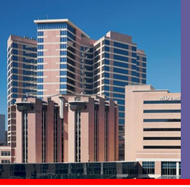

BRAF and *KRAS* Mutation Testing in Cell-Free DNA and Circulating Tumor Cells from Blood of Patients with Metastatic Cancers

Filip Janku, Ben Legendre, Katherine Richardson, Gerald S. Falchook, Aung Naing, Veronica R. Holley, David S. Hong, Ralph G. Zinner, Siqing Fu, Apostolia M. Tsimberidou, Vivek Subbiah, Daniel D. Karp, Sarina A. Piha-Paul, Jennifer J. Wheler, Vanda M. Stepanek, Goran Cabrilo, Rajyalakshmi Luthra, Funda Meric-Bernstam, Amy Kruempel, Jaclyn Pope, Courtney Cubrich, Grant Wu, Yanggu Shi, Marcia Lewis, Razelle Kurzrock

Investigational Cancer Therapeutics (Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, Houston, TX, USA; Transgenomic Inc., Omaha, NE; UCSD Moores Cancer Center, La Jolla, CA

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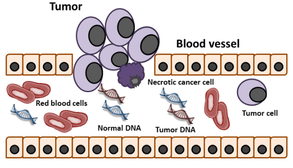
BACKGROUND

- 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 and circulating tumor cells (CTC) in individuals with cancer offers an easily obtainable, low-risk, and inexpensive source of material for mutation analysis

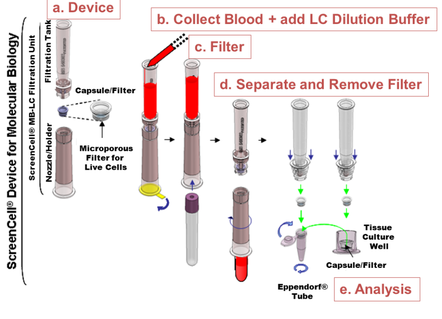


- Longitudinal assessment of cfDNA and CTC can be used for monitoring of molecular changes throughout cancer therapy.

METHODS

- Patients with advanced cancers, who were previously tested for *BRAF* V600 (30), *KRAS* G12/G13 (28), or both mutations (1) in the tumor samples (primary or metastatic) in a CLIA-certified laboratory during their clinical care were prospectively enrolled

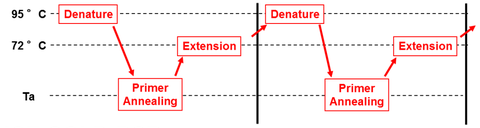
ScreenCell Device for CTC Collection



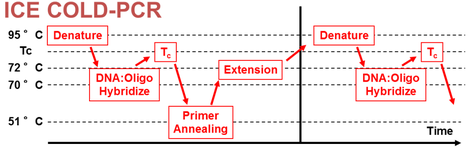
METHODS

- DNA from plasma (3-4ml) and CTC (7.5ml of blood) 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, and from matched CTCs collected using ScreenCell® MB kits (ScreenCell, Sarcelles, France)
- 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

Standard PCR



ICE COLD-PCR



RESULTS

Patient Characteristics

BRAF mutations

Histology	N (%)	*BRAF* mutation (CLIA)	*BRAF* wild-type (CLIA)
All	**31 (100)**	**30 (100)**	**1 (100)**
Melanoma	12 (39)	12 (40)	0 (0)
Colorectal	9 (29)	8 (27)	1 (100)
Non-small cell lung	5 (16)	5 (17)	0 (0)
Erdheim-Chester disease	2 (6)	2 (7)	0 (0)
Appendiceal	1 (<5)	1 (<5)	0 (0)
Papillary thyroid	1 (<5)	1 (<5)	0 (0)
Glioblastoma	1 (<5)	1 (<5)	0 (0)

KRAS mutations

Histology	N (%)	*KRAS* mutation (CLIA)	*KRAS* wild-type (CLIA)
All	**29 (100)**	**26 (100)**	**3 (100)**
Colorectal	24 (83)	21 (81)	3 (100)
Appendiceal	2 (7)	2 (8)	0 (0)
Non-small cell lung	2 (7)	2 (8)	0 (0)
Breast	1 (<5)	1 (<5)	0 (0)

Concordance Analysis: cfDNA vs. tissue

BRAF mutations

TESTED (N=31)	*BRAF* mutation CLIA	*BRAF* wild-type CLIA
***BRAF* mutation PLASMA**	20	0
***BRAF* wild-type PLASMA**	10	1
Observed agreements	21 (68%)	

KRAS mutations

TESTED (N=29)	*KRAS* mutation CLIA	*KRAS* wild-type CLIA
***KRAS* mutation PLASMA**	22	2
***KRAS* wild-type PLASMA**	4	1
Observed agreements	23 (79%)	

RESULTS

Concordance Analysis: CTC vs. tissue

BRAF mutations

TESTED (N=31)	*BRAF* mutation CLIA	*BRAF* wild-type CLIA
***BRAF* mutation CTC**	1	0
***BRAF* wild-type CTC**	29	1
Observed agreements	2 (6%)	

KRAS mutations

TESTED (N=29)	*KRAS* mutation CLIA	*KRAS* wild-type CLIA
***KRAS* mutation CTC**	3	0
***KRAS* wild-type CTC**	23	3
Observed agreements	6 (21%)	

Longitudinal Assessment of cfDNA Mutations

BRAF mutations

Patient	1	2	3	4	5	6
Histology	Melanoma	Melanoma	Melanoma	Melanoma	Erdheim-Chester disease	Papillary thyroid cancer
Prior therapy	BRAF inhibitor (response, intolerance)	BRAF inhibitor (response-> PD)	BRAF inhibitor -> BRAF and MET inhibitors (PD)	Ipilimumab and temodar	BRAF inhibitor (intolerance)	Sorafenib
Tissue (CLIA)	*BRAF* V600E	*BRAF* V600E	*BRAF* V600E	*BRAF* V600E	*BRAF* V600E	*BRAF* V600E
Baseline cfDNA	Wild-type	BRAF V600E	*BRAF* V600E	*BRAF* V600E	*BRAF* V600E	*BRAF* V600E
Treatment	Sirolimus + HCQ	Vemurafenib + chemotherapy	Vemurafenib + chemotherapy	Vemurafenib + chemotherapy	Peg-interferon	Vemurafenib + chemotherapy
Response	PD	SD (minor response)	SD (minor response)	SD (minor response)	SD (minor response)	SD
Follow up cfDNA	V600E	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type

Abbreviations: PD, progressive disease; HCQ, hydroxychloroquine; SD, stable disease

RESULTS

Longitudinal Assessment of cfDNA Mutations

KRAS mutations

Patient	1	2
Histology	Appendiceal carcinoma	Colorectal carcinoma
Prior therapy	Chemotherapy	Chemotherapy
Tissue (CLIA)	*KRAS* G13D	*KRAS* G12D
Baseline cfDNA	Wild-type	Wild-type
Treatment	Anti-VEGF	Chemotherapy
Response	PD	PD
Follow-up cfDNA	*KRAS* G13D	*KRAS* G12D

Abbreviations: PD, progressive disease

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
- ICE-COLD PCR detection of actionable mutations in *BRAF* and *KRAS* in CTC isolated from patients with advanced cancers using the ScreenCell device demonstrates low 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

fjanku@mdanderson.org