Plasma-Based Monitoring of BRAF Mutations During Therapy for Malignant Melanoma Using Combined Exosomal RNA and Cell-Free DNA Analysis

Sullivan RJ [1], O'Neill VJ [2], Enderle D [3], Valentino M [2], Koestler T [3], Spiel A [3], Blackmon S [1], Krug AK [3], Brinkmann K [3], Bentink S [3], Mueller R [3], Emenegger J [3], Noerholm M [3], Skog J [2], Berking C [4], Flaherty K [1]

(1) Massachusetts General Hospital, Boston, MA, USA | (2) Exosome Diagnostics Inc., Cambridge, MA, USA | (3) Exosome Diagnostics GmbH, Munich, Germany | (4) Ludwig-Maximilians University, Munich, Germany

Abstract #9017
Board #260

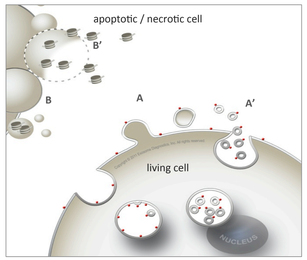
Background and Methods

Oncogenic mutations in BRAF (V600E/V600K) are present in 40-50% of patients with malignant melanoma (MM) and represent an important therapeutic target (e.g., vemurafenib). Blood-based, serial monitoring of BRAF and other mutations during therapy may be useful to inform time-critical treatment decisions. Plasma contains at least two sources of cell-free nucleic acids (NAs) where tumor derived mutations can be detected: exosomal RNA (exoRNA) and cell-free DNA (cfDNA). Circulating NAs provide an important opportunity to monitor cancer longitudinally to assess treatment response and disease progression. In patients with aggressive disease, such as MM, timely insights about treatment response and disease progression are paramount in order to help guide and adjust treatment decisions. Combining exoRNA and cfDNA maximizes the yield of genetic material from plasma, and may enable monitoring of both biologically important sources to enhance mutation detection sensitivity and help match patients to targeted therapies.

Blood was drawn from 12 metastatic MM patients for analysis prior to therapy initiation and up to 10 time points after. Subsequent purification of high-quality NAs using a novel, spin-column based method (EXO52) to co-isolate all exoRNA and cfDNA allowed analysis of mutations present in both NA fractions by ultra-deep sequencing. A custom next-generation sequencing (NGS) library preparation method with a novel bioinformatics pipeline to efficiently identify rare mutations (EXO1000 Solid Tumor Panel), was used to analyze a panel of nine mutation hotspots from six genes, including BRAF. Data were correlated with tissue-based mutational analysis, treatment information, and RECIST-defined response assessments.

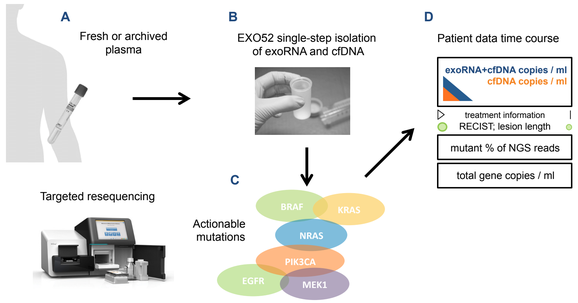
exoRNA and cfDNA: Two distinct sources of cell-free nucleic acids in plasma

- **Extracellular vesicles**, mainly 50-200 nm in size, are actively secreted by the cell and carry a snapshot of the body's transcriptome (exoRNA).
- **Circulating cell-free DNA** (cfDNA) is released by necrotic and apoptotic events in tumor and normal tissue.



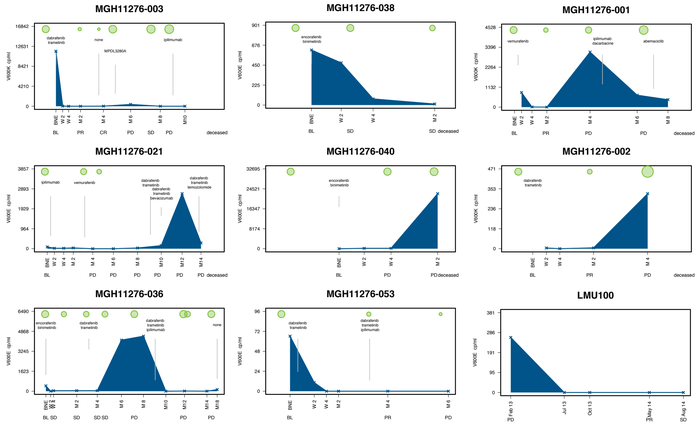
Extracellular RNA and DNA in plasma. Exosomes are actively released by living cells directly from the plasma membrane (A) or via the multivesicular body pathway (A'), carrying RNA into circulation (exoRNA). Apoptotic or necrotic cells may release cell-free DNA (cfDNA) in apoptotic vesicles (B) or as free-circulating nucleosomes (B').

Using the EXO1000 Solid Tumor Panel in monitoring circulating mutations of patients with MM



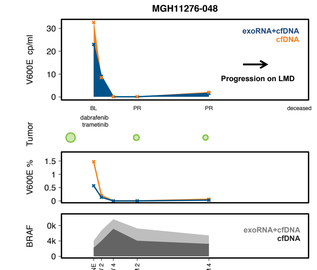
Workflow of the EXO1000 Solid Tumor Panel: EXO52 co-isolation of exoRNA and cfDNA from 0.5-4 mL of fresh or archived plasma, pre-filtered with 0.8 µm to exclude cellular material (**A** and **B**); Targeted enrichment, sequencing on the Illumina MiSeq™ platform and absolute quantification of input material; Bioinformatic analysis that includes noise correction and calculation of mutant copy numbers (**C**). Data is presented as outline of a single patient's longitudinal liquid biopsies during treatment (**D**). Shown are 4 panels which contain mutant copies per ml plasma (EXO52 copies depicted in blue, cfDNA copies depicted in orange), RECIST classification and tumor volume relative to baseline (green circles), the noise-corrected mutant %, and total gene copies per ml plasma (EXO52 copies depicted in light grey).

Plasma-based monitoring of BRAF mutations with EXO1000



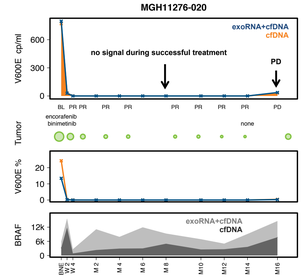
Monitoring BRAFi treatment with EXO1000. Simple outline of a single patient's longitudinal liquid biopsies during treatment. EXO52 mutant copies per mL plasma are plotted in blue and RECIST-define tumor lesion length is visualized as green circles.

Example 2: Low signal from patients with progressing LMD



Progression on leptomeningeal disease. Detailed outline of a single patient's longitudinal liquid biopsies during treatment. This patient is one out of two patients with systemic and leptomeningeal disease (LMD) which commenced on BRAFi/MEKi treatment. While both patients had treatment response per RECIST, improvement of LMD symptoms followed by an decrease in BRAF levels (no signal at first PR), only the displayed patient had a small but detectable mutation signal before progression of LMD.

Example 3: Monitoring of long-term treatment success



Successful therapy and relapse. Detailed outline of a single patient's longitudinal liquid biopsies during treatment. This patient showed continuous response to BRAFi/MEKi therapy, indicated by radiographic evidence and sustained low BRAF mutation levels (PR, arrow). Eventually, this patient elected to be removed from study due to quality of life reasons, and at time of next follow-up had an increase in mutant signal and that corresponded to progression of disease (PD).

EXO1000 analysis predicts disease progression in advance of clinical or radiographic progression

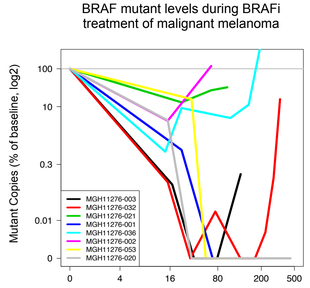
Complete BRAFi regimen		Vem	Dab + Tram	Enco + Bini
Total treated		2	5	5
Best response				
	PD	1	0	1
	SD	0	0	1
	PR	1	4	3
	CR	0	1	0
Eventual progression		2	4	4
BRAF MUT decreased on treatment		2	5	4
BRAF MUT increased @ PD		**2**	**3**	**4**
BRAF MUT increased before PD		**1**	**2**	**2**

BRAFi = BRAF inhibitor
MEKi = MEK inhibitor
PR = partial response
PD = progressive disease
CR = complete response
SD = stable disease

Dab + Tram = dabrafenib plus trametinib
Enco + Bini = encorafenib plus binimetinib
Vem = vemurafenib

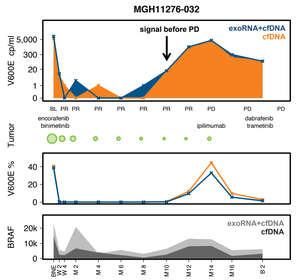
MUT = mutations

Treatment substantially decreases BRAF mutant levels until progression



Levels of BRAF V600E and V600K mutant during BRAFi treatment. Every colored line corresponds to a single patient in this study. Mutant BRAF copies relative to baseline (V600E or V600K) are shown starting at treatment onset (Day 0) to first indication of progression based on clinical or radiographical evidence (end of plotted line).

Example 1: BRAF mutant signal 4 months before clinical progression



Early detection of progression. Detailed outline of a single patient's longitudinal liquid biopsies during treatment. In this patient, two weeks after initiation of BRAFi/MEKi treatment (W2), the V600E mutant copies drop below detection level, following radiological response by RECIST (PR). A substantial mutant signal at month 10 of treatment predicts the clinical progression (PD) 4 months ahead (arrow).

Summary and Conclusions

- Using EXO52 spin columns and EXO1000 NGS analysis, we detected and quantified mutant BRAF levels in 12 of 12 patients with BRAF mutant melanoma.
- Mutant BRAF levels were reduced in 11 of the 12 patients in the setting of BRAFi therapy and in every one of the 10 RECIST-defined responders.
- Mutant BRAF levels were increased at time of PD to BRAFi in 9 of 10 patients.
- Mutant BRAF levels were increased in advance of PD in 5 of 10 patients.
- The two patients in whom mutant BRAF levels were not increased (or minimally increased) at time of PD had progressing leptomeningeal disease (LMD) in the setting of ongoing non-LMD response.
- **The EXO52 technology platform enabled longitudinal monitoring of BRAF mutant melanoma and demonstrated the ability to detect early disease progression prior to radiographic changes.**

This work was supported partly by the m4 spitzencluster initiative

Contact: Hannah@exosomedx.com