Monitoring Urinary Circulating Tumor DNA (ctDNA) *KRAS* for Treatment Response in Patients with Metastatic Colorectal Cancer (mCRC)

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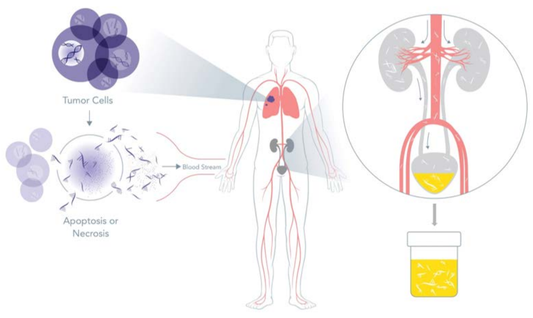
trovagene
precision cancer monitoring

Background

Colorectal cancer (CRC) is the third cause of cancer mortality in the United States. Despite advances in early detection, each year more than 50,000 patients are diagnosed with metastatic disease. Combination chemotherapy, targeted drugs, and surgical interventions have revolutionized the treatment landscape and improved survival of these patients. Clonal evolution is considered a major cause of drug resistance and non-invasive strategies to detect new and evolving mutations can impact the delivery of personalized treatment. Moreover, non-invasive techniques have the potential to transform the standard of response assessment in metastatic colorectal cancer (mCRC) and reduce the need for imaging in the management of CRC.



Liquid biopsy: urine as a source of ctDNA

Clinical Study

60 Metastatic CRC Patients

30 *KRAS* G12/13 mutant positive by tissue biopsy → Chemotherapy → Monitor Urine *KRAS* ctDNA for Response or Treatment Failure

30 *KRAS* wild-type by tissue biopsy → Anti-EGRF plus Chemotherapy → Monitor Urine *KRAS* ctDNA for Emergence of *KRAS* Mutations

- Interim analysis of 4 metastatic CRC patients with positive *KRAS* tissue status
  Metastases: liver, n = 3; lung, n = 1
- Patients were monitored for *KRAS* ctDNA during chemotherapy
  FOLFOX, n = 4; surgery, n = 2
- Urine was collected every two weeks on treatment (x6), and with each radiologic scan (at 6-8 weeks)

Results

Urinary *KRAS* G12/13 Assay

- Urinary ctDNA was extracted using Trovagene platform that preferentially isolates small fragmented DNA.
- Highly sensitive, quantitative mutation enrichment PCR-NGS (MiSeq) assay for the detection of *KRAS* codon 12/13 mutations in highly fragmented 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).

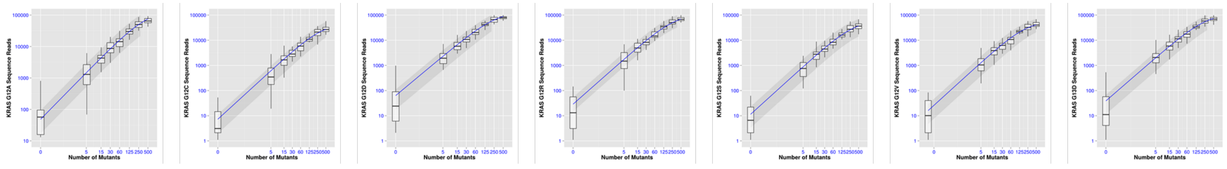
Lower Limit of Detection (LLoD) = 0.002%

Number of Mutant Copies	0/1	2+
Expected (95% CI) [2 copies/rep]	32 (21-46)	48 (35-64)
Observed G12A	36	44
Observed G12C	25	55
Observed G12D	31	49
Observed G12R	37	43
Observed G12S	24	56
Observed G12V	24	56
Observed G13D	45	35

Table 1. KRAS G12/13 ctDNA assay detects 2 copies of mutant KRAS DNA in a background of 100,000 copies of wild-type DNA (LLoD = 0.002%). LLoD verification testing using 80 replicates of DNA blends with mutant spike-in levels of 2 copies/100,000 geq demonstrates that the actual positive/negative hit distribution matches theoretical model for a Poisson distribution (LLoD = 0.002%).

Standard Curves for Quantification

Accurate quantitation of the input level of mutant KRAS ctDNA in analytical and clinical samples is achieved through a validated bioinformatics algorithm.

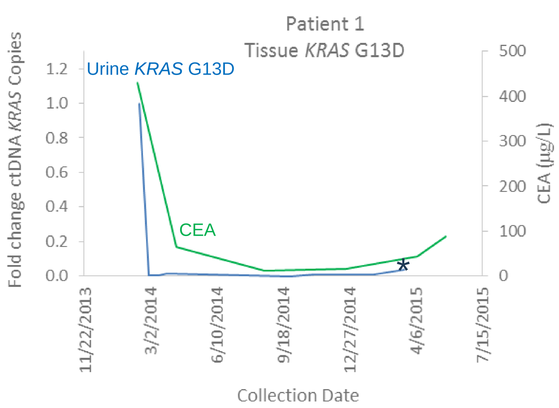


- Examples of master standard curves for *KRAS* exon 2 codons G12V/A/R/D/S/C and G13D.
- Standard curves were developed for each *KRAS* mutation using 288 independent enrichments reactions/curve with different amounts of spiked DNA input from 0-500 copies.
- Output is standardized to report number of input copies per 10^5 genome equivalents.

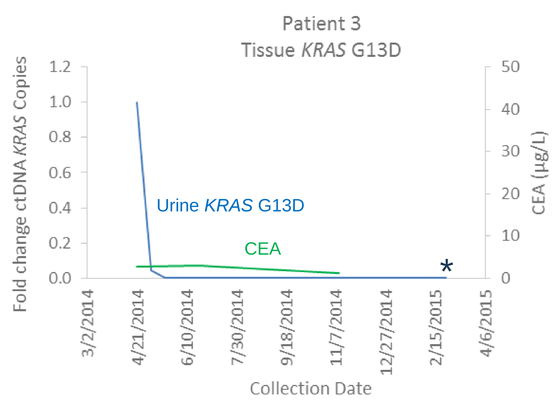
Monitoring ctDNA *KRAS* for Response to Chemotherapy

Decrease in *KRAS* G12/13 mutational burden after 2 weeks of chemotherapy correlates to treatment response

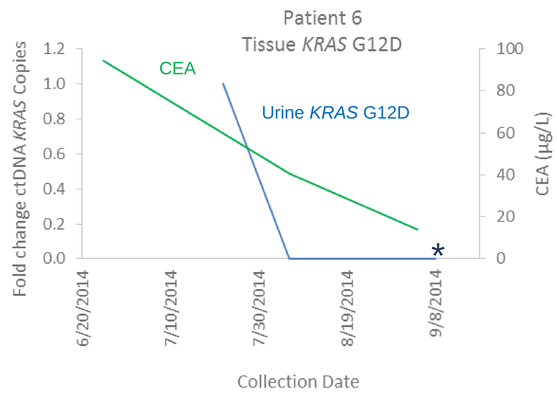
- A concordant *KRAS* G12/13 mutation was detected in urinary ctDNA in 3 of 4 mCRC patients. In 1 of 4 patients (patient with lung metastases), a different *KRAS* mutation was detected in urine after surgical resection of the primary tumor



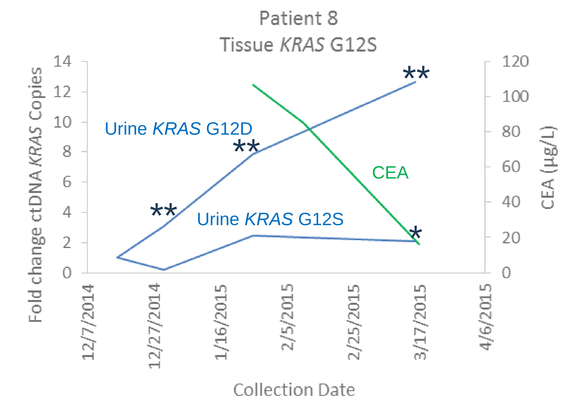
⁎ Last serial point for urine ctDNA *KRAS* G13D
Is **above** the assay detection threshold



⁎ Last serial point for urine ctDNA *KRAS* G13D
Is **below the** assay detection threshold



⁎ Last serial point for urine ctDNA *KRAS* G12D
Is **below** the assay detection threshold



⁎ All points for urine *KRAS* G12S are **below** threshold
⁎⁎ Urine *KRAS* G12D **above** threshold

Conclusions

- Dynamics of urinary ctDNA *KRAS* G12/13 mutational load correlated with clinical course in mCRC patients.
- Decrease in urine ctDNA *KRAS* G12/13 mutation levels after 2 weeks of chemotherapy detects molecular response in advance of radiographic response.
- In one patient (Patient 1), radiographic progression was detected 3 months after rising ctDNA *KRAS* mutation was observed in urine.
- The ctDNA KRAS G12/13 assay can be used to guide treatment decisions in mCRC patients.
- Given these results, expansion of this cohort is underway to investigate the clinical utility of *KRAS* mutation copy number correlated to a spectrum of various treatments for mCRC at different treatment stages.

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