Monitoring Minimal Residual Disease by Urinary or Plasma Circulating Tumor DNA of *KRAS* Mutation Burden in Colorectal Cancer Patients with Resectable Liver Metastases

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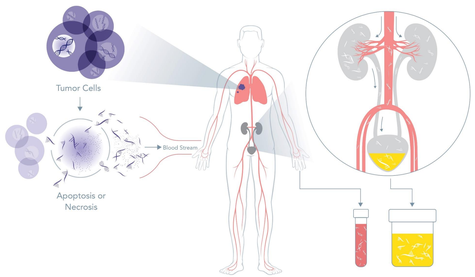
Abstract #: 5237

Background

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the third leading cause of cancer deaths. Over half of patients with CRC will develop liver metastases. Surgical resection, in combination with systemic therapies, greatly improves long-term outcomes. Around 40% of patients with resected liver limited disease are alive 5 years after diagnosis. While tumor staging and radicality of surgery are commonly used for prognostic assessment, better non-invasive markers are needed for monitoring chemo-responsiveness, following minimal residual disease (MRD), and guiding complex treatment decisions in these patients. This study evaluated the utility of quantitating *KRAS* mutation burden in urinary and plasma ctDNA as a means of monitoring MRD in surgical CRC patients with liver limited metastases.



Liquid biopsy: plasma and urine as sources of ctDNA

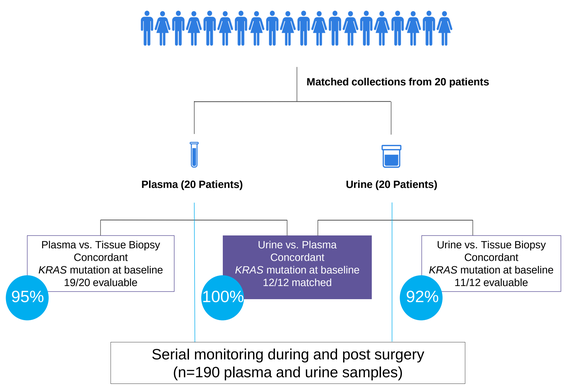
Clinical Study

- Samples were collected from 20 patients with Stage IV colorectal cancer and *KRAS* positive primary tumor.
- All patients were undergoing surgical treatment in combination with various systemic therapies including neoadjuvant radio/chemotherapy, adjuvant targeted therapy and adjuvant chemotherapy.
- Archived, matched longitudinal plasma and urine samples were collected and evaluated prior to surgery (baseline), as well as at time intervals throughout the course of the disease.
- In a blinded retrospective study, concordance between *KRAS* detection in archived tissue/plasma/urine samples was studied for baseline, and urine and plasma *KRAS* was monitored longitudinally.

Assay Design

- We developed a highly sensitive mutation enrichment assay for the detection of *KRAS* codon 12/13 mutations in highly fragmented urinary and plasma ctDNA.
- To achieve greater sensitivity in fragmented cfDNA, the assay utilizes a 31bp footprint and contains a selective enrichment step for mutated DNA fragments, while suppressing wild-type (WT) sequence amplification with a blocker. Barcoded adaptor primers are added for compatibility with next generation sequencing (MiSeq).
- Following sequencing, a proprietary analysis algorithm allows accurate quantitation of input level of mutant DNA.

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

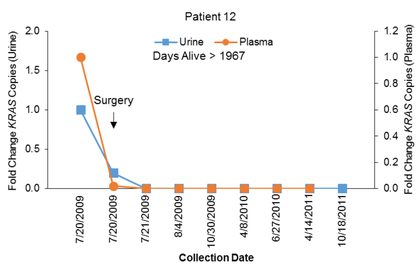
- Total of 190 plasma and urine samples archived for 3-5 years were tested.
- 101 of 101 plasma samples (100%) and 79 of 92 urine samples (86%) had sufficient DNA concentration and were deemed evaluable. The degree of DNA degradation in these archival urine samples is significantly elevated compared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

Association between Post-Operative *KRAS* Counts and Survival

mCRC Patients with Intent-to-Cure Surgery

Representative case of 5 patients 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.

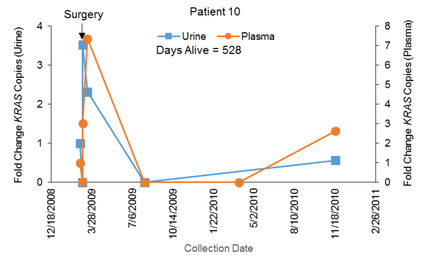


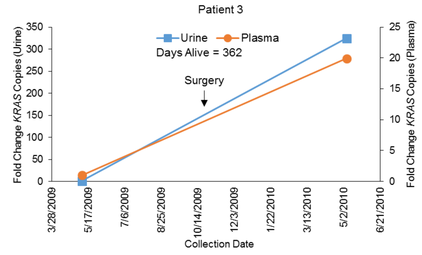
Monitoring during treatment
(total n=190 plasma and urine samples)

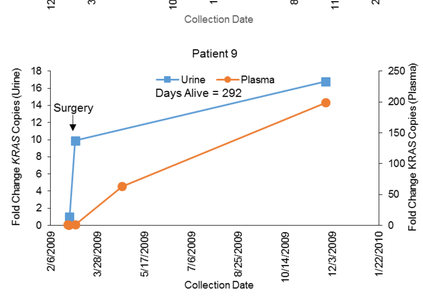
mCRC Patients with Palliative Surgery

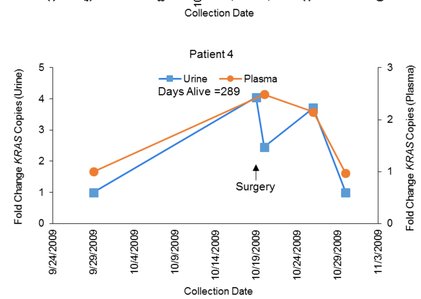
Representative cases of 5 patients shown

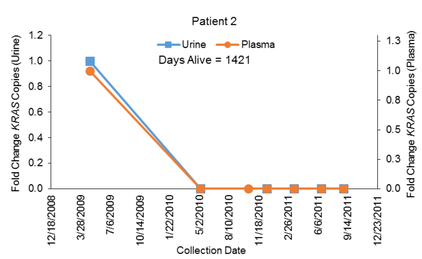
- In 10 of 12 patients with palliative surgery, the ctDNA *KRAS* signal in urine remained detectable post-surgery.
- In 9 of 12 patients, plasma *KRAS* remained detectable after surgery.
- Dynamics of *KRAS* signal in urine parallels the dynamics in plasma.

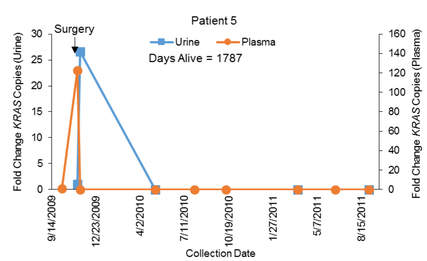












Conclusions

- A quantitative ctDNA assay using a next generation sequencing approach was developed to monitor ctDNA *KRAS* G12/13 mutational load in plasma and urine. The assay detects a single copy of mutant *KRAS* DNA with sensitivity of 0.006% mutant molecules in a background of wild-type DNA.
- 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.
- A prognostic significance of post-surgical *KRAS* levels and the overall survival in Stage IV CRC patients with liver metastases is being evaluated in a larger cohort.

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