Detection of *BRAF* Mutations in Urine and Plasma Cell-Free DNA: Application to the Diagnosis and Management of Histiocytic Disorder Patients

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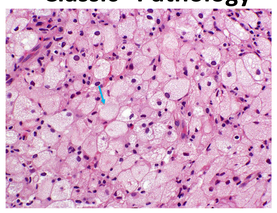
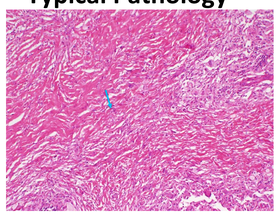
BACKGROUND

- 40-60% of patients with the systemic histiocytoses (including Langerhans Cell Histiocytosis (LCH) and Erdheim-Chester Disease (ECD)) have a *BRAF*V600E mutation.
- Treatment with RAF inhibitors have dramatic effects on the disease.
- However, contamination of stromal cells, low tumor content, and frequent use of bone biopsy lesions for molecular testing often impede effective detection of *BRAFV600E* mutations in clinical practice for these patients.
- We therefore sought to assess the utility of urinary and plasma cell-free DNA (cfDNA) analysis to (1) reliably detect *BRAF*V600E mutation and (2) dynamically monitor response to therapy in this unique subset of patients.

"Classic" Pathology

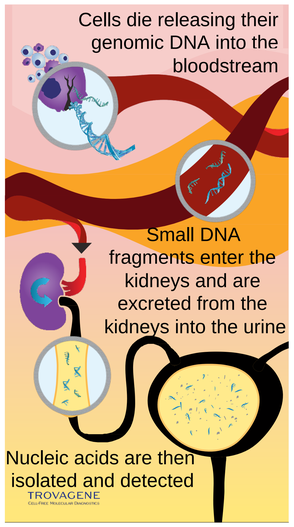


Typical Pathology



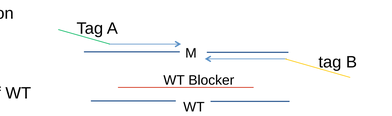
METHODS

- Between January 2013 and June 2014, 26 consecutive patients with LCH (n=5) and ECD (n=21) were enrolled from Memorial Sloan Kettering Cancer Center and MD Anderson Cancer Center.
- Urinary cfDNA analysis was performed in all patients and plasma cfDNA analysis in 19/26 patients.
- Serial urinary samples in 10 *BRAF*V600E mutant samples were also obtained to track disease burden with therapy.
- Urine and plasma cfDNA were quantified by a droplet digital PCR (ddPCR; QX-100, BioRad).

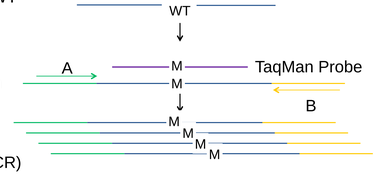


Two-Step Design for 31 bp *BRAF* V600E Assay

STEP ONE: Pre-amplification with wild-type suppression to decrease amplification of WT patient DNA



STEP TWO: Amplification with primers complimentary to A,B tags (droplet digital PCR)



RESULTS

- Of 26 patients, initial tissue *BRAF*V600E genotyping identified:
  - --11 patients to be *BRAF*V600E mutant (42.3%)
  - --6 patients as *BRAF*V600E wildtype (23.1%)
  - --9 patients with indeterminate initial tissue biopsy result (34.6%)
- Concordance between urinary cfDNA, plasma cfDNA, and tissue biopsy *BRAF*V600E genotyping results:

	Tissue biopsy (+)	Tissue biopsy (-)	Tissue biopsy (?)	Urine cfDNA Total
Urine cfDNA (+)	10	0	4	14
Urine cfDNA (-)	1*	6	4	11
Urine cfDNA indeterminate	0	0	1	1
Tissue biopsy total	11	6	9	

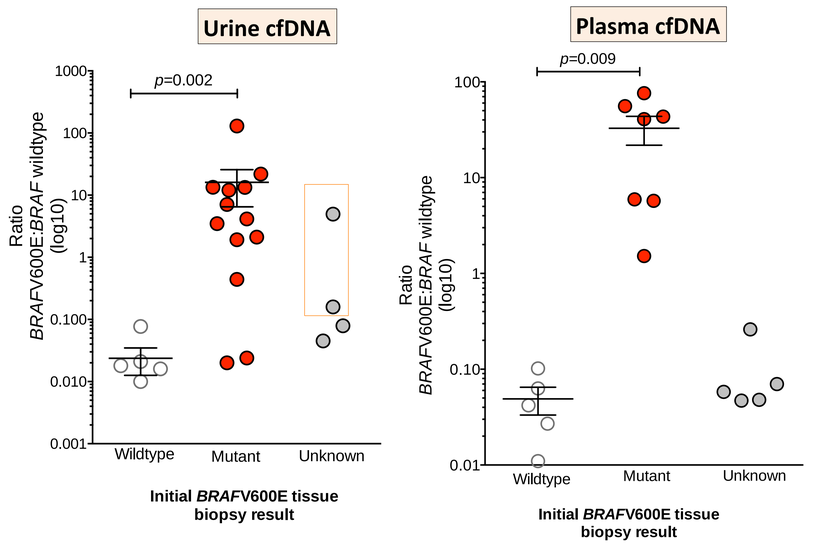
* Urine sample for this patient was acquired during therapy while tissue biopsy was performed pretreatment.

	Tissue biopsy (+)	Tissue biopsy (-)	Tissue biopsy (?)	Plasma cfDNA total
Plasma cfDNA (+)	8	1	2**	10
Plasma cfDNA (-)	0	3	5	9
Plasma cfDNA indeterminate	0	0	0	0
Tissue biopsy total	8	4	7	

** These 2 patients subsequently underwent repeat tissue biopsy which confirmed cfDNA test result and allowed the patients to enroll in phase II study of vemurafenib.

- cfDNA analysis (both plasma and urine) increased number of patients with known *BRAF*V600E genotype and identified new additional *BRAF*V600E mutant patients.

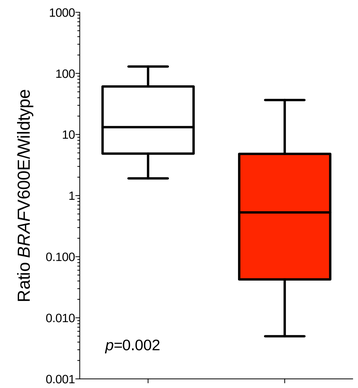
- Quantitative analyses of *BRAF*V600E mutation in urinary and plasma cfDNA reliably detects *BRAF*V600E mutation based on tissue genotype (analyses below include plasma and urine samples pretreatment and on treatment).



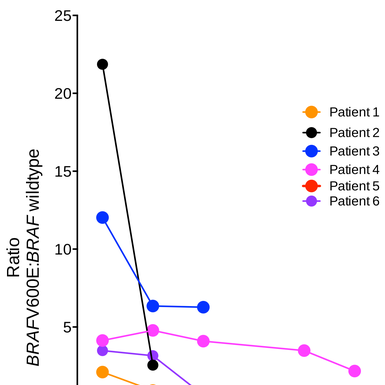
- Two patients with an indeterminate biopsy result but with clearly positive Urinary cfDNA *BRAF*V600E mutation subsequently were found to have *BRAF*V600E mutation in tissue with a repeat biopsy (orange box above).

RESULTS

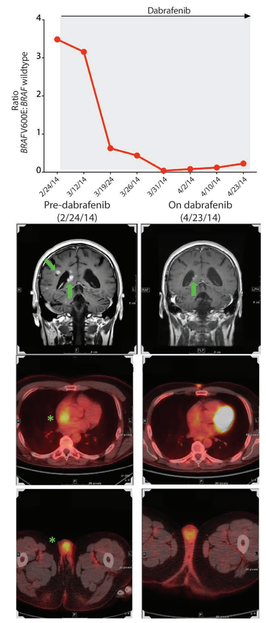
- Quantitative cfDNA analysis of *BRAF*V600E burden in urine in treatment naïve versus on treatment samples detects decreased mutational burden on therapy.



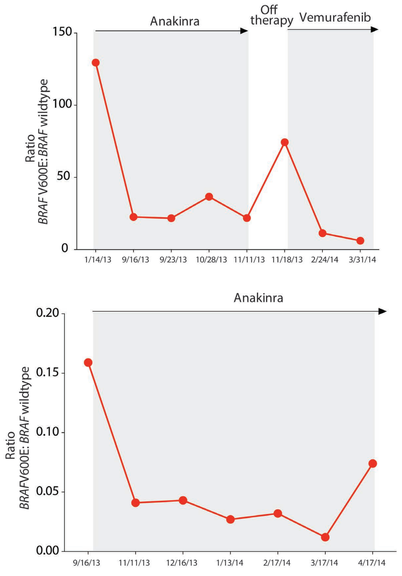
- Serial analysis of *BRAF*V600E mutation in urinary cfDNA of patients treated with vemurafenib reveals progressive decrement of *BRAF* mutant allele burden with therapy.



- *BRAF*V600E burden in urine in correlates with radiographic response.



- *BRAF*V600E allele burden in urine changes dynamically with therapy.



CONCLUSION

- *BRAF*V600E mutation detection in urine and plasma cfDNA provides a reliable means of detecting *BRAF*V600E mutations in histiocytosis patients.
- Serial monitoring of cfDNA BRAFV600E mutant allele burden from urine provides a convenient means of dynamically monitoring this unique disease.