# Identification of a CTC-based prognostic signature in mCRPC driven by Aurora Kinase A and Wnt signaling

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**Background:** Liquid approaches for assessing tumor behavior in men with metastatic castration-resistant prostate cancer (mCRPC) represent a major unmet clinical need. To facilitate non-invasive detection of genomic alterations and molecular expression in these men, we developed and implemented a circulating tumor cell (CTC) based platform for analysis of whole blood samples. Because CTCs likely represent cells from disparate metastatic sites, we hypothesized that understanding the expression of key genes of interest could have unique utility in precision medicine approaches to predict clinical outcomes in patients with advanced prostate cancer.

**Methods:** CTCs were isolated from 5 mL whole blood using anti-EpCAM-conjugated magnetic beads. Following cell lysis, mRNA from CTCs was purified, followed by reverse transcription. Libraries were generated and qPCR was performed to evaluate a panel of 96 genes. Blood processed from 27 healthy controls was used as a baseline referent. Gene expression data from prostate cancer cells (VCaP, PC3, and LNCaP) spiked into normal control blood was used for assay development, and CTCs from 41 patients with mCRPC were subsequently evaluated. The Halabi nomogram (JCO 2014) was utilized to account for baseline clinicopathologic variables (opioid analgesic use, disease site, ECOG PS, albumin, hemoglobin, alkaline phosphatase, and PSA). Cox regression was used to identify genes independently associated with overall survival (OS), and receiver operator curves were constructed to assess model performance.

**Results:** Of 41 patients with mCRPC, we identified 27 (63%) with detectable CTCs, defined by expression of EpCAM, EGFR, CDH1, CDH2, DSG2, KRT8, KRT18 and/or KRT19. There have been 21 deaths to date. The Halabi nomogram was strongly associated with OS (HR 1.74, 95%CI 1.20-2.54), and AURKA (HR 3.40, 95%CI 1.47-7.85), WNT5A (HR 2.71, 95%CI 1.43-5.13), and BMP7 (HR 2.10, 95%CI 1.25-3.52) were independently associated with OS after adjusting for the Halabi nomogram score ($p<0.01$, FDR<15%). A model including the Halabi nomogram, AURKA, BMP7, and WNT5A expression had an area under the curve (AUC) of 0.92 for predicting OS at 6 months in comparison with an AUC of 0.74 for the nomogram alone.

**Conclusions:** These data support the importance of Aurora Kinase A, a known driver of neuroendocrine prostate cancer, as a clinically relevant liquid-based biomarker in patients with mCRPC. Additionally, our study confirms previously reported RNAseq findings nominating CTC-based WNT5A expression as a marker of progression in mCRPC. Here, a score based on an established nomogram plus AURKA, WNT5A, and BMP7 expression in CTCs was highly prognostic. Additional patient cohorts are needed for validation.

**Conflict of interest:** none

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