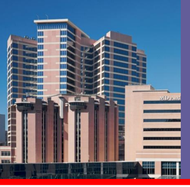

BRAF and *KRAS* Mutation Testing in Plasma Cell-Free DNA with ICE COLD-PCR in Patients with Advanced Cancers

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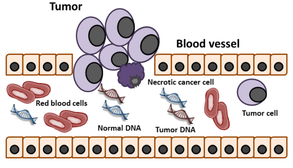
BACKGROUND

- Oncogenic mutations confer a survival and growth advantage to cancer cells
- Identifying oncogenic mutations in cancer can provide druggable targets for cancer therapies
- Plasma cell-free (cf) DNA in individuals with cancer offers an easily obtainable, low-risk, and inexpensive source of material for mutation analysis

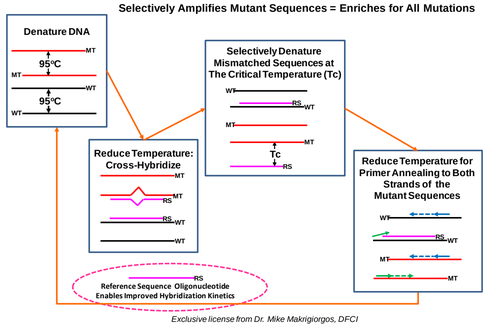


- Longitudinal assessment of cfDNA can be used for monitoring of molecular changes throughout cancer therapy.

METHODS

- Patients with advanced cancers, who were previously tested for *BRAF* V600 (42), *KRAS* G12/G13 (34), or both mutations (1) in the tumor samples (primary or metastatic) in a CLIA-certified laboratory during their clinical care were prospectively enrolled
- DNA from plasma (3-4ml) from patients with advanced cancers who progressed on systemic therapy were tested for *BRAF* V600 and *KRAS* G12 and G13 mutations using the ICE COLD-PCR platform
- ICE COLD-PCR, "Improved and Complete Enrichment COamplification at Lower Denaturation" selectively amplifies mutant DNA by exploiting differences in denaturation temperatures between mutant DNA duplexes and normal "wild-type" DNA duplexes
- *KRAS* Exon 2 and *BRAF* Exon 15 ICE COLD-PCR was performed on plasma samples
- Amplicons were analyzed by Sanger sequencing methods and results were compared to the mutation status of the archival primary or metastatic tumor tissue as determined in a CLIA-certified lab

METHODS



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RESULTS

Patient Characteristics

BRAF mutations

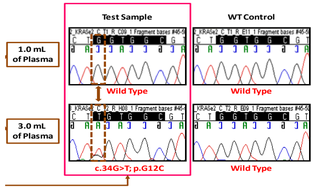
Histology	N (%)	*BRAF* mutation CLIA (%)	*BRAF* wild-type CLIA (%)
All	**43 (100)**	**40 (100)**	**3 (100)**
Melanoma	16 (37)	16 (40)	0 (0)
Colorectal	10 (23)	9 (23)	1 (33)
Non-small cell lung	5 (12)	5 (13)	0 (0)
Papillary thyroid	4 (<10)	4 (10)	0 (0)
Erdheim-Chester disease	3 (<10)	2 (<10)	1 (33)
Other	5 (12)	4 (10)	1 (33)

KRAS mutations

Histology	N (%)	*KRAS* mutation (CLIA)	*KRAS* wild-type (CLIA)
All	**35 (100)**	**29 (100)**	**6 (100)**
Colorectal	28 (80)	24 (83)	4 (66)
Appendiceal	2 (<10)	2 (<10)	0 (0)
Non-small cell lung	2 (<10)	2 (<10)	0 (0)
Other	3 (<10)	1 (<10)	2 (33)

RESULTS

Plasma Volume for Robust Mutation Detection



Concordance Analysis: cfDNA vs. tissue

BRAF mutations

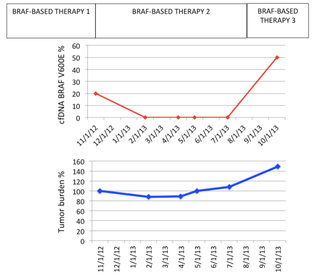
TESTED (N=43)	*BRAF* mutation CLIA	*BRAF* wild-type CLIA
***BRAF* mutation PLASMA**	30	0
***BRAF* wild-type PLASMA**	10	3
Observed agreements	33 (77%)	

KRAS mutations

TESTED (N=35)	*KRAS* mutation CLIA	*KRAS* wild-type CLIA
***KRAS* mutation PLASMA**	24	2
***KRAS* wild-type PLASMA**	5	4
Observed agreements	28 (80%)	

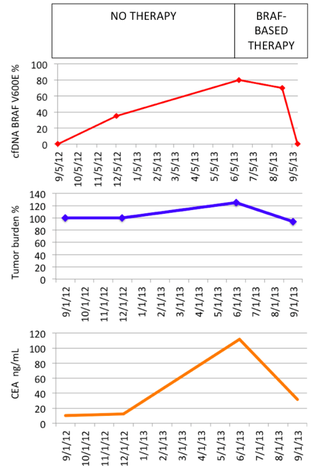
Longitudinal Assessment of cfDNA Mutations

Patient 45: Metastatic Melanoma with *BRAF* V600E Mutation

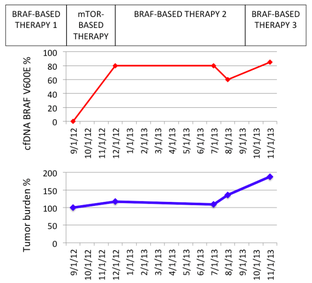


RESULTS

Patient 14: Metastatic Appendiceal Carcinoma with *BRAF* V600E Mutation

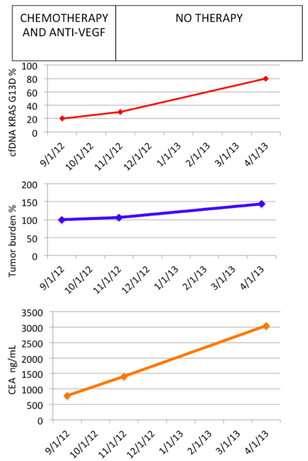


Patient 22: Metastatic Melanoma with *BRAF* V600E Mutation



RESULTS

Patient 18: Metastatic Sigmoid Cancer with *KRAS* G13D Mutation



CONCLUSIONS

- ICE COLD-PCR detection of actionable mutations in *BRAF* and *KRAS* in cfDNA from plasma of patients with advanced cancers is feasible with an acceptable level of concordance with mutation testing of tumor tissue in the CLIA laboratory
- Longitudinal assessment of cfDNA mutations can demonstrate changes in mutation status during therapy, which seem to be in agreement with clinical course

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