Blood-based detection of RAS mutations to guide anti-EGFR therapy in metastatic CRC patients: Concordance of results from circulating tumor DNA and tissue-based RAS testing

Frederick S. Jones¹, Dan Edelstein¹, Katharina Wichner², Carina Ross², and Frank Holtrup²

¹Medical Scientific Affairs, Sysmex Inostics Inc., 1 Sysmex Way, Mundelein, IL 60060, USA; ²Research & Development, Sysmex Inostics GmbH, Falkenried 88, 20251 Hamburg, Germany

BACKGROUND

Value of BEAMing and liquid biopsy approach in colorectal cancer EGFR antibody therapy selection

Evidence is building to support a key role for BEAMing (Beads, Emulsions, Amplification, Magnetics) technology for the rapid, accurate, and sensitive detection of clinically-actionable mutations in both therapeutic clinical trial patient stratification and oncology therapy selection applications.

- The clinical value of BEAMing to select metastatic colorectal cancer patients for anti-EGFR therapy is underscored by results from phase III trials. In these studies, RAS mutation detection by BEAMing resulted in superior overall survival for RAS wild-type metastatic colorectal cancer (mCRC) patients vs. RAS mutant patients who received treatment in first-line with EGFR antibodies. Notably, the BEAMing RAS 33 mutation panel was used to evaluate altogether 548 mCRC patients previously defined as KRAS exon 2 codon 12/13 WT in the cetuximab CRYSTAL and OPUS studies. Pre-amplification Emulsion PCR Hybridization & Flow Cytometry

METHODS

An analysis of combined data from two independent RAS mutation concordance studies using mCRC patient samples from European and Australian populations (7,8) comparing blood- vs. tissue-based RAS mutation testing. Plasma RAS mutation status was determined using the BEAMing RAS 33 mutation panel and compared to results obtained from SOC RAS DNA sequencing of FFPE tumor tissue samples.

- In both data sets, retrospective plasma and FFPE tumor tissue samples obtained from Stage IV CRC patients were compared. FFPE tissue originated from primary tumors of treatment-naive patients or metastatic sites in patients that progressed during chemotherapy.

OBJECTIVE

To evaluate the suitability of a blood-based RAS test for assessing eligibility of mCRC patients for anti-EGFR antibody therapy by establishing its concordance to SOC tissue-based RAS testing.

RESULTS

Concordance of Plasma and Tissue RAS Mutation Status in 76 mCRC Patients:

- 50 were from treatment-naïve mCRC patients — FFPE tumor tissue samples obtained from primary tumors (first-line anti-EGFR therapy candidates).
- 26 were from mCRC patients with >2 previous therapies at progression — FFPE tissue samples obtained from metastatic sites (later-line anti-EGFR therapy candidates).

<table>
<thead>
<tr>
<th>KRAS Mutation:</th>
<th>RAS 33 Mutation Panel:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon</td>
<td>Mutation</td>
</tr>
<tr>
<td>2</td>
<td>G12S</td>
</tr>
<tr>
<td>3</td>
<td>A59T</td>
</tr>
<tr>
<td>4</td>
<td>K117N</td>
</tr>
</tbody>
</table>

Plasma RAS result

<table>
<thead>
<tr>
<th>Tissue RAS result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>39</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>34</td>
<td>76</td>
</tr>
</tbody>
</table>

Overall Agreement = 71/76 = 93.4%

Positive Agreement = 39/42 = 92.9%

Negative Agreement = 32/34 = 94.1%

RAS mutation prevalence:

- Plasma — 54%
- Tumor tissue — 55.3%
- Both values are in accord with the known prevalence of extended RAS mutations observed in CRC patient populations.

- 3 cases were observed in which a RAS mutation was not detected in plasma, but was detected in tissue; the RAS mutation-positive status for 1 of these cases in tissue was confirmed by BEAMing; these results may be attributable to instances in which ctDNA was not shed into the circulation.
- 4 cases were observed in which a RAS mutation was detected in plasma, but not detected in tissue (see table in the next column).

CONCLUSION

The high overall agreement of plasma and tissue RAS testing results (93.4%) demonstrates that blood-based RAS mutation testing is a viable alternative to tissue-based testing for determining eligibility of CRC patients for anti-EGFR therapy.

REFERENCES

1. van Cutsem et al. 2015, J Clin Oncol 33, 692-700
2. Bokemeyer et al. 2015, Eur J Cancer 51(10), 1243-52
5. Morelli et al. 2015, Ann Oncol 26, 731-736
6. Tabernero et al. 2015, Lancet Oncol July 13 online
7. Hahn et al. 2015, ECC; Poster P-52
8. Scott et al. DOI:10.3252/pso.e17wgc15

This study was cooperatively funded by Sysmex Inostics and Merck KGaA.