition, i.e. those with left-sided tumors and absence of rare genomic alterations associated with primary resistance (HER2/MET amplification, gene fusions, PIK3CA mutations and microsatellite instability [MSI]).\(^2,3\) Tumor tissue profiling still represents the mainstay for the detection of such alterations, but it is limited by spatial and temporal genomic heterogeneity.

In the setting of acquired resistance to EGFR blockade, liquid biopsy (LB) overcomes such limitations as it allows to capture the dynamics of clonal evolution with greater precision compared to tumor re-biopsies.\(^4,5\) Specifically, the emergence of resistance alterations (more frequently involving RAS) under the selective pressure of therapy may be related to the expansion of pre-existing resistant tumor subclones, while their decay during post-progression treatments sustains the use of LB to select patients with potential benefit from anti-EGFR re-treatment.\(^6,7\)

Nevertheless, limited data are available on the role of LB to predict the outcomes of patients clinically eligible for an anti-EGFR-based upfront treatment (Supplementary Table 1).\(^8\) Indeed, some mechanisms of primary resistance to anti-EGFR therapy - such as HER2 amplification or gene fusions - are usually clonal alterations with the role of oncogenic drivers and may be easily assessed in tumor tissue. Other alterations - such as RAS and PI3KCA mutations - may be subclonal, with a variant allele fraction (VAF) below 5% or even 1%, and therefore they may be missed by several standard assays and only detectable by high-sensitivity techniques such as next generation sequencing (NGS) with high depth coverage.\(^3\) On top of this, such mutations may be highly heterogeneous also from a spatial point of view, with the chance of being detected only by means of LB. Therefore, genomic profiling of baseline circulating tumor DNA (ctDNA) may
increase the detection of anti-EGFR resistance alterations and it may allow to quantify their clonality and VAF, with potential association to treatment outcomes.\textsuperscript{4,8}

In this pre-specified exploratory analysis of the Valentino trial\textsuperscript{9}, we aimed at assessing the added value of baseline ctDNA NGS profiling in patients with molecularly hyperselected, left-sided mCRC receiving a panitumumab-based upfront treatment strategy.

**Methods**

**Patients’ cohort**

The Valentino study (NCT02476045) was a multicenter, randomized, open-label phase 2 trial that investigated the progression-free survival (PFS) non-inferiority of maintenance with s panitumumab (Arm B) versus panitumumab plus 5-FU/LV (arm A) following an induction treatment with panitumumab/FOLFOX-4 in patients with RAS wild-type mCRC.\textsuperscript{9} In patients signing an optional consent form, LBs were collected at baseline and every 8 weeks during treatment (independently of delays of treatment cycles) until disease progression, consent withdrawal or death.

In this pre-specified exploratory analysis, we included all clinically evaluable randomized patients with available baseline LBs, molecularly hyperselected (RAS and BRAF wild-type based on standard assays, HER2-negative and microsatellite stable status) and left-sided tumors, who are therefore highly enriched for EGFR-dependent cells in their tumors’ bulk.

RECIST 1.1 response, early tumor shrinkage (ETS) and depth of response (DoR) were assessed by blinded independent central review, as previously described\textsuperscript{10}. Available tumor profiling data included: HER2 status assessed by both immunohistochemistry and silver in-situ hybridization, MSI status by multiplex PCR and presence of RAS mutations with low VAF (i.e. <5% and therefore missed by several standard assays or usually filtered in standard NGS algorithms) and PIK3CA mutations by centrally-performed ultra-deep NGS.\textsuperscript{3}
Institutional review board approval was obtained from all participating Centers and all patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

**Next generation sequencing of plasma samples**

Cell-free DNA (cfDNA) was isolated and quantified from 1.5 mL of plasma using Maxwell RSC cfDNA plasma kit (Promega) and quantified using the Qubit® dsDNA HS Assay Kit (ThermoFisher). Targeted libraries were performed using Oncomine™ Colon cfDNA Assay (ThermoFisher) that amplifies 48 amplicons covering about 240 key hotspot mutations from 14 genes that are frequently mutated in colorectal cancers (AKT1, APC BRAF, CTNNB1, EGFR, ERBB2, FBXW7, GNAS, KRAS, MAP2K1, NRAS, PIK3CA, MAD4, TP53). An amount of 2-50 ng of cfDNA was used to prepare targeted libraries; briefly, each cfDNA molecule was assigned with unique molecular tag through a first PCR reaction in a Veriti™ thermal cycler and subsequently, tagged library fragments were amplified in a second round of PCR to produce independent barcoded libraries. Sequencing was carried out on the Ion GeneStudio™ S5 Prime system. For sensitive variant detection down to 0.1% VAF, we targeted a Median Read Coverage > 25000, Median Molecular Coverage > 2500 and Targets >0.8 Median Molecular Coverage >60%. Sequencing reads that shared the same molecular barcode information were grouped into a single family; to reduce potential false positives, a variant was called when at least two molecular barcode families shared the same mutation, corresponding to two independent mutated alleles.

**Statistical analysis**

No formal statistical hypothesis was done due to exploratory nature of this study. Progression-free survival (PFS) was defined as the time from randomization to documentation of progressive disease (PD)/death; overall survival (OS) was defined as the time from randomization to death from any cause. Both parameters were censored at the last follow-up for event-free subjects. Chi-squared and Fisher’s exact tests were used to test the distribution of categorical data. Mann-Whitney U test was used for the comparisons of continuous non-parametric data. Univariate cox regressions were used
to model right-censored variables. Unidimensional k-means algorithm was used to model the VAF cut-off of relevant resistance mutations and its correlation with outcomes. Data were imported and handled in R v 3.6.1, using ggplot2, dplyr, survminer, survival and finalfit packages.

**Results**

**Patients population**

Supplementary Figure 1 shows the patients’ flow chart in this study. A total of 120 patients with available and evaluable plasma samples had left sided, RAS and BRAF wild-type, HER2-negative and MSS cancers. Figure 1A shows the heatmap of all the alterations detected in ctDNA in the final cohort. Regarding genes with putative role in resistance to anti-EGFR therapy, 10 and 15 patients had RAS and PI3KCA mutations in LB, respectively, with only 4 patients among these showing concomitant RAS and PI3KCA mutations. Table 1 shows the specific RAS and/or PI3KCA mutations, as well as “founder”/clonal mutations such as TP53 or APC, with the VAF of each mutation in ctDNA, and RAS and PI3KCA status in matching tumor tissue. Supplementary Figure 2 reports the VAF distribution for each mutation, reaching the highest median value for TP53 and the lowest for RAS, while Supplementary Figure 3 shows the VAF distribution of relevant resistance alterations in RAS and PIK3CA, which was used to build the VAF cut-off for survival analyses. Supplementary Table 2 shows the patients’ and disease baseline characteristics in the final study cohort, without significant differences according to RAS or PI3KCA mutational status in LB.

**Prognostic impact of mutations in ctDNA**

Figure 1B and 1C resume the impact of each gene mutation detected in ctDNA in terms of PFS and OS, respectively. Regarding genes with putative role in resistance to anti-EGFR therapy, both PFS and OS were significantly worse in patients bearing a RAS mutation in ctDNA vs. RAS wild-type subgroup (mPFS: 8.0 vs. 12.8 months; HR=2.49, 95%CI: 1.28-4.81, p=0.007; mOS: 17.1 vs. 36.5 months; HR=2.26, 95%CI: 1.03-4.96, p=0.042; Figure 2A-B). Similar results were observed in
patients bearing a PIK3CA mutation in ctDNA vs. wild-type subgroup (mPFS: 8.5 vs 12.9 months; HR=2.86, 95%CI: 1.63-5.04, p<0.001; mOS: 21.1 vs 38.9 months; HR=2.18, 95%CI: 1.16-4.07, p=0.015; Figure 2C-D). Despite the low numerosity, a trend for a worse PFS and OS could be observed in patients bearing simultaneously PIK3CA and KRAS mutations compared to patients carrying only one of them (Supplementary Figure 4). Table 2 shows the impact of RAS and PI3KCA status in ctDNA in terms of tumor response dynamics; notably, the presence of RAS mutations in baseline ctDNA was significantly associated with overall response rate according to RECIST v1.1. and ETS, and trended to significance for association with DoR, while PI3KCA status was not associated with none of these parameters.

Comparison of liquid biopsy and tumor tissue NGS

All 120 patients had available tissue NGS deep-sequencing data, as previously reported.[3] Supplementary Figure 5 shows the concordance between liquid biopsy and tissue NGS in terms of RAS (panel A) and PI3KCA (panel B) mutations. Overall concordance was 109/120 (90.8%) for RAS status and 111/120 (92.5%) for PI3KCA status; however, positive concordance in mutated samples (in liquid biopsy and/or tumor tissue) was only 5/16 (31.3%) for RAS mutations and 8/17 (47.1%) for PI3KCA mutations. Patients with RAS or PI3KCA mutations found only in tumor tissue and not in liquid biopsy were very few and their outcomes could not be investigated as a unique subgroup, although they did not seem to show poorer individual outcomes (Supplementary Figure 6).

Role of RAS and PI3KCA variant allele fraction in liquid biopsy

Median VAF of RAS and PIK3CA mutations in LB were 1.3% (IQR, 1.1-14.3%) and 17.1% (IQR 1.3-29.3%), respectively. Expectedly, the median VAF of such mutations in LB was higher in patients with positive concordance with tissue NGS vs. those with undetectable tissue mutations (median RAS VAF: 17.8% vs 1.1% and median PIK3CA VAF: 27.8% vs 1.2%; Mann-Whitney test p=0.056 and p=0.004; Figure 3A,D). RAS VAF was also significantly associated with ETS.
(p=0.011, **Figure 3B**) and DoR (p=0.073, **Figure 3C**). Such associations for **PIK3CA** VAF status were not significant (**Figure 3E,F**).

Exploiting the VAFs cutoff derived upon their distribution, we then split patients with **RAS** and/or **PIK3CA** mutations with VAF of at least 5% in LB (“clonal” subgroup) versus those with mutations with VAF<5% (“subclonal” subgroup) and those without mutations in ctDNA. Compared to the all wild-type one, the “clonal” subgroup had the worst PFS (mPFS: 7.7 vs 13.1, HR: 4.02, 95%CI: 2.03-7.95, p<0.001; **Figure 4A**) and OS (mOS: 18.8 vs 38.9, HR: 4.07, 95%CI: 2.04-8.12, p<0.001; **Figure 4B**).

**Discussion**

Although genomic alterations of primary resistance to EGFR inhibition may be detected in ctDNA of patients with mCRC and potentially missed by genomic profiling of matched tumor tissue, their clinical significance and usefulness remains largely unknown. When focusing on **RAS** testing, pivotal cohort studies showed a good but not perfect concordance between plasma and tumor genotyping.\(^7, 12-19\) In fact, despite the use of tumor profiling assays with high sensitivity for the detection of subclonal alterations, only the use of LB may overcome the issue of spatial heterogeneity. On the opposite side, LB may have meaningful limitations in patients with low tumor burden and poorly-shedding cancers such as those with peritoneal/lymph nodal lesions and mucinous histotype. Exploratory analyses of the aforementioned studies suggested that **RAS** testing in liquid biopsy results in similar clinical outcomes when compared with tissue testing in patients treated with anti-EGFR-based regimens. However, such evidence was limited by the small sample size, the variety of treatment lines and regimens and, above all, the lack of survival comparison between subgroups.\(^12, 16-18, 20\) Moving forward from this background, Normanno et al. showed that, in a subgroup of 92 patients with **KRAS** exon 2 wild-type mCRC receiving upfront FOLFIRI plus cetuximab in the frame of CAPRI-GOIM trial, patients with **RAS** mutations detected in plasma or in
tumor tissue had significantly worse outcomes vs. wild-type counterparts, but without differences in
the discriminative ability of plasma vs. tissue testing. With the aim of providing new evidence on the potential added value of baseline LB genotyping in
the first-line setting, we took advantage of the all-RAS and BRAF wild-type patients’ population
enrolled in the Valentino study, who received FOLFOX plus panitumumab upfront strategy and
had been previously characterized by tissue deep-sequencing. Moreover, with the aim of
minimizing the potential confounding effects of the factors associated with primary resistance to
EGFR inhibition, we focused only on hyper-selected patients with left-sided, HER2-negative and
MSS cancers, who are currently considered as the optimal candidates for an upfront anti-EGFR-
based regimen. We showed that patients with RAS or PIK3CA mutations in baseline ctDNA have
worse outcomes in terms of PFS and OS compared to the wild-type subgroups, with RAS – but not
PIK3CA - mutations being also associated with poorer response dynamics in terms of RECIST
response, ETS and DoR. It should be acknowledged that the lack of significant association of
PIK3CA status with response parameters may be related to the low number of patients and to the
confounding effects of the associated chemotherapy. Finally, despite TP53 mutations are not an
established marker of resistance to anti-EGFR therapy, they had a negative prognostic value in our
cohort: since these mutations showed the highest median VAF across all samples and the highest
prevalence, we speculate that the detection of TP53 mutations in individual subjects may be at least
partly correlated with the higher amount of ctDNA and/or the higher tumor burden.

Regarding the specific negative predictive role of PIK3CA mutations in mCRC, their association
with other markers of primary resistance to anti-EGFR agents (such as RAS/BRAF mutations and
right-sidedness) has previously limited the clinical transferability of the results of initial
investigations. Our study showed the potential clinical usefulness of LB to assess PI3KCA status
in mCRC, in light of the high degree of heterogeneity of PIK3CA mutations in tumor tissue and the
available data in patients with other tumor types including advanced breast cancer. Notably, the
use of NGS assays for ctDNA profiling allowed us to concomitantly assess both PIK3CA and RAS status, as well as other genomic alterations with potential clinical actionability. 

RAS or PI3KCA mutations with low fractional abundance detected only in tumor tissue by ultra-deep sequencing, but not in ctDNA, may be subclonal and, on top of this, highly heterogeneous from a spatial point-of-view. The very few patients with this profile did not seem to show poorer individual outcomes, but larger datasets are needed to investigate such patients as a unique subgroup. Consistently with these results, the ULTRA study showed that RAS mutations in tumor tissue with a VAF<5% and only detectable thanks an ultra-sensitive assay (digital droplet PCR) were not associated with worse outcomes, further corroborating the current clinical use of validated assays with 5% limit of detection for RAS testing in tumor tissue.23 On the other side, mutations detected thanks to ctDNA genotyping may mirror the presence of clinically important tumor subclones and could be associated with the rapid emergence of acquired resistance rather than primary resistance to anti-EGFRs. In attempt to further characterize this phenomenon, we showed that patients with RAS and/or PIK3CA VAF >5% in cfDNA have the worst PFS and OS, while patients with RAS VAF >5% also showed worse dynamics of response including ETS and DoR. It should be pointed out that the VAF may be related to tumor burden and per-se prognostic. Moreover, VAF thresholds of resistance mutations in ctDNA, particularly after adjustment for the VAF of “founder” mutations (such as TP53 or APC) simultaneously identified by NGS, need validation thanks to pooled analyses of clinical trials and larger datasets. Our 5% VAF cut-off, which warrants further validation in similar settings, is based upon the VAF intrinsic distribution and is close to the values reported in previous datasets.16,24,25

Our study has the potential of clinical transferability in several settings. Prospective validation of our data may be achieved by randomized clinical trials investigating alternative upfront regimens or non-anti-EGFR-based maintenance strategies in patients with left-sided and molecularly hyperselected mCRC with baseline RAS mutations in ctDNA. Moreover, given the potential actionability of PIK3CA mutations when regarded as therapeutic targets rather than contributors to
the puzzle of negative hyper-selection, LB-guided proof-of-concept trials or pre-clinical studies of anti-EGFR agents combined with alfa-selective PIK3 inhibitors such as alpelisib could be planned in the population with “isolated” PIK3CA mutations. Clear limitations of our study include its exploratory nature, the lack of data on longitudinal monitoring of serial LB obtained during treatment and the lack of prospective validation of the mutational VAF in ctDNA. Moreover, the study design did not allow to discriminate between the prognostic value of specific gene mutations in ctDNA and their potential predictive role, since both study arms were panitumumab-based. On top of that, the low number of patients in the RAS/PIK3CA mutated subgroups did not allow us to properly assess the interaction with the two treatment arms. Finally, in light of the poor prognostic role of APC wild-type status in mCRC, 26,27 we acknowledge that the low frequency of APC mutations in our dataset is clearly related to the relatively low coverage of the gene sequence by the hotspots included in this custom panel.

In conclusion, we showed that baseline ctDNA genomic profiling may add value to tumor tissue profiling to refine the molecular hyperselection of patients with the highest likelihood of benefit from upfront anti-EGFR-based strategies. LB data should be incorporated in future clinical trials with targeted agents conducted in patients with mCRC.

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References


Figure Legends

**Figure 1:** A) Heatmap representing the gene mutations found in LB of patients with hyperselected mCRC included in the study cohort; bottom annotation shows the maximum VAF for each sample. B) and C) show the HRs for progression and death with respective confidence intervals for each gene mutation; ns: non-significant; *:p<0.05; **p<0.01; ***:p<0.001.

**Figure 2:** Kaplan-Meyer curves representing PFS and OS for, respectively, A-B) *RAS* mutated vs wild-type status in baseline ctDNA and C-D) *PIK3CA* mutated vs wild-type status in baseline ctDNA.

**Figure 3:** A) boxplot depicting *RAS* VAF according to the concomitant detection of *RAS* mutation in the matched tumor tissue; B) waterfall plot showing the relationship of ETS and *RAS* VAF; C) scatter plot showing the correlation of *RAS* VAF with DoR. D) boxplot depicting *PIK3CA* VAF according to the concomitant detection of *PIK3CA* mutation in the matched tumor tissue; B) waterfall plot showing the relationship of ETS and *PIK3CA* VAF; C) scatter plot showing the correlation of *PIK3CA* VAF with DoR.

**Figure 4:** Kaplan-Meyer curves representing PFS and OS for, respectively, A-B) patients with at least one *RAS/PIK3CA* mutation VAF>5% vs patients with *RAS/PIK3CA* mutation with VAF<5% vs patients with *RAS* and *PIK3CA* all-wild-type status in baseline ctDNA.
A

B

C

D

E

F

Figure 3

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**Figure 4**

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<td>Mutated (exon 9)</td>
<td>KRAS: G12V (c.35G&gt;T) PIK3CA: p.E545K (c.1633G&gt;A)</td>
<td>KRAS: 25.24%</td>
<td>KRAS: 25.24%</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>KRAS: G12V (exon 2) and PIK3CA: E545K (exon 9)</td>
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<td>Wild-type</td>
<td>G12D (c.35G&gt;A)</td>
<td>17.80%</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
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<td>Mutated (exon 2)</td>
<td>Mutated (exon 9)</td>
<td>NRAS: G13C (c.37G&gt;T) PIK3CA: p.E545K (c.1633G&gt;A)</td>
<td>NRAS: 0.22%</td>
<td>NRAS: 0.22%</td>
<td>TP53 (exon 7)</td>
<td>p.T230fs (c.688dupA)</td>
<td>8.10%</td>
<td>PIK3CA E545K (exon 9)</td>
<td>M</td>
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<tr>
<td>001-043</td>
<td>Mutated (exon 4)</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>p.A146T (c.436G&gt;A)</td>
<td>1.12%</td>
<td>APC (exon 15)</td>
<td>p.Q1291* (c.3871C&gt;T)</td>
<td>10.40%</td>
<td>Wild-type</td>
<td>P</td>
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<td>004-010</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Mutated (exon 9)</td>
<td>p.E542K (c.1624G&gt;A)</td>
<td>18.76%</td>
<td>APC (exon 15)</td>
<td>p.N235fsTer12 (c.705_706delCTinsA)</td>
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<td>H1047L (c.3140A&gt;T)</td>
<td>34.80%</td>
<td>TP53 (exon 5)</td>
<td>p.R175H (c.524G&gt;A)</td>
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<td>PIK3CA: H1047L (exon 20)</td>
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<td>Q61H (c.183A&gt;T)</td>
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<td>TP53 (exon 7)</td>
<td>p.S241del (c.1513_1523delTCC)</td>
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<td>Wild-type</td>
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<td>p.E545K (c.1633G&gt;A)</td>
<td>1.50%</td>
<td>TP53 (exon 8)</td>
<td>p.R282W (c.844C&gt;T)</td>
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<td>APC (exon 15)</td>
<td>p.Q1367* (c.4099C&gt;T)</td>
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<td>PIK3CA E542K (exon 9)</td>
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<td>Status 1 (Exon)</td>
<td>Status 2 (Exon)</td>
<td>Gene 1 (Exon)</td>
<td>Gene 2 (Exon)</td>
<td>Mutation</td>
<td>Allele 1</td>
<td>Allele 2</td>
<td>Allele 3</td>
<td>Allele 4</td>
<td>Allele 5</td>
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<td>c.1633G&gt;A</td>
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<td>Mutated</td>
<td>APC</td>
<td>p.Q1406*</td>
<td>c.4216C&gt;T</td>
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<td>Wild-type</td>
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<td>ERBB2</td>
<td>p.L313I</td>
<td>c.937C&gt;A</td>
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<td>(Exon 4)</td>
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<td>015-006</td>
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<td>Wild-type</td>
<td>Mutated</td>
<td>KRAS: G12D</td>
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<td>p.E1408*</td>
<td>c.4222G&gt;T</td>
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<td>(Exon 2)</td>
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<td>PIK3CA:</td>
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<td>TP53</td>
<td>p.R248Q</td>
<td>c.743G&gt;A</td>
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<td>c.3913delI</td>
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<td>p.R157H</td>
<td>c.524G&gt;A</td>
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<td>PIK3CA:</td>
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<td>p.G61H</td>
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<td>Wild-type</td>
<td>H1047R</td>
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<td>p.R157H</td>
<td>c.473G&gt;T</td>
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<td>(Exon 20)</td>
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</table>

mA%: mutation load; M: metastasis; NA: not applicable; NGS: next-generation sequencing; P: primary tumor.
Table 2: Summary of overall response rate according to RECIST v1.1, early tumor shrinkage (ETS) and depth of response (DoR) according to RAS and PIK3CA mutational status in baseline ctDNA.

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>RECIST response</th>
<th>No RECIST response</th>
<th>p§</th>
<th>ETS</th>
<th>No ETS</th>
<th>p§</th>
<th>Median DoR % reduction (IQR)</th>
<th>p*</th>
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<td><strong>RAS</strong></td>
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<td></td>
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</tr>
<tr>
<td>Wild-type</td>
<td>110 (91.7)</td>
<td>92 (86.0)</td>
<td>15 (14.0)</td>
<td>0.039</td>
<td>75 (75.0)</td>
<td>25 (25.0)</td>
<td>0.023</td>
<td>0.47 (0.38-0.60)</td>
<td>0.072</td>
</tr>
<tr>
<td>Mutated</td>
<td>10 (8.3)</td>
<td>5 (55.6)</td>
<td>4 (44.4)</td>
<td></td>
<td>3 (33.3)</td>
<td>6 (66.7)</td>
<td></td>
<td>0.17 (0.16-0.47)</td>
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<tr>
<td><strong>PIK3CA</strong></td>
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<tr>
<td>Wild-type</td>
<td>105 (87.5)</td>
<td>86 (85.1)</td>
<td>15 (14.9)</td>
<td>0.267</td>
<td>70 (74.5)</td>
<td>24 (25.5)</td>
<td>0.169</td>
<td>0.47 (0.38-0.59)</td>
<td>0.305</td>
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<tr>
<td>Mutated</td>
<td>15 (12.5)</td>
<td>11 (73.3)</td>
<td>4 (26.7)</td>
<td></td>
<td>8 (53.3)</td>
<td>7 (46.7)</td>
<td></td>
<td>0.43 (0.21-0.57)</td>
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</tbody>
</table>

Note: RECIST response was available for 116 of the 120 patients; ETS and DoR were available for 109 of the 120 patients.

§ χ² test or Fisher test as appropriate.
*Mann-Whitney test
Clinical Cancer Research

The added value of baseline circulating tumor DNA profiling in patients with molecularly hyperselected, left-sided metastatic colorectal cancer

Paolo Manca, Salvatore Corallo, Adele Busico, et al.

Clin Cancer Res  Published OnlineFirst February 5, 2021.

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