

Integrated high purity CTC RNA-Seq and ctDNA sequencing to define the intersection of high-risk genomic alterations and transcriptional phenotypes in metastatic prostate cancer via liquid biopsy

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Introduction: In the largest circulating tumor cell (CTC) RNA sequencing cohort in mPC to date, we previously defined four prognostic transcriptional subtypes: low proliferation (LP) and luminal (LumA) subtypes associated with favorable prognosis, and high-proliferation luminal (LumB) and neuroendocrine (NE) subtypes associated with poor prognosis and treatment resistance. Genomic mutation or copy number variation (CNV) of commonly altered prostate cancer genes—such as *AR*, *TP53*, *PTEN*, *PIK3CA*, *RB1*, and *MYC*—are also associated with poor clinical prognosis and treatment resistance. To elucidate the relationship between these genomic alterations and poor prognosis LumB and NE transcriptional subtypes, we integrated our high-purity circulating tumor cell (CTC) RNA-seq method with matched circulating tumor DNA (ctDNA) next-generation sequencing (NGS) to conduct a multi-omic liquid biopsy analysis of mPC patients. **Methods:** We collected 99 matched high purity CTC, cfDNA, and germline samples from 59 patients with mPC in a prospective biomarker trial. High-purity CTCs were isolated via immunomagnetic capture on a microfluidic platform, analyzed via RNA-seq, and assessed for transcriptional phenotypes and gene expression signatures. Cell-free DNA (cfDNA) was extracted from plasma and sequenced using a customized targeted NGS panel to identify ctDNA mutation and CNV profiles. **Results:** High-purity CTC RNAseq samples were classified as LP (n=19), LumA (n=32), LumB (n=43) or NE (n=5) subtype using a *k*-nearest neighbor classifier trained on our published CTC RNAseq cohort. Genomic analysis of matched ctDNA revealed a high prevalence of canonical prostate cancer alterations, led by *AR*, *TP53*, *PTEN*, *SPOP*, *FOXA1*, *PIK3CA*, *RB1*, and *MYC*. Patients were stratified based on the presence of a driver alteration (*AR* and *MYC* amplification, *PIK3CA* activating mutation, or *RB1/PTEN* mutation/deletion), a *TP53* mutation, or the simultaneous presence of both. We observed a progressive increase in the frequency of driver and *TP53* alterations moving from the more favorable LP and LumA to the poor prognosis LumB and NE transcriptional states (Fisher's exact test, p=0.00046). Functionally, cell-cycle, proliferation, and DNA repair pathway gene expression signatures were higher in CTCs with *TP53* and driver alterations in matched ctDNA, with the highest proliferation and DNA repair pathway signature scores observed in patients with co-occurring driver/*TP53* alterations. **Conclusions:** Our integrated multi-omic liquid biopsy analysis of mPC patients revealed that highly proliferative CTC phenotypes are associated with an increasing frequency and co-occurrence of canonical genomic drivers and *TP53* alterations observed in ctDNA. We are currently evaluating whether integrating driver/*TP53* alteration status with CTC transcriptional phenotypes can help guide treatment selection and intensification/de-intensification strategies in mCRPC.

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Conflicts of interest: KTH has a family member who is an employee of Epic Systems. MLB has a family member who is an employee of Luminex – A DiaSorin Company. SGZ reports unrelated patents licensed to Veracyte, and that a family member is an employee of Artera and holds stock in Exact Sciences. MNS reports consulting fees from AstraZenica and ArteraAI unrelated to the submitted work. JML is a consultant/AdvisoryBoard Member for Janssen, Pfizer, Astellas, Arvinas, AstraZenica, MacroGenics, Foundation Medicine, Bayer, Daiichi Sankyo, and Sanofi; receives institutional research funding from Immunomedics, reports a patent for Circulating tumor cell technology issued to Salus Discovery LLC and is a founder of Eolas Diagnostics Inc., which performs analysis of CTCs. RRM reports consulting fees from Ambrx, Arcus, AstraZenica, Aveo, Bayer, Bristol-Myers Squibb, Calithera, Caris, Dendreon, Exelixis, Johnson & Johnson, Lilly,

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