

# ANALYTICAL VALIDATION OF A LIVE-CELL PHENOTYPIC BIOMARKER - BASED DIAGNOSTIC ASSAY FOR THE PREDICTION OF ADVERSE PATHOLOGY IN PROSTATE CANCER FROM FIELD BIOPSY CORES

David M. Albala, MD, Michael S. Manak, PhD, Jonathan S. Varsanik, PhD, Stephen Zappala, MD, Grannum R. Sant, MD, Ashok C. Chander, PhD  
Cellanyx, LLC: info@cellanyx.com

## Abstract

**Background:** 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 or from a 'field sample.' Combining microfluidics, automated imaging and image analysis, the assay provides predictive scores for local aggressiveness, invasiveness, and the presence of adverse clinical pathologies.

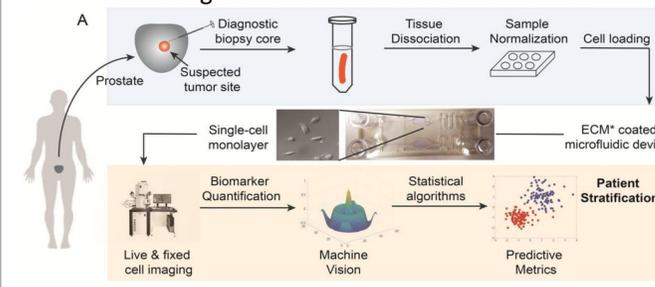
**Methods:** This clinical validation study was done on fresh prostate cancer samples (n=60) obtained at the time of radical prostatectomy in which both tumor tissue and adjacent normal tissue were procured. Patient cells were grown *ex vivo* (up to 72 h) to enable live-cell, label-free imaging of multiple 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 scores that report on the local aggressiveness, invasiveness, and general likelihood of adverse pathologies, respectively, are able to distinguish benign from malignant cells, risk stratify tumor or adjacent normal samples, and predict adverse pathology. Comparing our results with unblinded clinical pathology data, we can predict adverse pathology states from both fresh tumor samples and field samples with greater than 85% sensitivity and specificity.

**Conclusions:** Presented results support further validation of this novel assay to 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..

## High-Content, Ex Vivo Platform

First-in-class platform components include a proprietary extra-cellular matrix formulation, microfluidic device, live-cell phenotypic biomarkers, high-content machine vision analysis, and machine learning algorithms to generate clinically relevant predictive scores by interrogating patient biopsy cell behavior with single cell resolution over time *in vitro*.

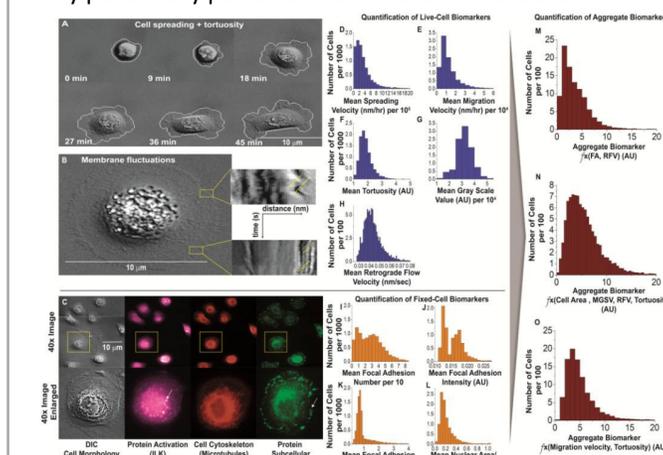


Prostate	PREDICTIVE METRICS		
	GAPP: General Adverse Pathology Potential	LAPP: Local Adverse Pathology Potential	MAPP: Metastatic Adverse Pathology Potential
	- Any of the six adverse pathologies (SVI, PSM, EPE, PNI, LNP, LVI)	- Seminal Vesicle Invasion (SVI) - Positive Surgical Margins (PSM) - Extra Prostatic Extension (EPE)	- Perineural Invasion (PNI) - Lymph Node Positive (LNP) - Lymph-Vascular Invasion (LVI)

The ability of the STRAT-AP platform to predict adverse pathology in prostate cancer is an important advance to traditional fixed-formalin-paraffin embedded tissue scoring systems / recently introduced genomic tests and improves tumor risk assessment and treatment decisions without interrupting the current pathology diagnostic workflow.

## Live-Cell Phenotypic Biomarkers

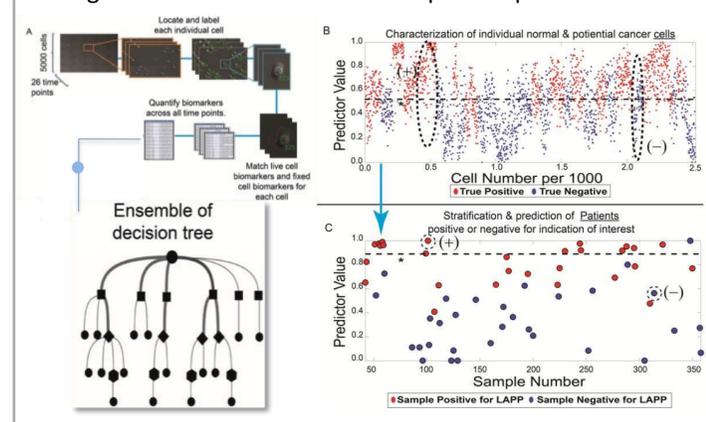
Phenotypic biomarkers have re-emerged to ameliorate the challenges of cancer diagnosis and risk stratification due to the inherent genetic heterogeneity in prostate cancer. Prior attempts to analyze dynamic biomarkers from single cells derived from primary biopsy tissue have been limited, leaving many potentially powerful biomarkers inaccessible.



The direct evaluation of dynamic phenotypic behavior of living single tumor cells grown in a controlled microenvironment could provide deeper insights into multiple and coordinated signaling pathways, and present an improved risk-stratification test and drug development tool.

## Machine Learning Algorithms

Machine vision and statistical analysis algorithms have the ability to process multiple biomarkers and accurately predict various pathological outcomes. Live cell images are collected on approximately 5000 cells for each of 26 time-points. Each cell is assigned a unique identifier. Phenotypic biomarkers are measured for each cell at multiple time-points, leading to an average of 42 million measurements per sample.



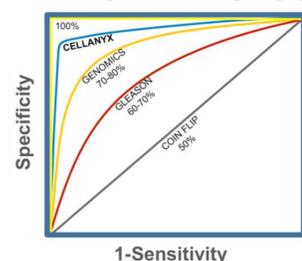
The decision trees are weighted to optimize algorithm accuracy. Single cells are stratified as negative and positive cells utilizing combinations of biomarkers dictated by decision tree analysis. Patient level predictions are obtained by summarizing cell level results.

## Personalized Treatment Guidance

It is estimated that 130,000 prostate cancer patients per year received suboptimal treatment in the U.S. due to inadequate clinical tools. Cellanyx's STRAT-AP platform addresses this unmet need and predicts prostate cancer adverse pathology features important for shared decision making and personalized treatment guidance with > 85% sensitivity and specificity. Cellanyx's live-cell phenotypic approach shows stronger performance than genomic and IHC methodologies.

### ABILITY TO PREDICT POST RADICAL PROSTATECTOMY ADVERSE PATHOLOGIES:

- 86% Sensitivity
- 96% Specificity
- AUC = 0.88
- n=238

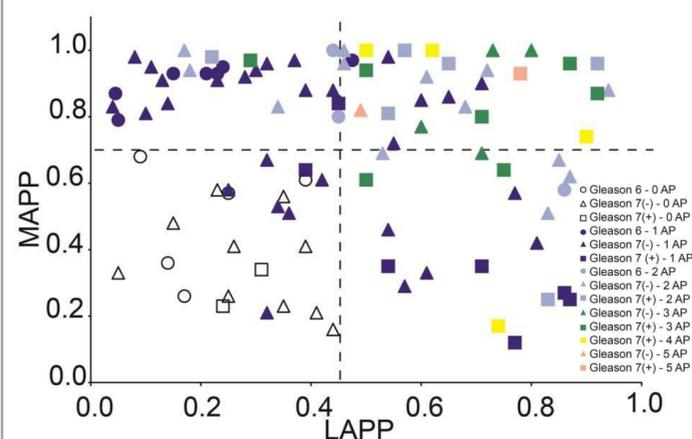


Predicted Adverse Pathology	Number of Samples per Adverse Pathology	Sensitivity	Specificity	AUC	Samples Positive for Adverse Pathology	Samples Negative for Adverse Pathology	True Positive	False Positive	True Negative	False Negative
GAPP	237	0.84	0.88	0.88	195	42	163	5	37	32
LAPP	237	0.80	0.79	0.85	118	119	94	25	94	24
MAPP	237	0.77	0.90	0.89	169	68	130	7	61	39

## Enhanced Risk Stratification

There is a significant need for better stratification tools to distinguish indolent from aggressive disease in men with low or intermediate grade prostate cancer – Gleason 6 or Gleason 7. Cellanyx's clinical scores can risk stratify these patients by predicting the gold-standard of prostate cancer diagnosis – surgical adverse pathology features – and determine which Gleason 6 or 7 patients are indeed low-risk or at risk for locally aggressive and / or metastatic disease.

### POWERFUL RISK STRATIFICATION OF CLASSICALLY DEFINED LOW- & INTERMEDIATE RISK PATIENTS

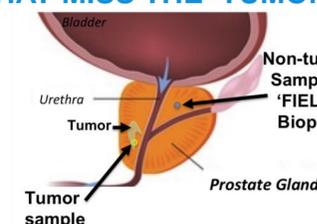


## Prognostics From Missed Biopsies

Roughly 700,000 men undergo repeat biopsies every year in the U.S., with 80% of those men being positively diagnosed upon repeat biopsy. Cellanyx's STRAT-AP can eliminate the need for repeat biopsies given its ability to predict surgical adverse pathology features from tumor-adjacent tissue found in biopsies that miss the tumor. This capability represents a significant advance in prostate cancer disease management via the reduction of unnecessary diagnostic and treatment procedures.

### ABILITY TO PREDICT ADVERSE PATHOLOGY FROM BIOPSIES THAT MISS THE TUMOR:

- 88% Sensitivity
- 93% Specificity
- AUC = 0.89
- n = 87



Predicted Adverse Pathology	Number of Samples per Adverse Pathology	Field Effect Algorithm Performance Statistics			+ for Adverse Pathology	(-) for Adverse Pathology	True Positive	False Positive	True Negative	False Negative
		Sensitivity	Specificity	AUC						
LAPP	89	0.84	0.86	0.90	45	44	38	6	38	7
MAPP	89	0.84	0.91	0.87	68	21	57	2	19	11
GAPP	87	0.88	0.93	0.89	73	14	64	1	13	9

## Conclusions & Prospective

1. The ability to rapidly culture primary human biopsy cells enables a powerful new class of live-cell phenotypic biomarkers to advance precision medicine tests and tools.
2. Live-cell phenotypic biomarkers provide dynamic spatiotemporal cell behavior information to discern the inherent heterogeneity of cancers such as prostate cancer with single cell resolution.
3. Leveraging automated and objective machine vision and machine learning, the live-cell phenotypic STRAT-AP platform can predict with strong accuracy (\*- AUCs > 0.85) adverse pathology features towards personalized treatment guidance.
4. STRAT-AP can risk stratify low- and intermediate-risk patients based on adverse pathology features.\*
5. STRAT-AP can predict adverse pathology and risk stratify patients even if biopsy misses the tumor.\*
6. Cellanyx's STRAT-AP platform is a next generation patient risk stratification test and drug development tool for precision medicine.