Introduction and Methods:

Circulating nucleic acids (RNA and DNA) in plasma are of growing interest due to the belief that these nucleic acids carry a snapshot of the host cell's RNA and DNA. Exosomes and other extracellular vesicles have been hypothesized to carry these nucleic acids. One of the best characterized exosomes is the exosome (exoRNA) that is released from neoplastic cells and contains the genome and proteome of cancer cells. This makes the exosome a potential source of non-invasive biomarkers that could serve as a diagnostic tool for melanoma. The main focus of our study was to analyze the presence and mutation in RNA and DNA of plasma from melanoma patients.

We present data from the first six melanoma patients with the following characteristics:

- Patient 1: 49 years old, male, stage IV, BRAF V600K mutation, BRAF V600K copy number, TMB 100 cp/ml, TMB 1000 cp/ml, PR PD
- Patient 2: 69 years old, male, stage IV, BRAF V600E mutation, BRAF V600E copy number, TMB 100 cp/ml, TMB 1000 cp/ml, PR PD
- Patient 3: 48 years old, female, stage IV, BRAF V600E mutation, BRAF V600E copy number, TMB 100 cp/ml, TMB 1000 cp/ml, PR PD
- Patient 4: 52 years old, female, stage IV, BRAF V600K mutation, BRAF V600K copy number, TMB 100 cp/ml, TMB 1000 cp/ml, PR PD
- Patient 5: 50 years old, male, stage IV, BRAF V600E mutation, BRAF V600E copy number, TMB 100 cp/ml, TMB 1000 cp/ml, PR PD
- Patient 6: 55 years old, female, stage IV, BRAF V600K mutation, BRAF V600K copy number, TMB 100 cp/ml, TMB 1000 cp/ml, PR PD

Each patient was treated with BRAFi/CDKi combination therapy. Complete reduction of mutation signal was observed after PR PD. A complete reduction of mutation signal was observed after 2 months of therapy. Complete reduction of mutation signal was observed after 2 months of therapy.

Conclusions:

- EXTRA2 platform provides a simultaneous single-step co-isolation method for exoRNA and cfDNA and, in this series of metastatic melanoma patients, yields substantially more mutant BRAF copies than cfDNA alone, increasing overall assay sensitivity.
- Molecular changes (mutant gene copy number) delineate clinical changes in disease response and progression, as defined by RECIST criteria.
- EXTRA2 platform provides a robust and highly sensitive means of detecting rare mutations in bloodplasma and has clear and immediate clinical application.
- Validation studies of several clinically relevant, actionable mutations and gene rearrangements in bloodplasma are ongoing in a number of tumour investigations.