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Analytical Validation of a Live-Cell Phenotypic Biomarker - Based Diagnostic Assay for the Prediction of Adverse Pathology in Prostate Cancer from Field Biopsy Cores

Introduction and Overall goal: Prostate cancer accounts for over 28% of total cancer cases in the United States. Current diagnostic approaches require biopsy cores that sample cancerous tissue within the prostate, and cannot effectively predict adverse pathology if a biopsy core samples tissue away from the tumor or lesion. To address this issue, a diagnostic assay was developed to differentiate indolent from aggressive tumors, objectively risk stratify patients and predict adverse pathology from tissue adjacent to suspected cancer tissue within the prostate, or 'field sample.' Here we describe a diagnostic platform that is based on the measurement of a panel of cellular and molecular phenotypic biomarkers in live biopsy-derived cells from tissue adjacent to tumor. Combining microfluidics, automated imaging and image analysis, the assay provides predictive scores for local aggressiveness, invasiveness, and the presence of adverse clinical pathologies.

Specific Aims: (1) Establish conditions to stably maintain live primary prostate cells for automated live-cell and fixed cell imaging and collection of data on phenotypic biomarkers. (2) Measure both cellular and molecular phenotypic biomarkers on a blinded 60 sample set in an automated high-through put manner. (3) Statistically analyze the 60 sample set in an unbiased objective way using automated algorithmic analysis and test the performance of the algorithmic analysis by comparative analysis of the unblinded data.

Rationale: Develop algorithms that can predict adverse pathologies from a sample using either tumor derived cells or cells from adjacent presumed-'normal 'tissue in order to capture the tumor behavior and 'field effect' the tumor has on cells from surrounding tissue not classically defined as tumor cells.

Methods: This clinical validation study was done on fresh prostate cancer samples (n=60) obtained at the time of radical prostatectomy in which both tumor tissue and adjacent normal tissue were procured. 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)," and "General Adverse Pathology Potential (GAPP)" report on the local aggressiveness, invasiveness, and general likelihood of adverse pathologies, respectively, are able to distinguish benign from malignant cells, risk stratify fresh tumor or adjacent normal samples, and predict adverse pathology. Comparing our results with known clinical pathology data, we can predict adverse pathology states from both fresh tumor samples and field samples with greater than 85% sensitivity and specificity. LAPP, MAPP, and GAPP 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.

Discussion and Conclusions: This novel live-cell phenotypic assay can quantitatively risk stratify patients with Gleason scores of 6, 3+4, and 4+3. Moreover this first-in-class diagnostic can predict adverse clinical pathologies from either tumor sample directly or given adjacent 'normal' tissue, namely 1) seminal vesicle invasion, 2) positive surgical margins, 3) extra prostatic extension, 4) perineural invasion, 5) vascular invasion and 6) lymph node invasion. The algorithms specifically designed to interrogate normal tissue adjacent to tumors resulted in GAPP metrics that give a reading about a patients adverse pathologies even when tumor sample is not directly collected producing a measure of the tumor 'field effect' on surrounding cells. These results suggest that this novel assay can accurately stratify low & intermediate risk cases and aid clinical decision-making to improve treatment outcomes whether a biopsy samples the tumor tissue or adjacent tissue.