Profiling Exosomal mRNAs in Patients Undergoing Immunotherapy for Malignant Melanoma

James Hurley[1], Lan Hu[1], Graham Brock[1], Ian Sinclair[1], Ryan J. Sullivan[2], Vincent J. O'Neill[1], Johan Skog[1], Keith Flaherty[3]

(1) Exosome Diagnostics, Cambridge, MA (2) Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA (3) Massachusetts General Hospital and Dana-Farber Cancer Institute, Boston, MA

Abstract – Poster # 452

Introduction

Traditionally, oncology biomarker discovery and development has required the use of material obtained from tissue biopsies. However, recent developments in the exosome field have allowed biomarker research in biofluids to evolve. Exosomes are highly stable microvesicles, approximately 30-200 nm in diameter, that are shed by cells into all biofluids, including blood, urine, and cerebrospinal fluid, carrying a rich source of intact protein and RNA (Fig.1). RNA can be efficiently isolated and addressed using technologies such as RT-qPCR and NGS. Here we demonstrate the use of RNA extracted from these vesicles to monitor transcriptional changes in response to an immunotherapy treatment for malignant melanoma.

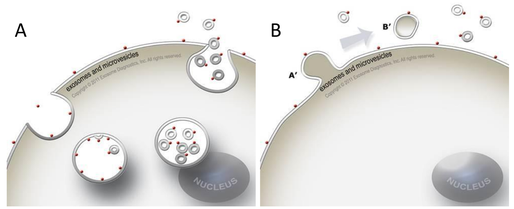

Fig.1 Exosomes and other vesicles can be released by (A) multi-vesicular body pathway or through (B) direct budding at the plasma membrane.

Methods

Exosomes were isolated, using exoRNeasy columns, from plasma collected from malignant melanoma patients treated with Ipilimumab. Patients were split in two groups, based upon either a positive or negative response to the therapy (5 responders, 11 non-responders, 16 patients in total). Responders were classified as patients with at least 6 months duration of response using the RECIST1.1 criterion. Plasma was collected pre-treatment (baseline) and the first post-treatment time point (week 2 or week 4). Exosomal RNA (exoRNA) was extracted using exoRNeasy kit (Fig.2). We screened the levels of 586 mRNAs associated with inflammation and 21 mRNA controls by RT-qPCR using the OpenArray® technology. The identified potential biomarker genes subsequently were verified independently.

- Bind vesicles to membrane & wash
- QIAzol lysis and release of RNA
- Phenol/Chloroform extraction
- Ethanol conditioning
- Bind to RNeasy column and wash
- Elute RNA

Fig.2 Workflow of exoRNA isolation with exoRNeasy platform. The platform allows scalable processing of 0.1mL to 4mL of plasma.

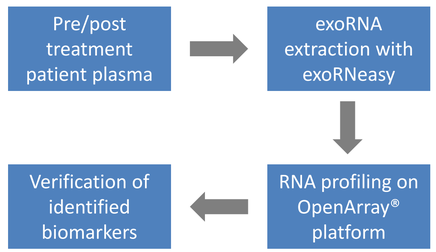

Fig.3 Workflow of plasma exoRNA isolation and expression profiling in malignant melanoma patients.

Results

Over 400 genes were detected across all samples and time-points. Eleven control mRNAs with robust detection in all samples were used for normalization. When compared in matched pre- and post-treatment time point patient samples, we identified 9 mRNA species with opposite expression changes. A number of mRNA species showed significant differential expression (p-value<0.05) in patients responding to the therapy compared to those that did not respond. By using high throughput PCR array screening, we have demonstrated the potential for exosome RNA profiling in longitudinal monitoring of treatment response. The signatures are being confirmed by individual assays run in 384-well format.

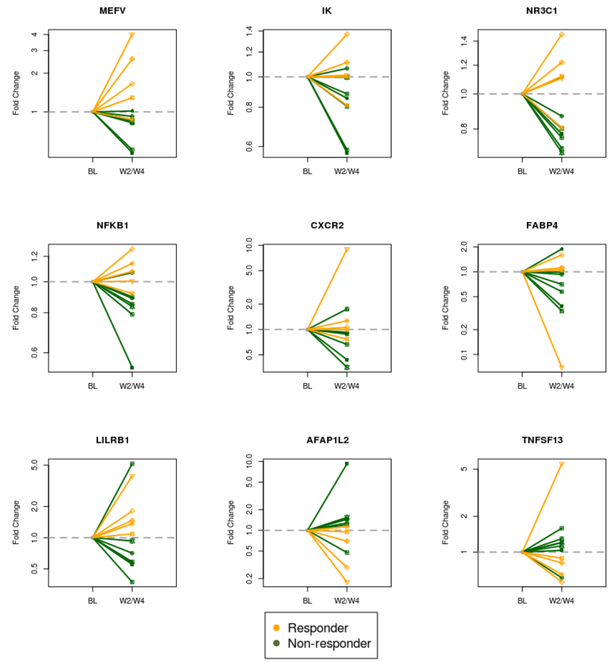

Fig. 4 Genes with opposite expression changes in responders and non-responders using the OpenArray® platform.
BL=baseline; W2/W4=week 2/week 4. The gray dash line at Fold change=1 means no change between time points. Same symbols indicate same patients.

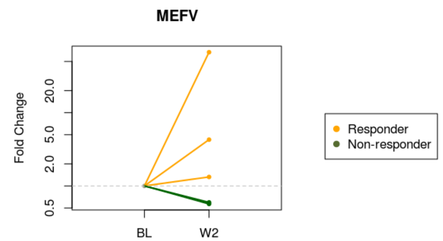

Fig. 5 Single assay PCR result of gene MEFV.
The single assay result is consistent with the OpenArray® platform screening result. BL=baseline; W2=week 2. The gray dash line at Fold Change=1 means no change between time points.

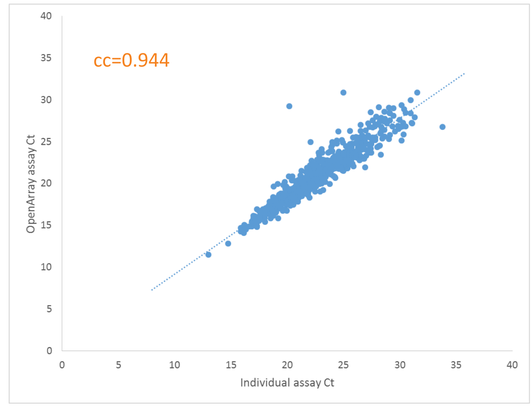

Fig 6. Comparison of OpenArray® and individual assay data. Using the OpenArray® as a means to screen large number of potential markers can be correlated to how the same assays will perform as individual assays. A subset of assays from the OpenArray were run as individual assays in a 384-well plates using the same cDNA samples. cc= correlation coefficient.

Conclusions

RNA expression profiling from circulating exosomes offers the ability to monitor changes in immune pathway genes serially during immunotherapy. Initial data suggest expression signature changes occur early during therapy, offering the potential for predicting response.

References

1. Brock, G. *et al.* (2015) Liquid biopsy for cancer screening, patient stratification and monitoring. *Translational Cancer Research*, 4(3), 280-290.
2. Enderle, D. *et al.* (2015) Characterization of RNA from Exosomes and Other Extracellular Vesicles Isolated by a Novel Spin Column-Based Method. *PLoS ONE*, 10(8): e0136133. doi:10.1371/journal.pone.0136133.
3. Skog, J. *et al.* (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumor growth and provide diagnostic markers. *Nature Cell Biology*, 10, 1470-1476.

Contact: voneill@exosomedx.com