Circulating Tumor DNA Assay Performance for Detection and Monitoring of *KRAS* Mutations in Urine from Patients with Advanced Cancers

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Introduction

- Noninvasive urinary ctDNA-based liquid biopsy approach can be used to detect and track cancer driver mutations for rapid diagnosis and disease monitoring.
- Using a highly sensitive ctDNA mutation detection platform, we examined detection of *KRAS* G12/13 mutations in urine obtained from patients with advanced cancers, assessed urine sample requirements, and compared the results with matched tumor tissue.

Study Design

TABLE 1: Patient demographics

	TOTAL (N=41)	# of patients (%)
Age, years – Median (range)	56 (38-77)	
Gender	Male	20 (48.8)
	Female	21 (51.2)
ECOG Performance status	0	4 (9.8)
	1	35 (85.4)
	2	1 (2.4)
	3	1 (2.4)
Cancer type	Colorectal cancer	29 (70.7)
	Non-small cell lung cancer	6 (14.6)
	Pancreatic cancer	2 (4.9)
	Ovarian cancer	2 (4.9)
	Others	2 (4.9)
KRAS mutation	G12C	7 (17.1)
	G12D	21 (51.2)
	G12R	3 (7.3)
	G12S	2 (4.9)
	G12V	6 (14.6)
	G13D	2 (4.9)

- Urine and plasma samples collected from 41 patients with advanced or metastatic cancers positive for *KRAS* G12/13 mutations in tissue (colorectal, n=26; lung, n=6; pancreatic, n=4; ovarian, n=2; melanoma, n=1; breast, n=1; other, n=1).

KRAS G12/13 Mutation Enrichment NGS Assay

- Mutant allele enrichment PCR, followed by NGS, utilizes a 31-bp footprint and selectively amplifies mutant DNA fragments while suppressing Wild-Type (WT) sequence amplification.
- Proprietary analysis algorithm allows accurate quantitation of mutant DNA by interpolation to standard curves.
- Mutation enrichment results in approximately 3000-fold increase in ratio of mutant over WT signal for low copy number inputs. For 5 mutant *KRAS* G12D copies spiked into 18,181 copies of WT DNA (0.0275%), the output sequencing library contains 94% mutant reads (~3418 fold enrichment; **Table 2**).

TABLE 2: Comparison between input ratio of mutant/WT *KRAS* copies and output ratio of mutant/WT *KRAS* sequencing reads

Input MT Copies/WT Copies (% Mutant)	Output Mutant Sequencing Reads/Wild Type Reads (% Mutant)		
	KRAS G12D	*KRAS* G12V	*KRAS* G13D
5/18,181 (0.0275%)	13447/858 (94%)	4269/410 (91.2%)	2318/748 (75.6%)
15/18,181 (0.0825%)	34363/1155 (96.7%)	9068/423 (95.5%)	15726/1053 (93.7%)
125/18,181 (0.688%)	156863/1855 (98.8%)	144666/1821 (98.8%)	170503/1348 (99.2%)
250/18,181 (1.375%)	309123/2307 (99.3%)	267933/2452 (99.1%)	331498/2216 (99.3%)
500/18,181 (2.750%)	508045/1442 (99.7%)	472491/2836 (99.4%)	585254/1807 (99.7%)

Similar results obtained for other variants (data not shown).

Results

Analytical Assay Sensitivity
Lower Limit of Detection (LLoD) = 0.002%

- LLoD was defined as the lowest number of copies for which frequency distribution of the copy number events, upon repeated measurements, falls within the 95% confidence interval of expected frequency distribution determined by Poisson statistics.
- *KRAS* G12/13 assay LLoD:
  - 1 mutant copy in a background of 18,181 copies of Wild-Type DNA (0.006%; **Table 3**).
  - 2 mutant copies in a background of 100,000 copies of Wild-Type DNA (0.002%; data not shown).

TABLE 3: Verification of *KRAS* G12/13 assay LLoD

Number of Mutant Copies	0 (Not-detected)	1+ (Detected)
Expected (95% CI) [1 copy/rep]	7 (2-14)	13 (6-20)
Observed G12A	2	8
Observed G12C	5	15
Observed G12D	3	17
Observed G12R	10	10
Observed G12S	6	14
Observed G12V	4	16
Observed G13D	3	17

The observed frequency distribution of copy number events falls within the expected distribution for the LLoD of 1 mutant copy in a background of 18,181 copies of WT DNA.

Clinical Sensitivity of *KRAS* G12/13 ctDNA Assay

- Of 41 patients with advanced cancers, 23 had pre-treatment baseline urine samples with urine volumes 40-110 mL available and 29 had pre-treatment plasma (1-4 mL) samples available.
- Urinary DNA yields ranged from 15 to 23059 ng (median, 1059 ng).
- Plasma DNA yields ranged from 12 to 1846 ng (median, 55 ng).

TABLE 4: Urine/tissue concordance for *KRAS* G12/13 detection

Colorectal Cancer	Urine (90-110 mL)	100% (4/4)
	Urine (40-110 mL)	78% (14/18)
	Plasma (1-4 mL)	100% (16/16)
Multiple Cancers	Urine (90-110 mL)	80% (4/5)
	Urine (40-110 mL)	70% (16/23)
	Plasma (1-4 mL)	83% (24/29)

- Positive percent agreement (PPA) for urinary *KRAS* G12/13 with tumor tissue as reference (**Table 4**):

  Colorectal Cancer
  - 100% (4/4) for urine with recommended volume of at least 90 mL.
  - 78% (14/18) for urine with all volumes (40-110 mL).

  Multiple cancers
  - 80% (4/5) for urine with recommended volume of at least 90 mL.
  - 70% (16/23) for urine with all volumes (40-110 mL).

- Positive percent agreement (PPA) for plasma *KRAS* G12/13 with tumor tissue as reference:

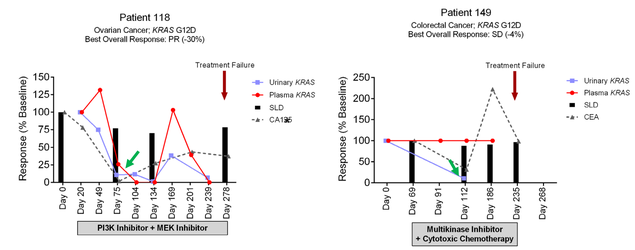
  Colorectal Cancer
  - 100% (16/16)

  Multiple cancers
  - 83% (24/29)

Longitudinal Monitoring by ctDNA *KRAS* G12/13

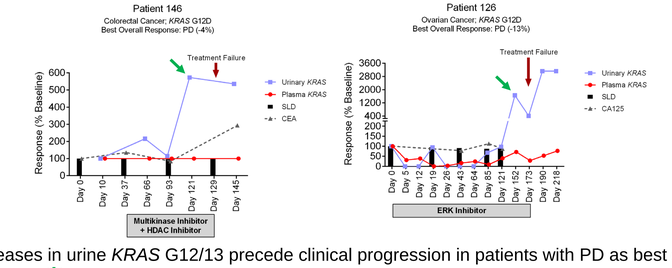
Representative cases showing dynamics of urine and plasma ctDNA *KRAS* G12/13 in patients with advanced or metastatic cancers on experimental therapies.

Patient with Partial Response (PR) or Stable Disease (SD)



- Early decreases from baseline (by 89-96%) are observed for urine *KRAS* G12/13 signal in patients with PR or SD as best overall response using RECIST 1.1 criteria (↓).

Patients with Progressive Disease (PD)



- Increases in urine *KRAS* G12/13 precede clinical progression in patients with PD as best overall response (↓).

**SLD, sum of the longest diameters of index lesions.*

Conclusions

- Mutation enrichment NGS assay for *KRAS* G12/13 mutation detection in ctDNA has a single copy analytical sensitivity (0.002%-0.006%).
- In a blinded study of 41 patients with advanced or metastatic cancers, including colorectal, pancreatic, ovarian, lung, melanoma, and breast cancers, *KRAS* G12/13 mutation concordant with tissue was detected in 80% of urine samples with recommended volume of at least 90 mL and 83% of plasma samples with volumes 1-4 mL. In a colorectal cancer cohort, *KRAS* G12/13 mutation detection sensitivity was 100% for both urine and plasma.
- Kinetics of *KRAS* G12/13 mutation signal in urine ctDNA corresponds to treatment outcomes.
- Analysis of urine and plasma may be a viable approach for diagnostic detection of *KRAS* mutations and therapeutic monitoring of patients with advanced cancers.

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