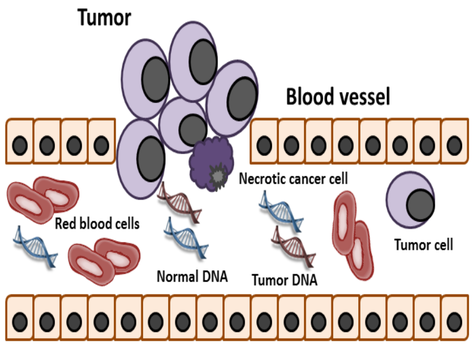
# *BRAF* and *KRAS* Mutation Testing in Plasma Cell-Free DNA with ICE COLD-PCR in Patients with Advanced Cancers (Selected Data from 2014 AACR Poster)

**Filip Janku, Ben Legendre, Katherine Richardson, Gerald S. Falchook, Aung Naing, Veronica R. Holley, Siqing Fu, David S. Hong, Sarina A. Piha-Paul, Jennifer J. Wheler, Ralph G. Zinner, Vivek Subbiah, Apostolia M. Tsimberidou, Daniel D. Karp, Vanda M. Stepanek, Goran Cabrilo, Rajyalakshmi Luthra, Funda Meric-Bernstam, Agop Y. Bedikian, Bryan K. Kee, Cathy Eng,Micahel J. Overman, Kevin B. Kim, Amy Kruempel, Jaclyn Pope, Courtney Cubrich, Grant Wu, 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**

## 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

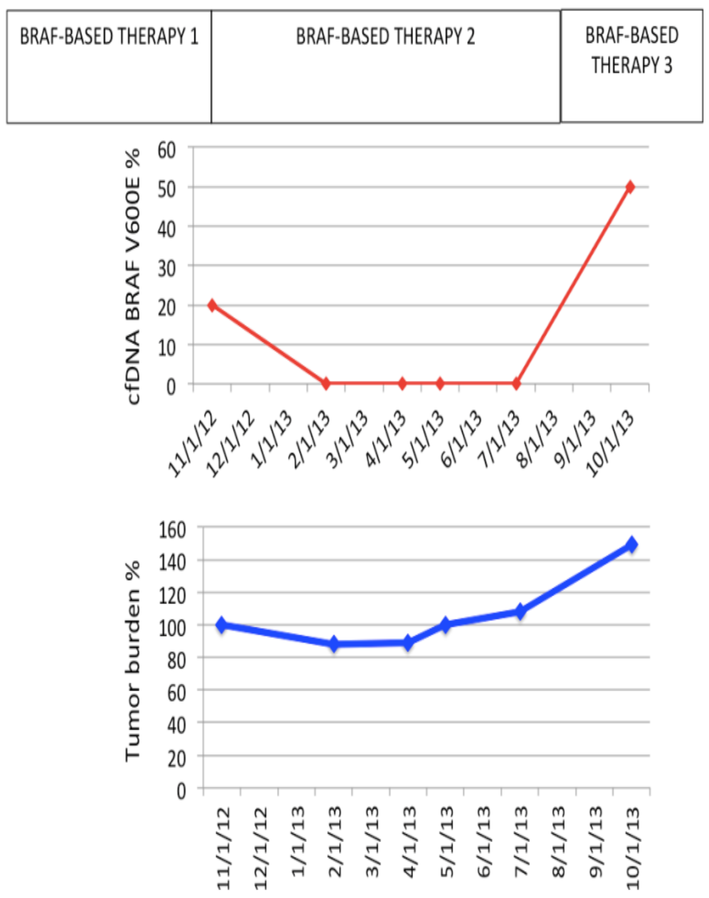
- Patients with advanced cancers, who were previously tested for *BRAF* V600 (42), *KRAS* G12/G13 (34), or both mutations (1) in the tumor samples (primary or metastatic) in a CLIA-certified laboratory during their clinical care were prospectively enrolled
- DNA from plasma (3-4ml) from patients with 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

## CONCLUSIONS

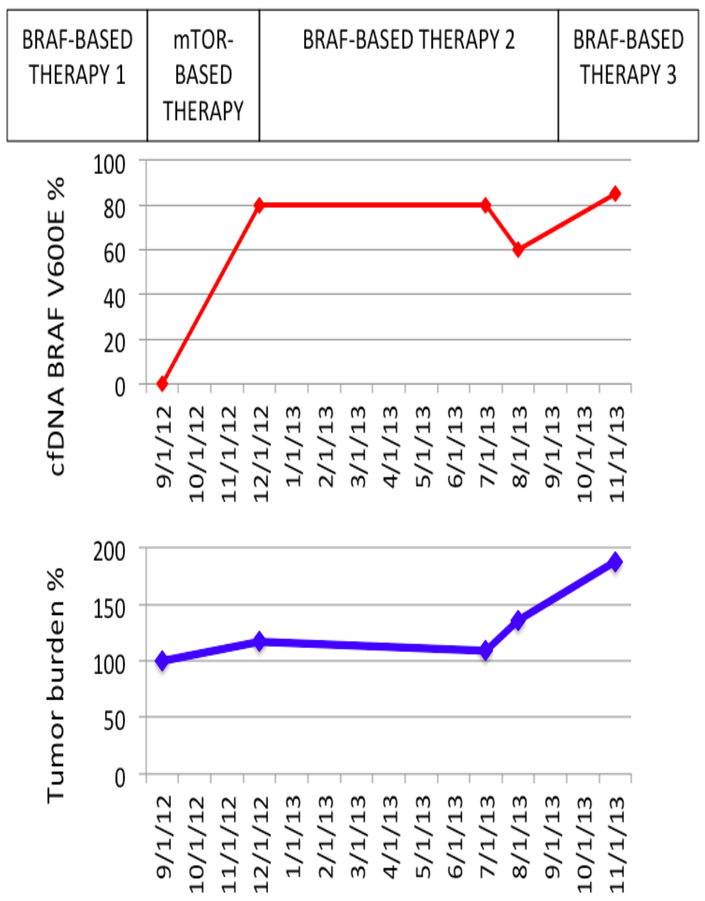
- ICE COLD-PCR detection of actionable mutations in *BRAF* and *KRAS* in cfDNA from plasma of patients with advanced cancers is feasible
- Longitudinal assessment of cfDNA mutations can demonstrate changes in mutation status during therapy, which seem to be in agreement with clinical course

## RESULTS: Longitudinal Assessment of cfDNA Mutations

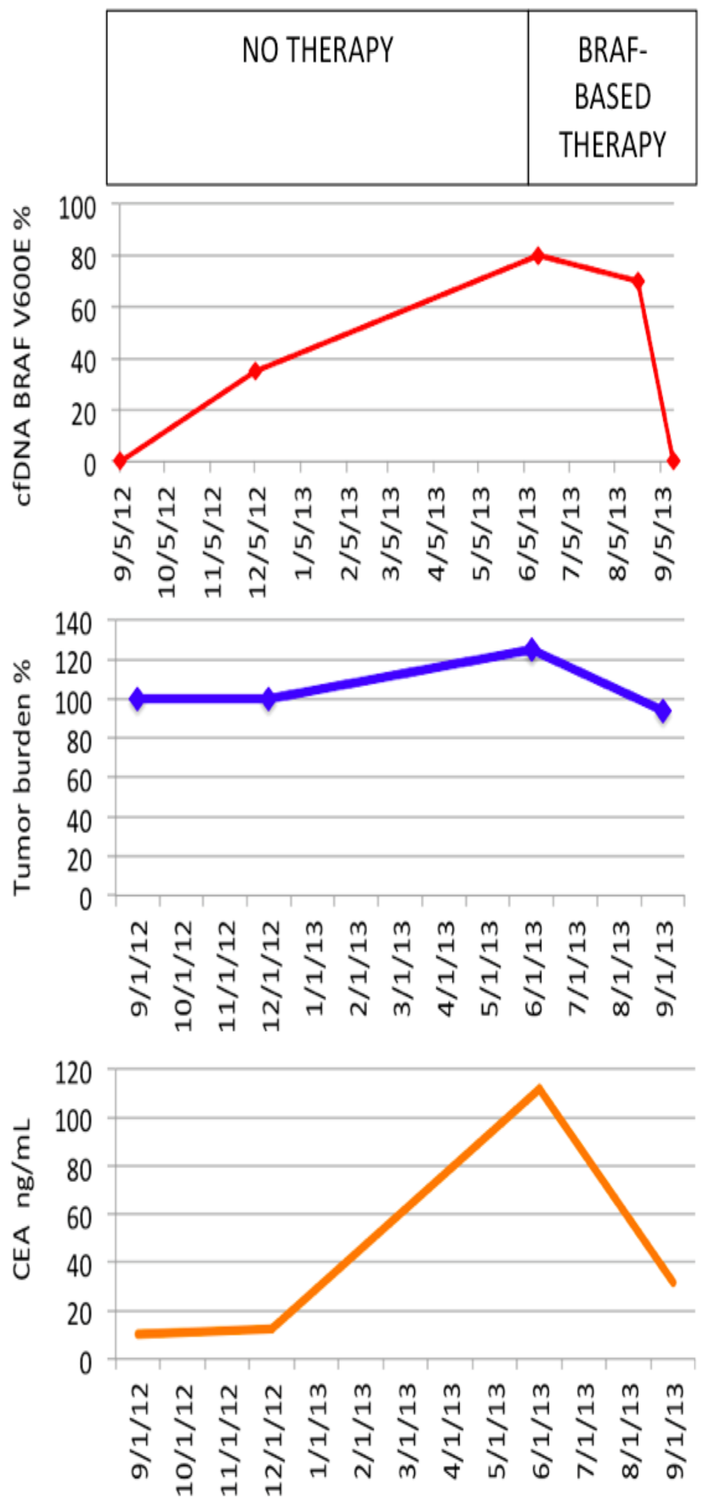
### Patient 45: Metastatic Melanoma with *BRAF* V600E Mutation




### Patient 22: Metastatic Melanoma with *BRAF* V600E Mutation




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




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