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

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BACKGROUND

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

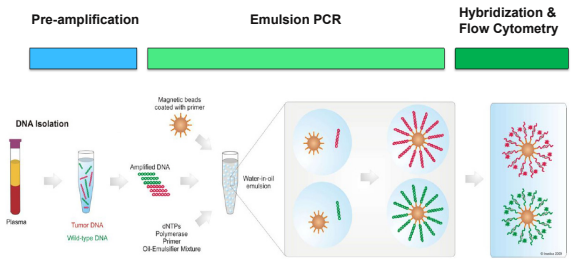
Evidence is building to support a key role for BEAMing (**B**eads, **E**mulsions, **A**mplification, **M**agnetics) technology for the rapid, accurate, and sensitive detection of clinically-actionable mutations in both therapeutic clinical trial patient stratification and oncology therapy selection applications.

- 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 when treated 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 and identified additional RAS mutations in 94 (17.2%) patients (1,2). Stratification of patients for 1st line cetuximab and chemotherapy based on this extended RAS panel improved patient outcomes compared to KRAS exon 2 analysis alone.
- Studies have showed that BEAMing liquid biopsy can reveal substantial differences in mutation status between the archival CRC tumor as compared to patient's current plasma mutation status. BEAMing has detected low frequency RAS mutations in primary tumors from CRC patients that were not detected by standard-of-care (SOC) methods; these patients had shorter PFS than those without detectable mutations by BEAMing. Differences in RAS mutation status between archival CRC primary tumor vs a current plasma RAS result in a metastatic CRC patient can also emerge as selective pressure is applied during multiple rounds of therapy. Therefore, liquid biopsy may be used to dynamically detect resistance to therapy in order to better inform subsequent treatment decisions (3-6).
- The accurate prescription of anti-EGFR therapy to RAS wild-type patients is of high clinical importance. BEAMing liquid biopsy can overcome current issues of RAS FFPE tumor tissue testing such as molecular heterogeneity, tissue availability/quality, and treatment history. The liquid biopsy approach also allows for detection of emergence/disappearance of genetic mutations linked to resistance/susceptibility to targeted therapies. This represents a distinct benefit to patient care.

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.

BEAMing digital PCR workflow



RAS 33 Mutation Panel:

KRAS

Exon	Mutation
2	G12S
	G12R
	G12C
	G12D
	G12A
	G12V
	G13D
3	A59T
	Q61L
	Q61H
	Q61H
4	K117N
	K117N
	A146T
	A146V

NRAS

Exon	Mutation
2	G12S
	G12R
	G12C
	G12D
	G12A
	G12V
	G13R
	G13D
	G13V
3	A59T
	Q61K
	Q61R
	Q61L
	Q61H
	Q61H
4	K117N
	K117N
	A146T

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 tested. FFPE tissue originated from primary tumors of treatment-naïve patients or metastatic sites in patients that progressed during chemotherapy.
- For BEAMing, A cut-off of 0.02% mutant fraction threshold was used for plasma. For SOC RAS testing, either 2% or 5% allelic fraction threshold was applied depending on the method.

RESULTS

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

- **50** were from treatment-naïve mCRC patients – FFPE 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)**.

		Tissue RAS result		
		Positive	Negative	Total
Plasma RAS result	Positive	39	2	41
	Negative	3	32	35
	Total	42	34	76

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 was not detected in tissue **(see table in the next column)**.

Tissue Source	Plasma RAS Result	Tissue RAS Results: Sequencing and BEAMing
Liver met	KRAS Codon146 (0.126%)	No result for KRAS Exons 3 & 4, no mutation detected at other codons
Primary tumor	NRAS Codon61 (0.258%)	No mutation detected; tissue not available for BEAMing
Primary tumor	KRAS Codon12 (0.111%)	No mutation detected; tissue not available for BEAMing
Primary tumor	KRAS Codon12 (0.425%)	No mutation detected; KRAS Codon12 (2.583%) detected by BEAMing

*mutant fraction percentages obtained by BEAMing are indicated.

- For 1 of these 4 cases, a KRAS Exon4 Codon 146 mutation was detected in plasma; however, tissue sequencing results for Exon4 was not obtained; given that the data were not complete, this case was excluded from the concordance analysis.
- For the next 2 cases, tissue was not available for re-examination of RAS status by BEAMing; thus these were determined to be cases of discordance in the overall analysis and may represent patients whose tumors exhibit molecular heterogeneity.
- In a final case, tissue was available for repeat RAS testing using BEAMing; this analysis identified the same KRAS mutation in the tissue specimen detected in plasma. This case was therefore included as concordant and highlights the variability in current SOC tissue techniques.

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
3. Diaz et al. 2012, Nature 486(7404), 537–540
4. Misale et al. 2012, Nature 486(7404), 532–536
5. Morelli et al. 2015, Ann Oncol 26, 731-736
6. Tabernero et al. 2015, Lancet Oncol July 13 online PMID: 26184520
7. Hahn et al. 2015, ECC ; Poster P-52
8. Scott et al. DOI:10.3252/pso.eu.17wcgc2015

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