Title: Development of Exosome-based Plasma RNA Biomarkers for High-Risk Prostate Cancer

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Introduction and overall goal: There is an unmet need for liquid biopsy tests to support the management of patients with intermediate and high-risk prostate cancer. In treatment naïve patients, risk stratification by standard clinical pathologic factors and tissue based genomic testing can be influenced by sampling errors inherent to prostate cancer biopsy. Exosome-based liquid biopsies are emerging as a clinically effective platform for minimally invasive and highly sensitive diagnostics in diverse types of cancer (Yu et al. 2021). In this project we undertake the development of a plasma exosome-based liquid biopsy with the goal to stratify patients with clinically significant prostate cancer that may circumvent the sampling challenges associated with tissue-based genomic tests.

Specific Aims: The aim of this pilot study is to identify plasma exosome RNA markers that differentiate treatment-naïve men diagnosed with NCCN low-risk vs high-risk prostate cancer as the first step in the development of a plasma exosome-based test to support the management of patients with intermediate and high-risk prostate cancer.

Rationale and Background: Exosomes are small double-lipid membrane vesicles that cells actively shed into various biofluids and provide packages for RNA, DNA and protein molecules. The mRNA and long non-coding RNA encapsulated in exosomes are of particular interest since most proven cancer diagnostic markers are long RNA-based. In this pilot project we take advantage of the well-developed ExosomeDx biomarker analysis platform and RNASeq to profile plasma exosomal RNA markers that differentiate treatment-naïve men with biopsy proven NCCN low risk vs high risk prostate cancer.

Materials and Methods: Our pilot biomarker discovery study compared plasma exosomal RNA profiles from 11 high-risk and 9 low-risk, treatment-naïve, biopsy-confirmed patients together with 4 healthy controls. The high-risk patients are enrolled in a radiotherapy trial (BLaStM NCT02307058) and the low-risk patients in an active surveillance trial (MAST NCT02242773). Exosomes were isolated from 1 ml plasma samples and exosomal RNA prepared using the ExosomeDx isolation platform ExoLution (Möhrmann et al 2018). A hybrid-capture-based Next Generation RNA Sequencing (RNAseq) analysis was performed on enriched transcripts of exons, 3' and 5' untranslated RNA and long non-coding RNA (lncRNA) to profile and identify differentially expressed long RNAs.

Results: We detected over 10,000 protein coding genes and ~400 lncRNAs in each plasma sample using exosomal RNAseq. 273 genes were differentially expressed in high-risk vs control but not in low-risk vs control. This pilot study identified four potential plasma exosomal biomarkers of high-risk prostate cancer. These include three protein-coding mRNA transcripts that showed >20 fold lower expression in plasma from high-risk in comparison to low-risk patients; TCGA data show that two of these mRNA markers are also downregulated in prostate cancer tissue in patients with worse survival. In addition, one lncRNA was identified that showed >20 fold higher expression in plasma from high-risk in comparison to low-risk patients. Together, these represent early data for potentially novel liquid biopsy RNA biomarkers for high-risk prostate cancer.

Discussion and Conclusion: Here we report preliminary results of a liquid biopsy biomarker discovery study for an exosome-based long RNA test to support the management of patients with intermediate and high-risk prostate cancer. Plasma exosomal long RNA provides a rich reservoir of currently untapped biomarkers for high-risk prostate cancer

References:

Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, Breakefield XO, Skog JK. Exosome-based liquid biopsies in cancer: opportunities and challenges. Ann Oncol. 2021 Apr;32(4):466-477. PMID: 33548389.

Möhrmann L, Huang HJ, Hong DS, Tsimberidou AM, Fu S, Piha-Paul SA, Subbiah V, Karp DD, Naing A, Krug A, Enderle D, Priewasser T, Noerholm M, Eitan E, Coticchia C, Stoll G, Jordan LM, Eng C, Kopetz ES, Skog J, Meric-Bernstam F, Janku F. Liquid Biopsies Using Plasma Exosomal Nucleic Acids and Plasma Cell-Free DNA Compared with Clinical Outcomes of Patients with Advanced Cancers. Clin Cancer Res. 2018 Jan 1;24(1):181-188. PMID: 29051321.