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Use of urinary circulating tumor DNA *KRAS* for monitoring treatment response in patients with metastatic colorectal cancer

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trovagene
precision cancer monitoring

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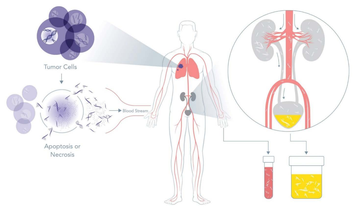
Background

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. Radiographic imaging in patients with mCRC is the current standard of care for monitoring responses. At least 40% of patients with mCRC have tumor associated *KRAS* mutations, and these may be detected in tumor DNA in plasma and urine. The aim of this study was to correlate the dynamics of *KRAS* mutational load in urinary ctDNA with clinical responses in patients with mCRC.



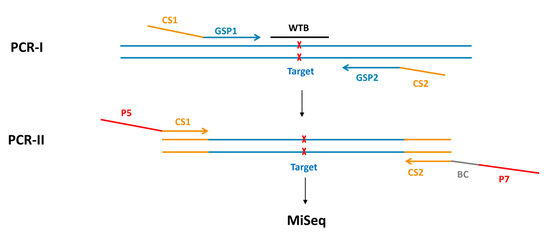
Liquid biopsy: urine and plasma as sources of ctDNA

Methods

ctDNA *KRAS* G12/13 assay (Figure 1)

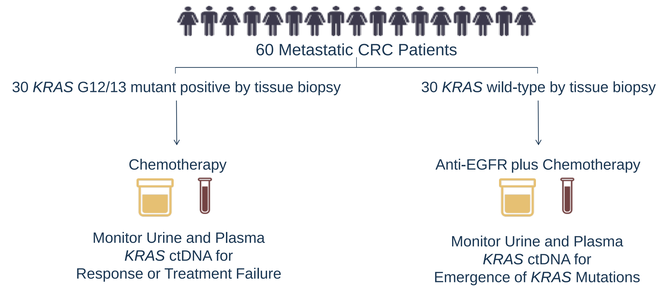
- Urinary ctDNA was extracted using the Trovagene platform that preferentially isolates small fragmented DNA.
- A quantitative mutation enrichment PCR- next-generation sequencing assay detects all *KRAS* codon 12/13 variants.
- For greater sensitivity in fragmented ctDNA, the assay utilizes a 31bp footprint. 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).

Figure 1. Enrichment PCR-NGS assay design



- **PCR-I**: Gene Specific Primers (**GSP1**, **GSP2**) with non-complementary "common sequence tails" (**CS1**, **CS2**), amplify target.
- Wild-type Blocker (**WTB**) limits WT amplification.
- Allele Specific Cycling Conditions (ASCC) limit WT amplification.
- **PCR-II**: Add flow cell adapters (**P5**,**P7**) and sample barcode (**BC**)

Clinical Study



- Patients were monitored for *KRAS* ctDNA during chemotherapy
  - o *KRAS* mutated patients: FOLFOX +/- Avastin (bevacizumab), n=7; surgical intervention, n=2 (One with curative resection and one with palliative resection)
  - o *KRAS* wild-type patients: FOLFIRI + Erbitux (cetuximab), n=5
- Urine was collected every two weeks on treatment (x6), and with each radiologic scan (at 6-8 weeks)

Results

ctDNA *KRAS* G12/13 assay performance (Table 1)

Table 1. ctDNA *KRAS* G12/13 assay performance

Performance Characteristics	*Performance*
ctDNA Source	Urine (specimen collection kit available, room temperature stability = 2 weeks) Plasma (Streck Whole Blood tube, BD Vacutainer K2 EDTA or CPT tube)
Input DNA	Accurate quantitation of mutant alleles with any DNA input. Standardized reporting indicates number of copies of mutant DNA per 10^5 genome equivalents (geq)
Analytical Sensitivity	***KRAS* G12/13** assay detects 1 copy of mutant DNA in a background of ~20,000 copies of wild-type DNA (0.006%) or 2 copies in 100,000 geq (0.002%)
Reportable Range	Verified linear quantitation range between 1 and 125 copies; 95% confidence intervals reported
Precision	2-4 fold discrimination within verified reportable range

ctDNA testing for *KRAS* G12/13 has high sensitivity and detects *KRAS* mutations concordant with tumor biopsy (Table 2)

- Seven (7) patients with *KRAS* G12/13 mutations and 5 patients with wild-type *KRAS* in tumor tissue were tested by ctDNA *KRAS* test in urine and plasma.
- ctDNA *KRAS* concordance between tissue and ctDNA:
  - Urine: Concordant *KRAS* G12/13 mutation was detected in 5 of 7 patients. In one additional patient (patient with lung metastases), a different *KRAS* mutation was detected in urine after surgical resection of the primary tumor.
  - Plasma: Concordant *KRAS* G12/13 mutation was detected in 6 of 7 patients.
  - Additional *KRAS* mutations were detected by ctDNA in 3 patients (Table 2, *), which may be explained by tumor tissue heterogeneity.

Table 2. Concordance between tissue, urine and plasma *KRAS* G12/13

Patient ID	*KRAS* G12/13 Result		
	Tissue	Urine	Plasma
1	G13D	G13D	G13D
2	Wild-type	Wild-type	Wild-type
3	G13D	G13D*	G13D
4	Wild-type	Wild-type	Wild-type
5	Wild-type	Wild-type	Wild-type
6	G12D	G12D	G12D
7	G13D	Not Detected	G13D
8	G12S	Not Detected	G12S
9	G12D	G12D	G12D*
10	Wild-type	Wild-type	Fail
11	Wild-type	Wild-type	Wild-type
13	G12D	G12D*	Discordant (G12A)

Results

Monitoring molecular response to chemotherapy by urinary *KRAS* (Figures 3, 4, 5)

- Five patients with positive *KRAS* G12/13 status in urine at baseline were monitored on treatment (FOLFOX).
- Clinical responses determined by RECIST 1.1 criteria. Urinary ctDNA assessed at Cycle 4 Day 1, contemporaneously with CT scans (**Figure 3**).
  - Best Response by imaging: Partial Response (PR) was observed in 3 patients, Stable Disease (SD) was observed in 2 patients.
  - Molecular response in urinary *KRAS*: *KRAS* mutation burden in urine decreased by more than 90% at Cycle 4 Day 1 in all five patients who responded to FOLFOX (**Figure 3**).
- Decrease in urinary *KRAS* is observed after 2 weeks of therapy in patients with PR and SD (**Figure 4,** representative patients shown).
- Monitoring by urinary *KRAS* allows to detect response and progression in advance of imaging (**Figure 5**).

Figure 3. Changes in urinary *KRAS* at Cycle 4 Day 1 correlate with Clinical Response

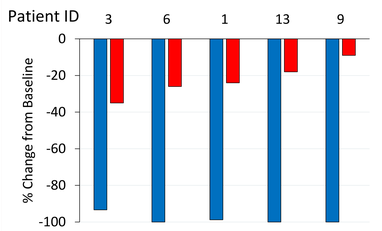


Figure 4. Patients responding to therapy have a significant decrease in urinary *KRAS* signal after the first treatment cycle

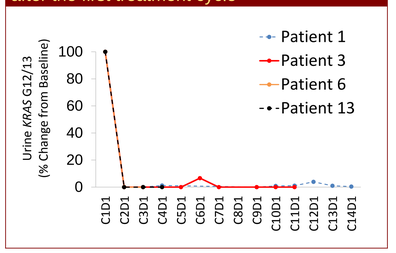
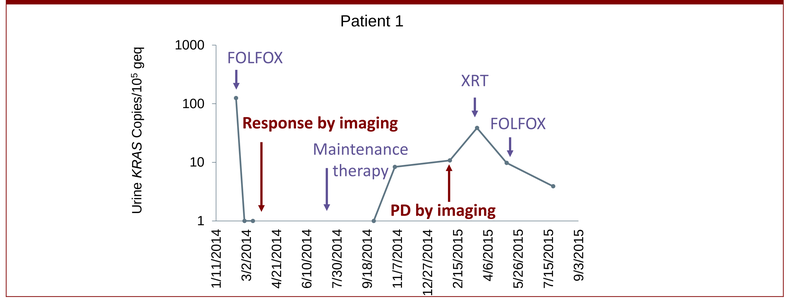


Figure 5. Dynamics of urinary *KRAS* predicts clinical response in advance of imaging



Conclusions

- ctDNA testing by urine and plasma has high sensitivity and detects *KRAS* mutations concordant with tumor biopsy.
- Dynamics of urinary ctDNA *KRAS* G12/13 mutational load correlated with clinical course in mCRC patients.
- Decrease in urine or plasma 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 2-3 scan cycles after rising ctDNA *KRAS* mutation was observed in urine.
- Expansion of this cohort is underway to further investigate the clinical utility of monitoring urinary *KRAS* to inform treatment decisions for mCRC patients.

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