Nanofluidic digital PCR and extended genotyping of RAS, BRAF and PI3KCA for improved selection of metastatic colorectal cancer patients to anti-EGFR therapies


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Introduction: Concomitant detection of mutations in downstream effectors, mainly KRAS (exon 2), of the EGFR pathway improves the selection of candidate metastatic colorectal cancer (mCRC) patients that will respond to anti-EGFR therapy. The clinical significance of minor mutant clones remains unclear. The aim of our study was to evaluate if the addition of the mutational analysis of NRAS, BRAF, and PI3KCA to the conventional standard KRAS (exon 2) mutational analysis and the use of highly sensitive and quantitative technology may improve the prediction of response to anti-EGFR therapy.

Methods: A panel of 38 hotspots including RAS (KRAS exon 2/3/4 and NRAS exon 2/3/4), BRAF (V600E) and PI3KCA (exon 20) were analyzed in primary tumor FFPE tissues from 102 mCRC patients treated with anti-EGFR therapy both as monotherapy and in combination with chemotherapy. Highly sensitive nanofluidic digital PCR (dPCR) was compared to conventional quantitative PCR (qPCR). Response was evaluated by RECIST 1.1 criteria and progression free survival (PFS) after anti-EGFR treatment was analyzed according to the mutational status and the mutated allele fraction.

Results: Twenty-three of 102 (23%) patients were positive for one mutation with qPCR with no multiple mutations detected. All these tumors were scored as positive with dPCR and the percentage of mutated alleles ranged from 2.1% to 66.6% (median: 35.84%). Analysis by dPCR increased the number of patients bearing mutations to 49/102 (48%) and identified multiple mutant alleles in 13 cases. The percentage of mutated alleles in this set of 26 patients reclassified as positive ranged from 0.04% to 10.8% (median: 0.85%). We observed an inverse correlation between the proportion of mutated allele (main clone) and the anti-EGFR response (P = 0.0001). Analysis of PFS taking into account only KRAS exon 2 hotspots showed that patients with more than 1% of mutated allele fraction (n = 21) (as described in Laurent-Puig et al, Clin Cancer Res 2014) presented a hazard ratio of 2.92 (CI95% [1.1-6.04], P = 0.0059) compared to wild-type patients or with less than 1% of mutated allele fraction (n = 81). When we extended the analysis to RAS, BRAF and PI3KCA, the patients with more than 1% of mutated allele fraction (n = 40) presented a hazard ratio of 3.96 (CI95% [1.98-7.93], P = 0.0001). We obtained similar results in terms of PFS (hazard ratio 3.61, P = 0.0003) considering qualitative mutation detected by dPCR. In this context we favour the use of a cut-off of 1% that increased the number of wt patients eligible for treatment (n = 9) that may benefit from the anti-EGFR treatment (See attached Table).

Conclusion: Analysis of an extended gene panel including RAS, BRAF and PI3KCA with highly sensitive nanofluidic dPCR improved the prediction of response to anti-EGFR therapy and/or prognosis in mCRC patients adequately classifying a significant proportion of cases.

Table: O-012

<table>
<thead>
<tr>
<th>KRAS mut status</th>
<th>dPCR mutation allele fraction</th>
<th>qPCR mutation allele fraction</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (n=81)</td>
<td>0.00% (median: 0.00%)</td>
<td>0.00% (median: 0.00%)</td>
<td>0.00% (median: 0.00%)</td>
</tr>
<tr>
<td>Mutant (n=21)</td>
<td>&gt;1% (median: 1%)</td>
<td>&gt;1% (median: 1%)</td>
<td>&gt;1% (median: 1%)</td>
</tr>
</tbody>
</table>

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