Monitoring Minimal Residual Disease (MRD) by *KRAS* Mutation Burden in Urinary or Plasma Circulating Tumor (ct) DNA in Colorectal Cancer (CRC) Patients with Resectable Liver Metastases

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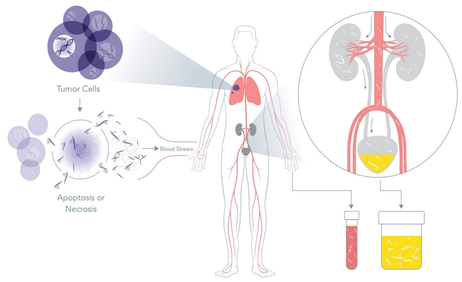
Background

Over half of patients with CRC will develop liver metastases. Surgical resection greatly improves outcomes in these patients. Non-invasive markers are needed to better monitor treatment responses and guide complex treatment decisions. This study evaluated the utility of quantifying *KRAS* mutation burden in urine and plasma ctDNA for monitoring MRD in surgical CRC patients with liver metastases.



Liquid biopsy: urine and plasma ctDNA sources

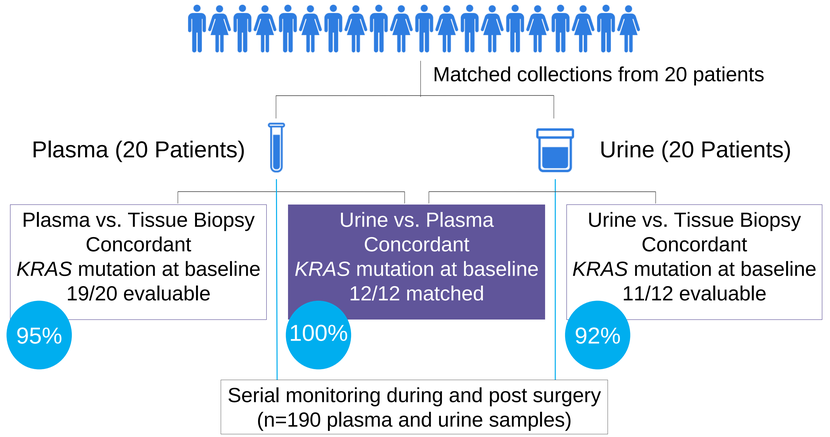
Clinical Study Design

- Samples were collected from 20 patients with Stage IV colorectal cancer and *KRAS* positive primary tumor.
- All patients had undergone surgical treatment in combination with various systemic therapies including neoadjuvant radio/chemotherapy, adjuvant targeted therapy and adjuvant chemotherapy.
- In a blinded retrospective study, concordance between *KRAS* detection in archived tissue/plasma/urine samples was studied for baseline. Matched urine and plasma *KRAS* were monitored longitudinally.

Assay Design

- Highly sensitive mutation enrichment assay for the detection of *KRAS* codon 12/13 mutations in highly fragmented urinary and plasma ctDNA.
- For greater sensitivity in fragmented ctDNA, the assay utilizes a 31bp footprint. A selective enrichment step for mutated DNA fragments suppresses wild-type (WT) sequence amplification with a blocker. Barcoded adaptor primers are added for compatibility with next generation sequencing (MiSeq).
- The Lower Limit of Detection (LLoD) of the ctDNA *KRAS* G12/13 assay is 0.002% mutant alleles in a background of wild-type DNA (2 mutant copies in 100,000 wt copies background)

Concordance Study



PLASMA: *KRAS* mutation concordant with tumor tissue detected in 19 of 20 evaluable baseline plasma samples (95%).

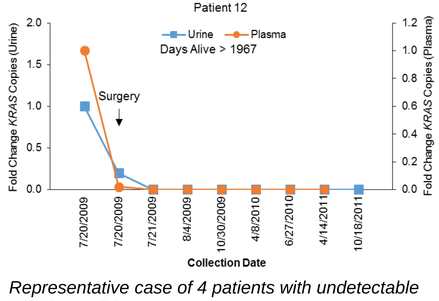
URINE: *KRAS* mutation concordant with tissue detected in 11 of 12 evaluable baseline urine samples (92%).

SAMPLE QUALITY:

- Total of 190 plasma and urine samples archived for 3-5 years were tested.
- 101 of 101 plasma samples (100%) and 79 of 92 urine samples (86%) had sufficient DNA concentration and were deemed evaluable. The degree of DNA degradation in these archival urine samples is significantly elevated compared to typical clinical samples.

Correlation between Post-Operative *KRAS* Counts and Surgical Radicality

mCRC Patients with Intent-to-Cure Surgery



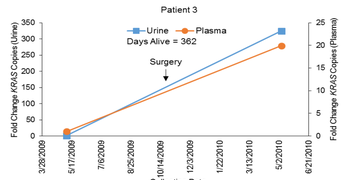
Representative case of 4 patients with undetectable KRAS and R0 surgical radicality shown

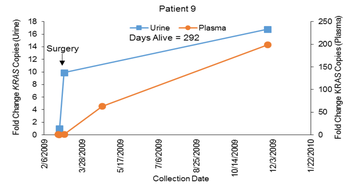
- In 4 out of 5 patients with curative intent surgery, ctDNA *KRAS* levels were undetectable in urine or plasma at all time points measured after surgery.

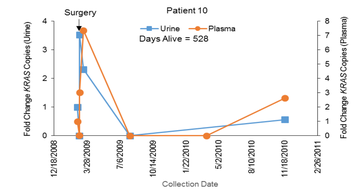
Correlation between Post-Operative *KRAS* Counts and Surgical Radicality

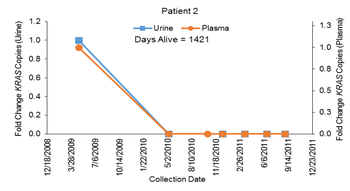
mCRC Patients with Palliative Surgery

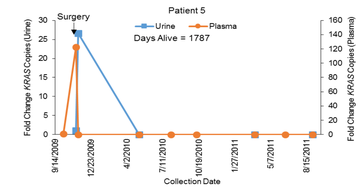
Representative cases of 12 patients shown











Conclusions

- In a blinded study of colorectal cancer patients with known *KRAS* mutational status in tumor tissue, a correct *KRAS* mutation was identified in 95% of archival plasma and 92% of archival urine specimens.
- Clear correlation and compatible fold change was demonstrated between the dynamics of plasma and urinary ctDNA *KRAS* changes on treatment (surgery and adjuvant).
- In 4 of 5 patients with curative intent surgery, ctDNA *KRAS* levels were undetectable in urine or plasma at all points post-surgery. In contrast, in cases with palliative surgery, the ctDNA *KRAS* signal remained detectable after surgery for 10 of 12 patients in urine and 9 of 12 patients for plasma.
- We demonstrate clinical applicability of assessing the MRD post-surgery in CRC patients with liver metastases by quantitative monitoring of urinary ctDNA *KRAS* with single molecule sensitivity.

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