Detection and Monitoring of *BRAF* and *KRAS* Mutations in Cell-Free Urinary DNA of Metastatic Cancer Patients by Droplet Digital PCR

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CELL-FREE MOLECULAR DIAGNOSTICS

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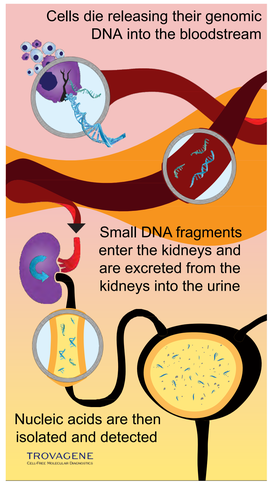
BACKGROUND

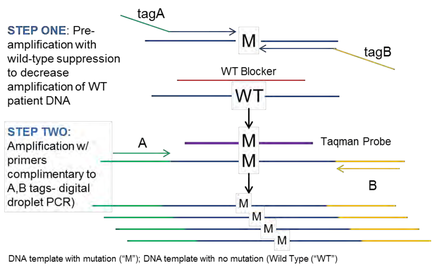
- *BRAF* and *KRAS* mutations confer a survival and growth advantage to cancer cells and can be used for selection of targeted therapies
- Cell-free (cf) DNA detected in the urine of individuals with cancer offers an easily obtainable, low-risk, and inexpensive source of biologic material for mutation analysis
- Longitudinal assessment of *BRAF* and *KRAS* mutations in urinary cfDNA can be used for monitoring of molecular changes throughout cancer therapy



METHODS

- A total of 27 patients with advanced cancers and 5 patients with Erdheim-Chester disease (histiocytic disorder with high prevalence for *BRAF* mutations), who were previously tested for mutations in *BRAF* and/or *KRAS* a CLIA-certified laboratory were prospectively enrolled
- Single or multiple sequential urine samples (90-110ml or 24 hour urine collection) for cfDNA mutation analysis were obtained at baseline and during therapy

Two-Step Assay Design for 28-30 bp footprint

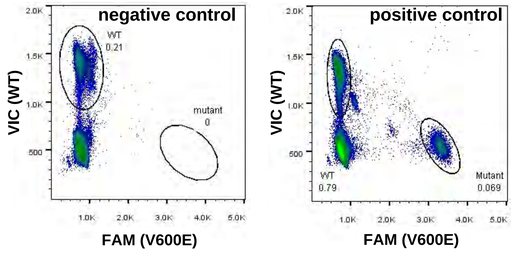


- Assays for quantitative assessment of *BRAF* V600E, *KRAS* G12D and G12V mutations in urinary cfDNA were developed using droplet digital PCR (RainDance, Billerica, MA) with enrichment of mutant DNA fragments by pre-amplification of *BRAF* and *KRAS* genes
- Concordance between mutation analysis results from urinary cfDNA and tumor tissue from the CLIA laboratory was determined
- Whenever possible longitudinal assessment of mutation status in sequential urine samples was performed

RESULTS

- Cell lines with respective mutations (*BRAF* V600E, *KRAS* G12D or G12V) were used as a positive control
- Thresholds for mutation detection were determined by assessing data from 50 healthy controls and 39 patient samples using a classification tree. Minimizing the percentage of false negatives was given a higher importance than minimizing false positives. Thresholds were defined as no detection – wild-type (<0.05%), borderline (0.05% - 0.107%), and detected - mutation (>0.107%)

Positive and negative controls for *BRAF* V600E mutation



Urinary cfDNA BRAF Mutations in Cancers

Detection limits for *BRAF* V600E mutation in urinary cfDNA

- V600E mutation: > 0.107% of mutant DNA
- V600E borderline: 0.05%-0.107% of mutant DNA
- V600E wild-type: <0.05% of mutant DNA

Agreement rate (CLIA V600E vs. urinary cfDNA V600E mutation or borderline mutation): 19/20 (95%)

Tumor Type	Tumor (CLIA)	Urinary cfDNA V600E *BRAF* mutation (%)*
Non-Small Cell Lung Cancer	V600E	V600E (0.17)
Papillary Thyroid Carcinoma	V600E	V600E (0.17)
Non-Small Cell Lung cancer	V600E	V600E (1.08)
Melanoma	V600E	V600E (37.9)
Non-Small Cell Lung Cancer	V600E	V600E (0.68)
Colorectal Cancer	V600E	V600E (21.12)
Melanoma	V600E	V600E (0.13)
Colorectal Cancer	V600E	V600E (1.49)
Glioblastoma	V600E	V600E (5.36)
Melanoma	V600E	Borderline V600E (0.07)
Melanoma	V600E	Wild-type (0.04)
Melanoma	V600E	V600E (0.15)
Adenocarcinoma of Unknown Primary	V600E	Borderline V600E (0.07)
Colorectal Cancer	V600E	V600E (416.58)
Non-Small Cell Lung Cancer	V600E	V600E (2.93)
Melanoma	V600E	V600E (0.97)
Papillary Thyroid Cancer	V600E	V600E (1.66)
Melanoma	V600E	V600E (1.01)
Ovarian Cancer	V600E	Borderline V600E (0.08)
Appendiceal Cancer	V600E	V600E (3.43)

** In patients with several sequential urine collection samples with highest mutant fraction are recorded*

RESULTS

Urinary cfDNA BRAF Mutations in Erdheim-Chester Disease

Erdheim-Chester Disease Involvement	Tissue (CLIA)	Urinary cfDNA V600E *BRAF* mutation (%)
Bones, cardiac, CNS, kidneys	V600E*	V600E (129.50)
Bones, kidneys	Unknown	Wild-type (0.02)
Skin	NTRK1 rearrangement*	Wild-type (0.01)
Bones	Unknown	V600E (0.16)
Bones	Unknown	V600E (4.94)

** Molecular analysis was done with Targeted Next-Generation Sequencing (Foundation One, Foundation Medicine, Cambridge, MA)*

Urinary cfDNA KRAS Mutations in Cancers

Detection limits for KRAS G12 mutations in urinary cfDNA

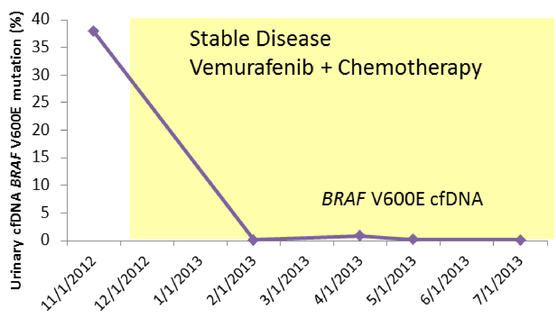
- G12 healthy control: 234 mutant fragments
- G12 mutation: 489-2825 mutant fragments

Agreement rate (CLIA G12 vs. urinary cfDNA G12 mutation): 7/7 (100%)

Tumor Type	Tumor (CLIA)	Baseline G12 *KRAS*-mutant urinary cfDNA (mutant fragments)
Colorectal Cancer	G12D	G12D (489)
Colorectal Cancer	G12D	G12D (563)
Colorectal Cancer	G12D	G12D (1935)
Colorectal Cancer	G12D	G12D (2825)
Colorectal Cancer	G12V	G12V (1168)
Non-Small Cell Lung Cancer	G12V	G12V (1083)
Appendiceal Cancer	G12D	G12D (1231)

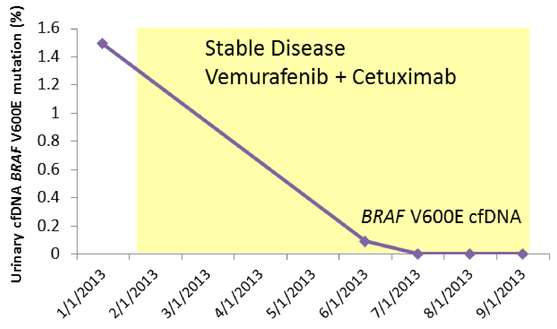
Longitudinal Assessment of cfDNA Mutations

Patient 5: Melanoma

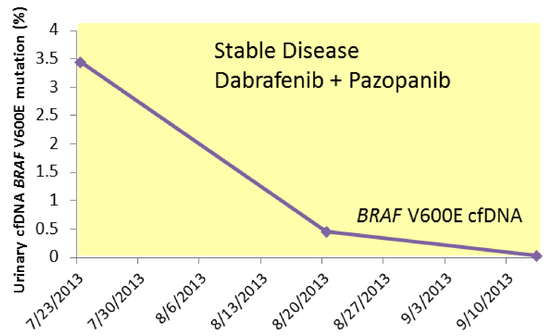


RESULTS

Patient 16: Colorectal Cancer



Patient 44: Appendiceal Cancer



CONCLUSIONS

- Detection of actionable *BRAF* and *KRAS* mutations with droplet digital PCR in urinary cfDNA from patients with advanced cancers is feasible with good preliminary concordance with mutation testing of tumor tissue in the CLIA laboratory
- *BRAF* mutations were detected in urine from patients with Erdheim-Chester disease including patients who did not have adequate tissue for molecular analysis
- Mutations in urine cfDNA should be further investigated for longitudinal assessment of effects of anticancer therapies

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