Low Frequency *KRAS* G12/13 Mutations in Urine Cell-Free (cf) DNA from Patients with *BRAF* V600E-Mutant Advanced Cancers

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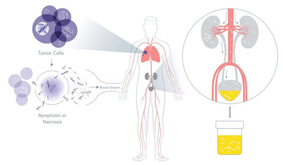
Background

Tumor heterogeneity and clonal selection contribute to resistance to molecular targeted therapies. Dynamic tracking of urinary cell-free DNA mutations can offer a noninvasive tool for monitoring therapeutic efficacy.



Liquid biopsy: urine as a source of cfDNA

Clinical Study Design

34 metastatic cancer patients

Positive tissue BRAF V600E

Treatment with BRAF or MEK targeted therapy

Evaluate urinay cfDNA *BRAF* V600E *and KRAS G12/13 at* baseline

Evaluate urinary cfDNA *BRAF* V600E and *KRAS* G12/13 on treatment

Patients

Cancer Types: melanoma, n=11; colorectal cancer, n=8; thyroid cancer, n=5; non-small cell lung cancer, n=5; other, n=5.

Treatment: BRAF targeted therapy, n=26; MEK targeted therapy, n=1; no therapy, n=7.

cfDNA KRAS and BRAF Assays

- cfDNA *BRAF* V600E was levels were detected by mutant enrichment ddPCR (RainDance). LLoD = 0.03% mutant in the background of wild-type DNA.
- cfDNA *KRAS* G12/13 levels were monitored using highly sensitivity mutant enrichment NGS assay. LLoD = 1 copy in 18,180 wild-type genome equivalents (geq) (0.006%), or 2 copies in 100,000 geq (0.002%).

KRAS G12/13 assay LLoD = 0.002% (2 copies in 100,000 geq)

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. *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%).*

Results

Detection of low frequency urinary *KRAS* G12/13 Mutations in Patients with *BRAF* V600E mutation in Tumor Tissue

Urinary cfDNA *BRAF* V600E

- 32 (94%) of 34 patients with *BRAF* V600E mutation in the tumor had detectable *BRAF* V600E mutation in urine (mutant, n=24; low-mutant, n=8). In 25 of 34 patients with longitudinal time points, changes in ctDNA *BRAF* V600E copies correlated with percentage changes in target lesions on imaging (r=0.68, p<0.001).

		Urine cfDNA *KRAS* G12/13															
		Pretreatment								Posttreatment							
Patient ID	Cancer Type	Not Tested	Not Detected	KRAS G12A	KRAS G12C	KRAS G12D	KRAS G12R	KRAS G12S	KRAS G12V	KRAS G13D	Not Tested	Not Detected	KRAS G12A	KRAS G12C	KRAS G12D	KRAS G12R	KRAS G12S	KRAS G12V	KRAS G13D
Patient 01	Non-Small Cell Lung Cancer																		
Patient 02	Papillary Thyroid Cancer																		
Patient 03	Non-Small Cell Lung Cancer											no treatment							
Patient 05	Melanoma	X																	
Patient 06	Papillary Thyroid Cancer*	X																	
Patient 09	Colorectal Cancer											no treatment							
Patient 11	Melanoma		ND																
Patient 16	Colorectal Cancer																		
Patient 26	Temporal Glioblastoma																		
Patient 29	Melanoma																		
Patient 30	Melanoma	X																	
Patient 32	Non-Small Cell Lung Cancer		ND								X								
Patient 37	Non-Small Cell Lung Cancer	X																	
Patient 38	Melanoma											no treatment							
Patient 39	Papillary Thyroid Cancer											no treatment							
Patient 40	Melanoma											no treatment							
Patient 43	Ovarian Carcinoma		ND																
Patient 44	Appendiceal Adenocarcinoma		ND																
Patient 46	Colorectal Cancer		ND									ND							
Patient 48	Melanoma											no treatment							
Patient 49	Melanoma		ND																
Patient 54	Cholangiocarcinoma		ND																
Patient 59	Ovarian Carcinoma		ND																
Patient 61	Colorectal Cancer		ND								X								
Patient 75	Colorectal Cancer		ND								X								
Patient 76	Melanoma		ND																
Patient 77	Papillary Thyroid Cancer		ND																
Patient 79	Colorectal Cancer		ND									ND							
Patient 86	Papillary Thyroid Cancer		ND									ND							
Patient 89	Colorectal Cancer**		ND																
Patient 93	Melanoma		ND																

Table 2. *Heterogeneous KRAS G12/13 mutations frequently detected in urine of patients on BRAF/MEK1 targeted therapy. *Patient with tissue positive KRAS G12/13 by ddPCR test (1.3% mutant allele fraction).**Patient with tissue positive KRAS G12/13 by CLIA test.*

Urinary cfDNA *KRAS* G12/13 mutations

- 25 (81%) of 31 patients tested had urinary *KRAS* G12/13 mutations in at least one time point; 11 patients had detectable urinary *KRAS* mutations pre-treatment and 19 on treatment.

Plasma *KRAS* G12/13 mutations

- 17 of 25 patients with urinary *KRAS* G12/13 mutations were tested for *KRAS* mutations in plasma and 11 (65%) had *KRAS* G12/13 mutations

Tumor tissue *KRAS* G12/13 mutations

- Of 25 patients with urinary *KRAS* mutations, 21 had *KRAS* tumor tissue testing (wild-type, n=20; G13D mutation, n=1). We retested 8 available samples with droplet digital PCR and found one additional low frequency (1.3%) *KRAS* mutation .

Urine Contains 10x Amounts of cfDNA as Compared to Plasma

- In a separate cohort of *KRAS* G12/13 tissue positive patients, the number of total DNA fragments was found to be higher in urine as compared to plasma samples. Volume of urine specimens, 70 mL; volume of plasma specimens, 2 mL.

	Total ng DNA per sample		Total number of mutant *KRAS* copies per sample*	
	urine	plasma	urine	plasma
Median	989.5	61.6	317.4	27.6
Range	28.9-11,165	2.1-3,270	5.7-1,685,000	1.0-338.6
10% Percentile	177.2	25.0	18.8	1.5
90% Percentile	4411.0	752.4	27,789	197.8
Number of samples	58	43	34	23

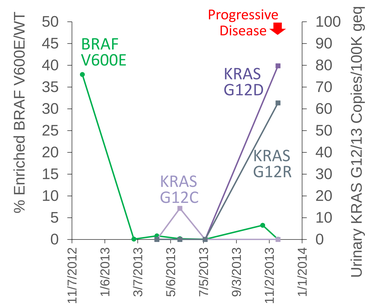
Table 3. *Urine and plasma samples from Stage IV KRAS-positive colorectal cancer patients at any time point on treatment (chemotherapy). *Samples with detectable KRAS G12/13 in urine or plasma.*

Results

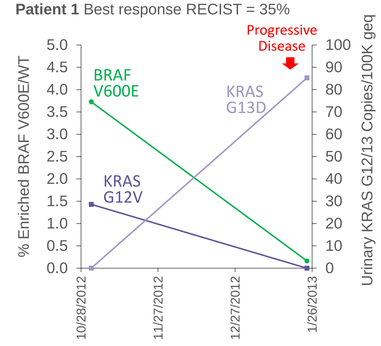
Urinary cfDNA Detects Acquisition of *KRAS* G12/13 in Patients Treated with BRAF or MEK Targeted Therapy

Representative patient cases showing dynamics of urinary cfDNA *BRAF* V600E and *KRAS* G12/13 on BRAF or MEK targeted therapy

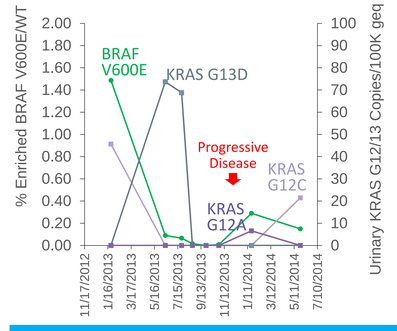
Patient 5 Best Response RECIST = -11%



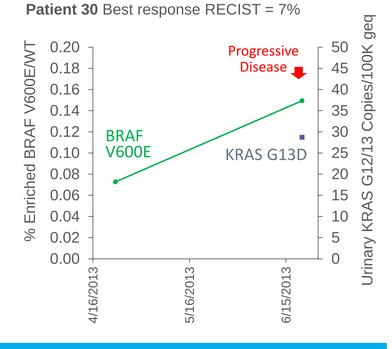
Patient 1 Best response RECIST = 35%



Patient 16 Best Response RECIST = -3%



Patient 30 Best response RECIST = 7%



Conclusions

- 81% of patients with advanced cancers and *BRAF* V600E mutation in tumor tissue have low frequency *KRAS* G12/13 mutations in urine cfDNA undetected in tumor samples by standard CLIA technologies.
- Low frequency *KRAS* mutations can plausibly drive resistance to BRAF targeting agents, and these may be detected in urine cfDNA.

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