**RAS testing of liquid biopsy correlates with the outcome of metastatic colorectal cancer patients treated with first-line FOLFIRI plus cetuximab in the CAPRI-GOIM trial**

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**Background:** Liquid biopsy is an alternative to tissue for RAS testing in metastatic colorectal carcinoma (mCRC) patients. Little information is available on the predictive role of liquid biopsy RAS testing in patients treated with first-line anti-EGFR monoclonal antibody-based therapy.

**Patients and methods:** In the CAPRI-GOIM trial, 340 KRAS exon-2 wild-type mCRC patients received first-line cetuximab plus FOLFIRI. Tumor samples were retrospectively assessed by next generation sequencing (NGS). Baseline plasma samples were analyzed for KRAS and NRAS mutations using beads, emulsion, amplification, and magnetics digital PCR (BEAMing). Discordant cases were solved by droplet digital PCR (ddPCR) or deep-sequencing.

**Results:** A subgroup of 92 patients with available both NGS data on tumor samples and baseline plasma samples were included in this study. Both NGS analysis of tumor tissue and plasma testing with BEAMing identified RAS mutations in 33/92 patients (35.9%). However, 10 cases were RAS tissue mutant and plasma wild-type, and additional 10 cases were tissue wild-type and plasma mutant, resulting in a concordance rate of 78.3%. Analysis of plasma samples with ddPCR detected RAS mutations in 2/10 tissue mutant, plasma wild-type patients. In contrast, in all tissue wild-type and plasma mutant cases, ddPCR or deep-sequencing analysis of tumor tissue confirmed the presence of RAS mutations at allelic frequencies ranging between 0.15% and 1.15%. The median progression-free survival of RAS mutant and wild-type patients according to tissue (7.9 versus 12.6 months; P = 0.004) and liquid biopsy testing (7.8 versus 13.8 months; P < 0.001) were comparable. Similar findings were observed for the median overall survival of RAS mutant and wild-type patients based on tissue (22.1 versus 35.8 months; P = 0.016) and plasma (19.9 versus 35.8 months; P = 0.013) analysis.

**Conclusion:** This study indicates that RAS testing of liquid biopsy results in a similar outcome when compared with tissue testing in mCRC patients receiving first-line anti-EGFR monoclonal antibodies.

**Key words:** colon carcinoma, liquid biopsy, KRAS, NRAS, anti-EGFR monoclonal antibodies
Introduction

With the term liquid biopsy, we refer to the possibility to perform tumor molecular profiling by using tumor-derived biomarkers that can be isolated from body fluids of cancer patients, including peripheral blood [1]. Among the different applications of liquid biopsy, the analysis of circulating tumor DNA (ctDNA) is the only employed in clinical practice. In particular, ctDNA testing allows the detection of genetic alterations associated with sensitivity or resistance to targeting agents [2].

The ctDNA is usually a small fraction of the cell-free DNA (cfDNA) that can be extracted from the plasma of cancer patients, being diluted in genomic DNA deriving from normal dividing cells [2]. For this reason, highly sensitive techniques are required for cfDNA testing. This is of particular relevance when testing for KRAS and NRAS mutations in patients with metastatic colorectal carcinoma (mCRC) in order to avoid false negative results. In this respect, different studies have shown the feasibility to assess RAS mutations in cfDNA from mCRC patients using the beads, emulsion, amplification, and magnetics digital PCR (BEAMing) technique [3–6]. The OncoBEAM RAS CRC assay is an in vitro diagnostic (CE-IVD) kit that detects 34 mutations in exons 2, 3, and 4 of the KRAS and NRAS genes. Therefore, it covers the most frequent RAS mutations to be tested in mCRC patients candidate to treatment with anti-EGFR monoclonal antibodies (MoAbs) [7].

Liquid biopsy has several advantages over tissue testing, including the low invasive procedure, the fast turnaround time and the ability to provide a more comprehensive molecular portrait of the disease when compared with the analysis of a small fragment of a single tumor lesion. Nevertheless, tissue testing is still considered the golden standard for RAS testing in mCRC. This is in part due to the lack of data formally demonstrating a correlation between plasma RAS testing and outcome in mCRC patients. In fact, few data are available on the predictive value of plasma RAS testing, and they are limited to patients treated in second and third line with anti-EGFR agents [4, 5].

In this study, we evaluated the predictive value of liquid biopsy RAS testing with the BEAMing technology in mCRC patients who received first-line cetuximab plus FOLFIRI within the CAPRI-GOIM trial.

Study design and patient population

The CAPRI-GOIM trial is a non-profit academic, open-label, multicenter study carried out by the GOIM cooperative group (Eudract number: 2009-014041-81). KRAS exon-2 wild-type mCRC patients, according to local pathology assessment, received first-line FOLFIRI plus cetuximab [8].

Next generation sequencing analysis of tumor tissue

Tumor samples were analyzed with the Ion AmpliSeq™ Colon and Lung Cancer Panel (Thermofisher, Milan, Italy) using Ion Torrent semiconductor sequencing, as previously described [8]. The sensitivity of the method was set at 2% allelic frequency. Deep-sequencing analysis was carried out using the Oncomine Comprehensive Tumor Panel (Thermofisher), with a coverage >2000x.

Beads, emulsion, amplification, and magnetics

Plasma samples were analyzed with BEAMing digital PCR technology using the OncoBEAM™ RAS CRC Kit (Sysmex Inostics, Milan, Italy), which allows the detection of 34 mutations in the exons 2, 3, and 4 of KRAS and NRAS genes (detailed protocol in the supplementary material, available at Annals of Oncology online).

Droplet digital PCR

The discordant cases between tissue and plasma testing were analyzed using the QX200 Droplet Digital PCR (ddPCR) System and the KRAS Screening Multiplex Kit (Bio-Rad, Milan, Italy) that is able to detect the most frequent mutations in KRAS codons 12 and 13 (detailed protocol in the supplementary material, available at Annals of Oncology online).

Statistical analysis

The Kaplan–Meier method was used to estimate median progression-free survival time (mPFS) and median overall survival (mOS) time. P-values were calculated using log-rank tests at a significance level of 5%. Differences between categorical data were measured using χ² and Fisher’s exact test, when adequate. All statistical analyses were carried out using IBM-SPSS statistics version 22.0. Differences between continuous variables were investigated by Mann–Whitney U-test.

Results

Patients

We selected for this study a subgroup of patients enrolled in the CAPRI-GOIM study with available both next generation sequencing (NGS) data on tumor samples and baseline plasma samples. Tumor samples from 182/340 (53.5%) KRAS exon-2 wild-type mCRC patients enrolled in the first-line of the CAPRI-GOIM trial were retrospectively analyzed by NGS [8]. Baseline plasma samples were available for a subgroup of 92 patients from the NGS cohort. The clinical and pathological features of these subgroups are described in supplementary Table S1, available at Annals of Oncology online.

Tissue and plasma analysis for RAS mutations

Retrospective analysis of tissue specimens with NGS identified 33 KRAS or NRAS mutations in 33/92 patients (35.9%) (Table 1). Plasma-derived cfDNA analysis with BEAMing detected 34 RAS mutations in 33 cases, thus resulting in an identical rate of RAS mutant cases (Table 1 and supplementary Table S2, available at Annals of Oncology online). However, 10 cases were mutant on

| Table 1. Tissue and plasma KRAS and NRAS status |
|-----------------|------------------------|
| **Tissue KRAS/NRAS mutational status, n** | **Plasma KRAS/NRAS mutational status (n)** |
| Mutated | 33 | 23 |
| Wild-type | 59 | 10 |
| **Total** | **92** | **33** |
| Mutated | 10 |
| Wild-type | 49 |


tissue and wild-type on plasma, whereas additional 10 cases were wild-type on tissue and mutant on plasma (Table 1).

The concordance rate of plasma and tissue testing was only 78.3%, with a specificity of 83.1% and a sensitivity of 69.7% for plasma testing (supplementary Table S3, available at Annals of Oncology online). The positive predictive value (PPV) of liquid biopsy RAS testing was 69.7% and the negative predictive value (NPV) 83.1% (supplementary Table S3, available at Annals of Oncology online).

Clinical and pathological features of tissue mutant/plasma wild-type cases

The clinical and pathological features of the 10 cases that were RAS mutant in tissue and wild-type in plasma are described in Table 2. Five tumors showed a frequency of mutant RAS allele in the tissue ≤10% although the fraction of neoplastic cells was high in most cases. In 9/10 patients the plasma volume available for analysis was suboptimal (<3 ml), although the input cfDNA was above the suggested thresholds. In 5/10 patients, the recurrence of the disease occurred at least 1 year after surgery. In 7/10 cases, the primary tumor was surgically removed before blood drawing. Finally, in six cases the only sites of recurrence were lung and/or lymph nodes. Analysis of plasma samples with ddPCR identified RAS mutations only in two cases.

Clinical and pathological features of tissue wild-type/plasma mutant cases

The frequency of mutant beads detected by BEAMing in the 10 cases that were RAS wild-type on tissue ranged between 0.026% and 5.619% (Table 3). In agreement with previous findings [5], these levels were lower when compared with cases with concordant RAS status in tissue and plasma, although this difference was not statistically significant (median RAS mutant beads 1.04% and 3.45%, respectively; P = 0.39). In all cases, the frequency of mutant beads was above the suggested threshold of the specific assays. Nine patients within this subgroup had liver metastases and five had multiple metastatic sites. Analysis of tumor tissue with ddPCR or with deep-sequencing identified the same RAS mutations found in plasma by BEAMing in all cases (Table 3). The tissue allelic frequency of RAS mutations ranged between 0.1% and 1.15%, below the 2% limit of detection of NGS. Conversely, no KRAS mutations were detected by ddPCR in the tumor tissue of 10 cases that were RAS wild-type according to both tumor and plasma analysis (data not shown).

Clinical outcomes of FOLFIRI plus cetuximab according to RAS status in tissue and liquid biopsy

Among the cohort of 92 patients, the mPFS of RAS mutant and wild-type patients according to tissue analysis with NGS were 7.9 months (CI 95% 5.9–9.8 months) and 12.6 months (CI 95% 9.9–16.2 months; P = 0.004), respectively (Figure 1A). These findings are in line with our previous report of the entire NGS cohort of the CAPRI trial, in which we observed an mPFS of 8.9 months in KRAS/NRAS mutant mCRC patients and of 11.1 months in the wild-type group [8].

Patients with an RAS mutant liquid biopsy analysis had an mPFS of 7.8 months (CI 95% 6.7–8.9 months), whereas RAS wild-type patients had an mPFS of 13.8 months (CI 95% 10.5–17 months; P < 0.0001) (Figure 1B).

Similar findings were observed for mOS. Tissue testing for RAS mutations identified a subgroup of mutant patients with an mOS of 22.1 months (CI 95% 17–27.2 months), which was significantly inferior to the mOS of 35.8 months (CI 95% 29.2–42.5 months) of RAS wild-type patients (P = 0.016). Analogously, the mOS of RAS mutant patients according to plasma testing was 19.9 months (CI 95% 15.6–24.2 months), whereas wild-type patients had an mOS of 35.8 months (CI 95% 31–40.6 months; P = 0.013).

The response rate within the RAS mutant and wild-type cohorts according to tissue and plasma analysis were identical: 17 responses were observed among the 33 RAS mutant patients (51.5%), while 35/59 (56%) RAS wild-type patients responded to cetuximab plus FOLFIRI (supplementary Table S4, available at Annals of Oncology online).

No statistically significant differences in the second line therapies received by patients according to tissue (P = 0.12) or plasma (P = 0.30) RAS status were observed (supplementary Table S5, available at Annals of Oncology online).

Discussion

Our study indicates that RAS testing of liquid biopsy results in a similar outcome when compared with tissue testing in mCRC patients receiving first-line anti-EGFR MoAbs. Previous studies suggested that RAS testing of cfDNA correlates with benefit from anti-EGFR treatment. However, Grasselli et al. assessed the predictive value of liquid biopsy RAS testing in a cohort of RAS wild-type patients (N = 52) treated with anti-EGFR MoAbs in second or third line [4]. Vidal and collaborators evaluated the impact of RAS detection in plasma in predicting response to anti-EGFR based therapy in 34 RAS wild-type patients treated with different anti-EGFR MoAbs, in monotherapy or plus chemotherapy [5]. In contrast, the patients prospectively enrolled in the CAPRI-GOIM trial received first-line cetuximab plus FOLFIRI [8]. In addition, tumor tissues from patients included in this investigation were all analyzed by NGS, thus representing a homogeneously treated and molecularly characterized cohort. Nevertheless, we acknowledge that our study has several limitations being a retrospective and unplanned evaluation of a small cohort of patients, representing only 27% of the initial cohort of 340 patients enrolled in the CAPRI-GOIM trial.

The concordance between tissue and plasma RAS testing was lower in this study when compared with previous reports [3–5]. We hypothesize that this difference might be due to chance because of the relatively low number of patients included in these investigations.

A subgroup of patients were RAS mutant on tissue and wild-type on liquid biopsy. This cohort was enriched of patients that had recurrence of the disease after surgical resection of the primary and a lower tumor burden, with metastatic lesions often localized in the lung and lymph nodes. These features have been indeed associated with lower levels of cfDNA and inferior sensitivity of RAS plasma testing [3–5, 9]. An exploratory analysis showed that this subgroup of patients had a favorable outcome with an mPFS of 13.8 months and an mOS of 35.8 months. While
Table 2. Clinical and pathological features of RAS tissue mutant/plasma wild-type cases

<table>
<thead>
<tr>
<th>CAPRI CODE</th>
<th>Tissue NGS results (% allelic frequency)</th>
<th>Plasma volume (ml)</th>
<th>Results BEAMing</th>
<th>Results ddPCR plasma (% allelic frequency)</th>
<th>Time tissue - plasma (months)</th>
<th>Neoplastic cell fraction (%)</th>
<th>Tissue source</th>
<th>Prior surgery</th>
<th>T location</th>
<th>Grading</th>
<th>Site of metastasis</th>
<th>Second-line treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C141</td>
<td>KRAS: p.A146T (8)</td>
<td>2</td>
<td>WT</td>
<td>WT</td>
<td>1</td>
<td>50</td>
<td>Biopsy</td>
<td>No</td>
<td>SN</td>
<td>G2</td>
<td>Liver</td>
<td>FOLFOX (Arm B)</td>
</tr>
<tr>
<td>C118</td>
<td>KRAS: p.G12V (8)</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>38</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G3</td>
<td>Lung</td>
<td>FOLFOX (Arm B)</td>
</tr>
<tr>
<td>C159</td>
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<td>WT</td>
<td>WT</td>
<td>22</td>
<td>60</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Lung</td>
<td>FOLFOX (Arm B)</td>
</tr>
<tr>
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<td>2</td>
<td>WT</td>
<td>WT</td>
<td>2</td>
<td>50</td>
<td>Biopsy</td>
<td>No</td>
<td>SN</td>
<td>G1</td>
<td>Liver and lymph node</td>
<td>FOLFOX (Arm B)</td>
</tr>
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<td>WT</td>
<td>WT</td>
<td>1</td>
<td>60</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver and lung</td>
<td>FOLFOX + cetuximab (Arm A)</td>
</tr>
<tr>
<td>C106</td>
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<td>2</td>
<td>WT</td>
<td>WT</td>
<td>13</td>
<td>40</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Lung and lymph node</td>
<td>FOLFOX (Arm B)</td>
</tr>
<tr>
<td>C116</td>
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<td>2</td>
<td>WT</td>
<td>WT</td>
<td>5</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Lung and lymph node</td>
<td>FOLFOX (Arm B)</td>
</tr>
<tr>
<td>C135</td>
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<td>WT</td>
<td>NA</td>
<td>1</td>
<td>70</td>
<td>Biopsy</td>
<td>No</td>
<td>SN</td>
<td>G2</td>
<td>Liver and lymph node</td>
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</tr>
<tr>
<td>C71</td>
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<td>1</td>
<td>WT</td>
<td>KRAS: p.G12D (2S1)</td>
<td>12</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Lung</td>
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<td>WT</td>
<td>KRAS: p.G12D (Q1)</td>
<td>35</td>
<td>60</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Lung and lymph node</td>
<td>FOLFOX (Arm B)</td>
</tr>
</tbody>
</table>

*aBefore CAPRI enrollment and blood drawing.
*bTest carried out on bioptic specimens.
*cCAPRI-GOIM Arm.

NA, Not amplified.
Table 3. Clinical and pathological features of RAS tissue wild-type/plasma mutant cases

<table>
<thead>
<tr>
<th>CAPRI CODE</th>
<th>Tissue NGS results</th>
<th>Tissue ddPCR or deep sequencing results (% allelic frequency)</th>
<th>Plasma volume (ml)</th>
<th>Result BEAMing</th>
<th>% Mutant beads</th>
<th>Time tissue-plasma (months)</th>
<th>Neoplastic cell fraction (%)</th>
<th>Tissue source</th>
<th>Prior surgery</th>
<th>T location</th>
<th>Grading</th>
<th>Site of metastasis</th>
<th>Second-line treatment</th>
</tr>
</thead>
<tbody>
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<td>C60 WT</td>
<td>KRAS: p.G12X (0,10)</td>
<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>0.857</td>
<td>2</td>
<td>80</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver</td>
<td>FOLFOX + bevacizumab</td>
</tr>
<tr>
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<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>0.078</td>
<td>2</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver and spleen</td>
<td>FOLFOX + bevacizumab</td>
</tr>
<tr>
<td>C40 WT</td>
<td>KRAS: p.Q61H (1)♭</td>
<td>Mutation codon 61. KRAS</td>
<td></td>
<td></td>
<td>0.242</td>
<td>5</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver</td>
<td>FOLFOX + cetuximab</td>
</tr>
<tr>
<td>C84 WT</td>
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<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>5.619</td>
<td>1</td>
<td>10</td>
<td>Biopsy♭</td>
<td>No</td>
<td>SN</td>
<td>G2</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>C24 WT</td>
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<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
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<td>2</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver, lung lymph node</td>
<td>FOLFOX + cetuximab (Arm A)</td>
</tr>
<tr>
<td>C2 WT</td>
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<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>0.488</td>
<td>3</td>
<td>60</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver, lung lymph node</td>
<td>FOLFOX + cetuximab (Arm B)</td>
</tr>
<tr>
<td>C81 WT</td>
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<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>2.372</td>
<td>22</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver, lymph node and adrenal gland</td>
<td>Unknown</td>
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<tr>
<td>C82 WT</td>
<td>KRAS: p.G12X (0,15)</td>
<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>0.133</td>
<td>6</td>
<td>60</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver</td>
<td>Unknown</td>
</tr>
<tr>
<td>C117 WT</td>
<td>KRAS: p.G12X (0,18)</td>
<td>Mutation codon 12. KRAS</td>
<td></td>
<td></td>
<td>0.056</td>
<td>2</td>
<td>80</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>DX</td>
<td>G3</td>
<td>Liver and lymph node</td>
<td>FOLFOX + cetuximab (Arm A)</td>
</tr>
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<td>Mutation codon 12. KRAS</td>
<td></td>
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<td>2</td>
<td>70</td>
<td>Primary tumor</td>
<td>Yes</td>
<td>SN</td>
<td>G2</td>
<td>Liver</td>
<td>FOLFOX + cetuximab (Arm A)</td>
</tr>
</tbody>
</table>

*Prior to CAPRI enrollment and blood drawing.
♭Deep-sequencing.
♭♭CAPRI-GOIM Arm.
♭♭♭Test carried out on bioptic specimens.
these data need to be cautiously interpreted because of the small sample size of this subgroup (n = 10), they might indicate that a negative liquid biopsy reflects a lower aggressiveness of the disease. In this regard, previous studies have demonstrated that mCRC patients with lower levels of cfDNA have a better prognosis when compared with patients with higher levels [6, 10, 11]. In agreement with these findings, patients with RAS mutant allelic frequency <0.1 in the liquid biopsy had a worse outcome when compared with patients RAS wild-type or with RAS mutations allelic frequency >0.1 [4].

Ten cases were RAS wild-type on tissue and mutant on plasma. Analysis of tissue with ddPCR or deep-sequencing confirmed the presence of RAS mutations at a low allelic frequency in all discordant cases, suggesting that in these tumors RAS variants are likely to be subclonal. We and other research groups have previously reported that subclonal RAS mutation can occur in colon carcinoma [12, 13]. It is still debated which is the minimum level of RAS mutations in tumor tissue that is associated with resistance to anti-EGFR MoAbs. Laurent Puig et al. [14] suggested that patients with KRAS mutations at an allelic frequency >1% have an outcome similar to RAS wild-type patients when treated with anti-EGFR MoAbs. Data from the retrospective analysis of tumor tissue with BEAMing in the CRYSTAL study also support the hypothesis that patients with RAS mutations at low allelic frequency might benefit therapy with anti-EGFR agents [15]. However, these analyses were limited to the primary tumor tissue. Several studies have shown that disagreement for KRAS mutational status can occur between primary tumor and metastases. Whereas a discordance rate of 5% between liver metastases and primary CRC has been shown [16], the disagreement is significantly higher for lymph node and lung localizations that were quite frequent in our cohort [17, 18].

Figure 1. PFS of RAS wild-type and mutant mCRC patients according to RAS test carried out on tissue (A) or liquid biopsy (B). OS of RAS wild-type and mutant mCRC patients according to tissue (C) or liquid biopsy (D) RAS mutational status.
subgroup the primary tumor was removed before enrollment in the CAPRI-GOIM trial and blood drawing. Therefore, liquid biopsy in these patients reflected the mutational status of the metastatic lesions, whereas tissue testing was carried out on the resected primary tumor. Importantly, patients with RAS wild-type tumor and mutant liquid biopsy showed an mPFS of 8 months, which was similar to the mPFS of patients with RAS mutant tumor tissue (7.9 months).

In conclusion, our study suggests that RAS testing of liquid biopsy is a suitable tool to select mCRC patients who might benefit anti-EGFR MoAbs treatment, although a number of variables including tumor burden and heterogeneity of the disease are likely to affect the results of plasma testing. Whereas liquid biopsy is currently limited to patients with no tissue available for molecular testing, our data indicate that cfDNA testing might produce information complementary to tissue analysis that might be relevant for patients’ stratification. In fact, in patients with RAS mutations detected in the tumor tissue, the liquid biopsy analysis might provide prognostic information. In patients with RAS wild-type primary tumor tissue and multiple metastatic lesions, liquid biopsy can better recapitulate the heterogeneity of the disease and identify patients resistant to anti-EGFR therapies. We acknowledge that further studies in larger cohorts of patients are required to confirm these latter hypotheses.

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References


Appendix

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