High purity CTC RNA sequencing identifies poor prognosis lineage states in castrate resistant prostate cancer.

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Background: Metastatic prostate cancer is an androgen receptor (AR) driven disease for which multiple AR targeted therapies are FDA approved, leading to improvements in patient outcomes. However, development of treatment resistance remains universal, driven by AR alterations as well as lineage state transitions that bypass AR signaling and culminate in neuroendocrine prostate cancer (NEPC) with poor prognosis. Liquid biopsies could enable longitudinal molecular analysis to identify such lineage transitions, but bulk RNA sequencing of circulating tumor cells (CTCs) has been limited by the challenge of isolating sufficiently pure samples for global signaling pathway analysis. We developed a novel approach to CTC purification that results in purity comparable to tissue biopsies and performed the first large-scale CTC RNA-seq study in a multi-institutional cohort of patients with metastatic prostate cancer. Using this approach, we identified multiple prostate cancer lineage states associated with prognosis. **Methods:** 273 blood samples were collected from 117 patients with histologically confirmed metastatic prostate cancer treated at the University of Wisconsin Carbone Cancer Center, William S. Middleton Memorial Veterans Hospital, UC San Diego Moores Cancer Center and Dana Farber Cancer Institute. CTCs were isolated with our automated microfluidic technology integrating negative and positive selection for CTC enrichment. CTCs were captured immunomagnetically followed by RNA isolation on chip and RNA sequencing. A modification of the ESTIMATE algorithm was used to infer sample tumor purity, and CIBERSORTx was used to infer immune content. Overall survival (OS) was defined as date of death or last contact relative to first CTC sample collection. **Results:** 173 samples (63%) recovered adequate RNA for sequencing, and 146 samples from 70 patients met our >50% purity threshold for gene expression pathway analysis. Single sample pathway analysis identified four CTC transcriptional phenotypes: luminal A (high AR signaling, intermediate proliferation), luminal B (high AR signaling, high proliferation), low proliferation, and neuroendocrine. Compared to patients with low CTC burden/low tumor purity (median OS NR, n=63), patients with luminal A (n=21) and low proliferation (n=12) phenotypes had similar survival, while patients with luminal B (n=18) and NE (n=3) had markedly shorter survival (LumB: median OS 6 months, HR 9.1, log rank $p<0.0001$, NE: median OS 3.7 months, HR 11.8, log rank $p=0.0019$). **Conclusion:** We report the largest CTC RNA sequencing cohort of patients with metastatic prostate cancer and demonstrate that CTC RNA-seq identifies prostate cancer lineage states mirroring those described in tissue profiling. In addition to an NE phenotype concordant with tissue histology and associated with markedly inferior overall survival, we identified a more common luminal B CTC subtype defined by persistent AR signaling and high proliferation and associated with poor prognosis comparable to NEPC.

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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. SMD reports consulting relationships with BMS, Oncternal therapeutics, Janssen R&D/J&J and a grant from Pfizer/Astellas/Medivation (the grant was submitted to Medivation, ultimately funded by Astellas and then moved to Pfizer). FYF reports personal fees from Janssen Oncology, Bayer, PFS Genomics, Myovant Sciences, Roivant Sciences, Astellas Pharma, Foundation Medicine, Varian, Bristol Myers Squibb (BMS), Exact Sciences, BlueStar Genomics, Novartis, and Tempus; other support from Serimmune and Artera outside the submitted work. MS reports speaker fees from Astellas, Scientific Advisory board for Veracyte/Adelphi Targis. MNS reports other support from Novartis unrelated to the submitted work.