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\textbf{Title:} A novel live cell diagnostic platform measuring phenotypic biomarkers using objective algorithmic analysis enables further risk stratification for intermediate-risk prostate cancer patients.

Due to the inconsistencies of existing molecular, genomic, and pathophysiologic markers for patient risk stratification, effective prostate cancer diagnostics and treatment remains a challenge in clinical practice. Therefore, the development of a diagnostic platform that differentiates cancer patients who have clinically significant disease from those who have a low risk of progression is an important area of interest. In this study, we tested a diagnostic platform that combines a scalable microfluidic device, enabling an automated live cell assay, that measures \textit{phenotypic biomarkers} [as defined here as functional biophysical and molecular biomarkers], via objective machine vision algorithms to evaluate both local invasive and metastatic potential of prostate cancer.

An analytical validation study was performed on fresh prostate cancer samples (n=100) obtained at the time of radical prostatectomy (RP). The diagnostic platform enables: 1) growth of patient cells \textit{ex vivo} on extra cellular matrix formulations supporting adhesion/survival for 72 hours post biopsy 2) high-throughput imaging of multiple \textit{phenotypic biomarkers} such as morphology, cytoskeleton dynamics, and protein subcellular localization & modification states and 3) objective quantification of biomarkers via machine vision analysis. Patient samples were imaged over a three hour period capturing live-cell biophysical biomarkers. After three hours cells were fixed and stained for molecular biomarkers. Machine vision technology was then utilized to analyze \textit{phenotypic biomarkers} to yield specific metrics (Oncogenic potentials and Metastatic potentials (OP and MP)), that correlated with RP specimen pathologic findings.

Analysis of quantified \textit{phenotypic biomarkers} distinguished normal from cancer cells. Interestingly, OP and MP metrics demonstrated statistical significance in distinguishing Gleason 6 from Gleason 7 prostate cancer with 85\% sensitivity and 80\% specificity (n=100) and concordance with relevant RP pathology findings.

In conclusion, OP and MP derived from defined \textit{phenotypic biomarker} metrics, demonstrate the ability to differentiate Gleason 6 and 7 scores and correlate with, 1) seminal vesicle invasion, 2) positive RP surgical margins, 3) vascular Invasion, and 4) lymph node involvement. This novel functional-live-cell diagnostic platform allows for the measurement of a biomarker panel that further stratify patients to improve prostate cancer treatment, clinical decision-making, further risk stratify intermediate prostate cancer populations, and potentially predict actionable pathological findings.