

Development of a Pipeline for Analysis of Extracellular Vesicles in Health and Disease

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Background: For patients who present with or develop progressive, metastatic disease, new immunotherapies offer significant hope of remission or cure. However, many of the most effective immunotherapies and cancer vaccines are often effective in fewer than 50% of patients treated, and assessment of treatment responses is only feasible after multiple weeks or months of treatment, a period of time during which other more effective treatment options or combinations might have been selected instead for the non-responding patients. Extracellular Vesicles (EVs), which are continuously released, carry surface receptors and RNA/DNA cargo related to the state of their cell of origin, may enable near-real-time clinical blood and/or urine tests for early screening, risk stratification, and monitoring of cancer immunotherapies.

Methods: To extract the information that is carried by EVs, our lab has developed a first-in-class pipeline to characterize EV heterogeneity and provide high-sensitivity quantification of informative EV subsets by combining multiplex assays with high-resolution, single EV flow cytometric methods together into a **Multiplex-to-Single EV Analysis (Mt-SEA)** pipeline.

Results: We have preliminary data from ongoing clinical trials, combining checkpoint blockade with conventional chemotherapy and cancer vaccine therapy, wherein we have identified EV subset changes that correspond to responses to treatment. Moreover, we find that it is possible to interrogate several dimensions of EV cargo to further improve the utility and impact of this approach. Also, the methods and tools that we have developed are being made available for use in the wider research community

Conclusions: We hypothesize that identification of sensitive and specific EV-associated biomarkers for responses to immunotherapies would significantly advance efforts to improve treatment outcomes and quality of life for advanced cancer patients. The Mt-SEA Pipeline of methods developed by the NCI Translational Nanobiology lab establishes a foundation for interrogating different genetic and molecular profiles in tumor-derived and immune cell-derived EVs in urine and/or blood within 1-2 weeks of the start of treatment with immunotherapies.

Conflict of Interest: No conflicts to declare. All authors are employed by the National Cancer Institute, and patents have been filed by NCI for Mt-SEA Pipeline- related methods, reagents, and software. NCI has a collaborative research and development agreement (CRADA) with Beckman Coulter for development of EV flow cytometry tools from the Mt-SEA Pipeline.

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