Detection of EV-based signatures in prostate cancer using microflow cytometry and machine learning

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Microflow Cytometry (μFCM) has recently emerged as a viable technology to enumerate and quantify individual EVs from complex biological fluids. This method is a promising alternative to biopsy collection because it is highly sensitive and allows for longitudinal assessment of patients. EVs are highly abundant and analysis by μFCM allows for sophisticated multi-parametric analysis of organ-specific, disease-specific and outcome-specific proteins, genes or metabolites. Detection of EVs with μFCM requires very little sample, yet provides exceptional specificity and sensitivity for low abundance events. By combining μFCM analysis of EVs with advanced machine learning approaches, we have developed a platform technology called ClarityDX to generate EV fingerprints that significantly improve the diagnosis of diseases such as prostate cancer using a few drops of blood. Here I will discuss the development and validation of new blood tests for prostate cancer that utilize the ClarityDX platform.

Prostate cancer is the most commonly diagnosed cancer and the third leading cause of cancer deaths in men. Screening has enabled the early detection of many latent prostate cancers, and it is estimated that up to 50% of new prostate cancer diagnoses detect a tumour that was unlikely to surface clinically in the absence of PSA screening. In the majority of cases, the treatment of indolent cancer causes a patient more harm than good. Consequently, there is an urgent need for a non-invasive test to detect clinically-significant prostate cancer, or perhaps even more importantly – to detect metastatic disease.

ClarityDX Prostate is a μFCM-based test to predict clinically-significant prostate cancer prior to a prostate biopsy. A prospective clinical validation of ClarityDX Prostate was performed in a cohort of 377 Albertan men. The overall average AUC for ClarityDX Prostate in this population was 0.83, which was significantly higher than PSA alone. While PSA alone provided only 17% specificity for aggressive prostate cancer at a 95% sensitivity, ClarityDX Prostate was 56% specific for aggressive prostate cancer. This suggests that up to 40% of unnecessary biopsies could be avoided by incorporating ClarityDX Prostate into routine prostate cancer screening. We are also developing a second test, ClarityDX Metastasis, to improve the detection of metastatic disease after an initial diagnosis of prostate cancer. This test utilizes a μFCM approach to enumerate circulating extracellular vesicles (EVs) in serum that express prostate membrane specific antigen (PSMA) and/or tetraspanin CD151. A pilot study in a 66 patient cohort indicates that an elevation of CD151+ EVs is significantly associated with metastatic disease, and our efforts are now concentrated on whether elevated CD151+ EVs can predict future metastasis in patients subsequent to diagnosis.

Overall, we have established a robust μFCM EV platform for the development of novel and clinically-viable diagnostic tests.