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**Clinical Validation of a live-cell phenotypic biomarker - based diagnostic assay for**

**the prediction of adverse pathology in Prostate Cancer**

Introduction and Objective: Prostate cancer accounts for over 28% of total cancer cases in the United States. Current screening and diagnostic approaches lack the sensitivity to objectively assess the aggressiveness of tumors. To address this issue, a diagnostic assay was developed to differentiate indolent from aggressive tumors, objectively risk stratify patients and predict adverse pathology. Here we describe a diagnostic platform that is based on the measurement of a panel of phenotypic and molecular biomarkers in live biopsy-derived cells. Combining microfluidics, automated imaging and image analysis, the assay provides predictive scores for local aggressiveness, invasiveness and the presence of adverse clinical pathologies.

Methods: This clinical validation study was done on fresh prostate cancer samples (n=250) obtained at the time of radical prostatectomy. Patient cells were grown ex vivo (up to 72 h) to enable live-cell, label-free imaging of multiple phenotypic biomarkers. Cells were then stained & imaged for molecular markers. Data were objectively quantified by machine vision algorithms to evaluate cellular behavior, and machine learning analysis to generate predictive metrics.

Results: The developed predictive dynamic biomarker metrics of adverse pathology: “Local Adverse Pathology Potential (LAPP)” & “Metastatic Adverse Pathology Potential (MAPP),” report on the local aggressiveness and invasiveness, respectively, are able to distinguish benign from malignant cells, risk stratify fresh tumor samples, and predict adverse pathology. Comparing our results with known clinical pathology data, we can distinguish Gleason 6 from Gleason 7 and Gleason (3+4) from Gleason (4+3) with greater than 90% sensitivity and specificity. LAPP and MAPP metrics can also predict the likelihood of six different adverse clinical pathologies with high accuracy as characterized by Receiver Operator Curves with Area Under the Curve (AUC) values >0.80.

Conclusions: This novel live-cell phenotypic assay can quantitatively risk stratify patients with similar Gleason scores. Moreover this first-in-class diagnostic can predict adverse clinical pathologies, namely 1) seminal vesicle invasion, 2) positive surgical margins, 3) extra prostatic extension, 4) perineural invasion, 5) vascular invasion and 6) lymph node invasion. These results suggest that this novel assay can accurately stratify low & intermediate risk cases and aid clinical decision-making to improve treatment outcomes.