Droplet Digital PCR Mutant and Longitudinal Monitoring of BRAF Mutations in Cell-Free Urinary DNA of Patients Metastatic Cancers or Erdheim-Chester Disease

Filip Janku, Gerald S. Falchook, Sarina A. Piha-Paul, Aung Naing, Veronica R. Holley, David S. Hong, Vivek Subbiah, Daniel D. Karp, Ralph G. Zinner, Siqing Fu, Jennifer J. Wheler, Funda Meric-Bernstam, Vanda M. Stepanek, Rayjalakshmi Luthra, Lorieta Leppin, Latifa Hassaine, Karena Kosco, Jason C. Poole, Cecile Rose T. Vibat, Mark G. Erlander

Department of Investigational Cancer Therapeutics and Molecular Diagnostic Laboratory, The University of Texas MD Anderson Cancer Center, Houston, TX; Trovagene Inc., San Diego, CA

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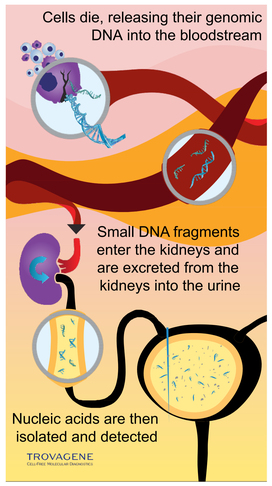
Abstract #: LB170

BACKGROUND

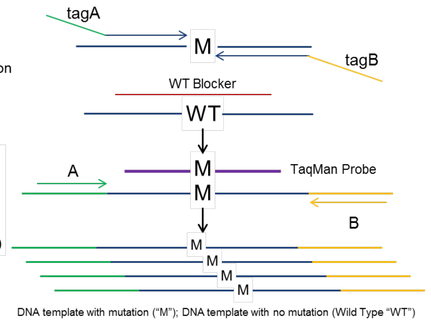
- *BRAF* mutations confer a survival and growth advantage to cancer cells and can be used for selection of targeted therapies
- Cell-free (cf) DNA detected in the urine of individuals with cancer offers an easily obtainable, low-risk, and inexpensive source of biologic material for mutation analysis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



METHODS

- Quantitative assessment of *BRAF* V600E in urinary cfDNA was accomplished by droplet digital PCR (RainDance, Billerica, MA) with enrichment of mutant DNA fragments by pre-amplification of *BRAF* mutant alleles
- Concordance was determined between mutation analysis results from urinary cfDNA and tumor tissue obtained during routine diagnostic or therapeutic procedures for testing in the CLIA laboratory
- Longitudinal assessment of mutation status in sequential urine samples was performed whenever possible

Two-Step Assay Design for 28-30 bp footprint



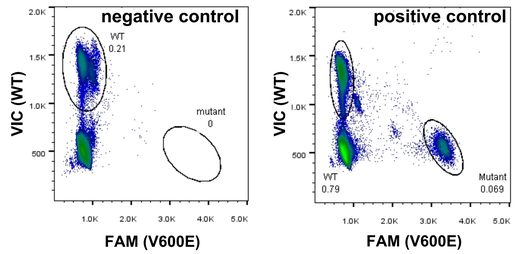
Urinary cfDNA Mutant limits for *BRAF* V600E mutations

- V600E Mutant: > 0.107% of mutant DNA
- V600E Low Mutant: 0.05%-0.107% of mutant DNA
- Wild type: <0.05% of mutant DNA

Mutation thresholds were determined by assessing data from 50 healthy controls and 39 advanced cancer patient samples using a classification tree. Minimizing the percentage of false negatives was given a higher importance than minimizing false positives.

METHODS

Positive and negative controls for *BRAF* V600E mutation



- A total of 33 patients with diverse advanced cancers or Erdheim-Chester disease (histiocytic disorder with high prevalence for *BRAF* mutations), who were previously tested for *BRAF* V600E mutations in tumor tissue by 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

RESULTS

Urinary cfDNA *BRAF* V600E Analysis

Tumor Type	Tissue (CLIA)	Baseline Urinary *BRAF* V600E cfDNA Detection	Longitudinal Urinary *BRAF* V600E cfDNA Detection
Appendiceal Adenocarcinoma	*BRAF* V600E	Mutant	Mutant
Cholangiocarcinoma	*BRAF* V600E	Mutant	Mutant
Colorectal Cancer	*BRAF* V600E	Low Mutant	Not Done
Colorectal Cancer	*BRAF* V600E	Low Mutant	Not Done
Colorectal Cancer	*BRAF* V600E	Low Mutant	Not Done
Colorectal Cancer	*BRAF* V600E	Low Mutant	Not Done
Colorectal Cancer	*BRAF* V600E	Mutant	Mutant
Colorectal Cancer	*BRAF* V600E	Mutant	Mutant
Colorectal Cancer	*BRAF* V600E	Mutant	Not Done
Colorectal Cancer	*BRAF* V600E	Mutant	Not Done
Erdheim-Chester Disease	*BRAF* V600E	Mutant	Mutant
Melanoma	*BRAF* V600E	Low Mutant	Low Mutant
Melanoma	*BRAF* V600E	Low Mutant	Mutant
Melanoma	*BRAF* V600E	Low Mutant	Not Done
Melanoma	*BRAF* V600E	Mutant	Mutant
Melanoma	*BRAF* V600E	Mutant	Mutant
Melanoma	*BRAF* V600E	Mutant	Mutant
Melanoma	*BRAF* V600E	Mutant	Not Done
Melanoma	*BRAF* V600E	Mutant	Not Done
Melanoma	*BRAF* V600E	Wild-Type	Low Mutant
Melanoma	*BRAF* V600E	Wild-Type	Not Done
Melanoma	*BRAF* V600E	Wild-Type	Wild-Type
Non-Small Cell Lung Cancer	*BRAF* V600E	Low Mutant	Mutant
Non-Small Cell Lung Cancer	*BRAF* V600E	Low Mutant	Not Done
Non-Small Cell Lung Cancer	*BRAF* V600E	Mutant	Mutant
Non-Small Cell Lung Cancer	*BRAF* V600E	Wild-Type	Mutant
Ovarian Cancer	*BRAF* V600E	Wild-Type	Low Mutant
Papillary Thyroid Cancer	*BRAF* V600E	Low Mutant	Low Mutant
Papillary Thyroid Cancer	*BRAF* V600E	Low Mutant	Mutant
Papillary Thyroid Cancer	*BRAF* V600E	Mutant	Not Done
Papillary Thyroid Cancer	*BRAF* V600E	Wild-Type	Not Done
Papillary Thyroid Cancer	*BRAF* V600E	Wild-Type	Not Done
Temporal Glioblastoma	*BRAF* V600E	Wild-Type	Mutant

RESULTS

Concordance of *BRAF* V600E Tissue (CLIA) to Baseline Urine cfDNA

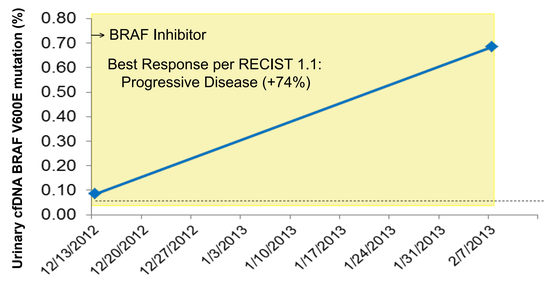
Tested (N-33)	*BRAF* Mutation Urine	*BRAF* Wild-Type Urine
BRAF Mutation CLIA	25	8
BRAF Wild-Type CLIA	0	0
Observed Agreements	25 (76%)	

Concordance of *BRAF* V600E Tissue (CLIA) to Any Assessed Point of Urine cfDNA

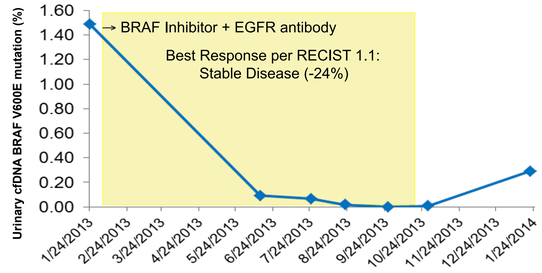
Tested (N-33)	*BRAF* Mutation Urine	*BRAF* Wild-Type Urine
BRAF Mutation CLIA	29	4
BRAF Wild-Type CLIA	0	0
Observed Agreements	29 (88%)	

Longitudinal Assessment of Urine cfDNA Mutations

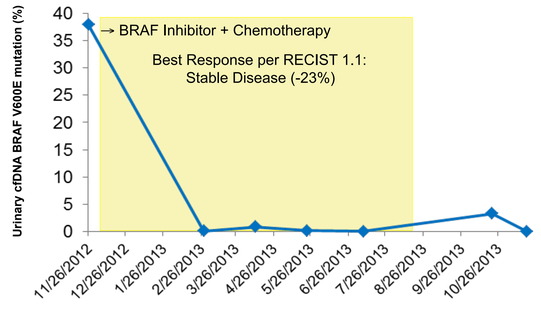
Patient 8: Metastatic Non-Small Cell Lung Cancer



Patient 16: Metastatic Colorectal Cancer

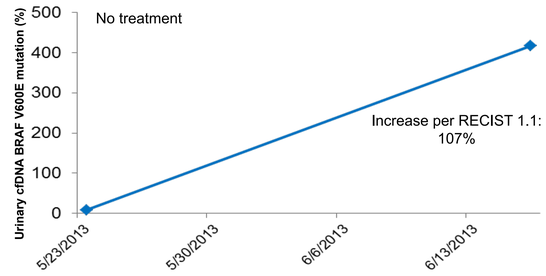


Patient 5: Metastatic Melanoma

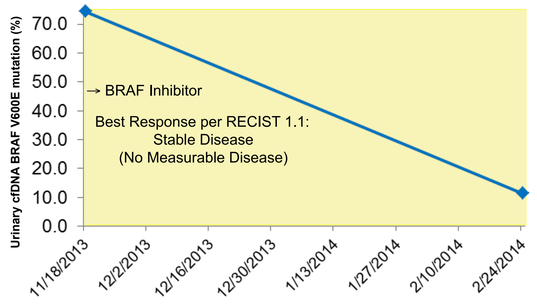


RESULTS

Patient 36: Metastatic Non-Small Cell Lung Cancer



Patient 15: Erdheim-Chester Disease



- Longitudinal analysis of *BRAF* V600E in 17 of 32 metastatic cancer patients and one Erdheim-Chester disease patient was performed by testing serially collected urine. Dynamics of urinary cell-free BRAF V600E correlated with response to therapy in 13 of 17 advanced cancer patients (76%)

CONCLUSIONS

- Detection and monitoring of actionable *BRAF* V600E mutations with droplet digital PCR in urinary cfDNA from patients with advanced cancers and Erdheim-Chester disease display acceptable preliminary concordance with mutation testing of tumor tissue in the CLIA laboratory
- Urine-based cell-free DNA *BRAF* mutation testing may offer a non-invasive alternative to mutational analysis in tumor tissue
- Mutations in urine cfDNA should be further investigated for monitoring mutation status during anticancer therapies

FOR MORE INFORMATION PLEASE CONTACT
Filip Janku | fj/anku@mdanderson.org