Novel live tumor cell diagnostic test using biophysical and molecular biomarkers for assessment of tumor burden and metastatic potential in prostate cancer.

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Abstract:

Background: 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, automated live cell assay, and objective machine vision algorithms to measure *phenotypic biomarkers* [defined here as functional biophysical and molecular biomarkers], which evaluate both local growth and metastatic potential of prostate cancer.

Methods: 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 *ex vivo* on extra cellular matrix formulations supporting adhesion/survival for 72 hours 2) high-throughput imaging of multiple *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 *phenotypic biomarkers* to yield specific metrics that quantified local tumor growth (Oncogenic Potential-OPs) and invasive potential of the tumor to other tissues (Metastatic Potential- MPs) that correlated with RP specimen pathologic findings.

Results: Analysis of quantified *phenotypic biomarkers* distinguished normal cells from cancer cells. The OP and MP metrics demonstrated statistical significance in distinguishing Gleason 6 (low-risk) from Gleason 7 (intermediate-risk) prostate cancer with 80% sensitivity and 80% specificity and concordance with relevant RP pathology findings.

Conclusions: Specifically, OP and MP derived from defined *phenotypic biomarker* metrics, demonstrated the ability to differentiate Gleason 6 and 7 scores and correlated 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 stratifies patients to improve prostate cancer treatment, clinical decision-making, further risk stratification of intermediate prostate cancer populations, and potentially predict actionable pathological findings leading to improved treatment outcomes for prostate cancer patients.

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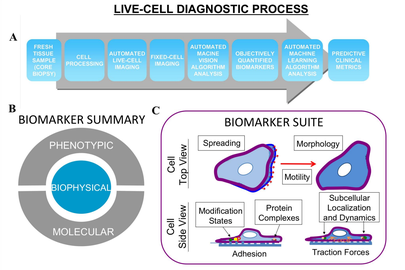
INTRODUCTION:

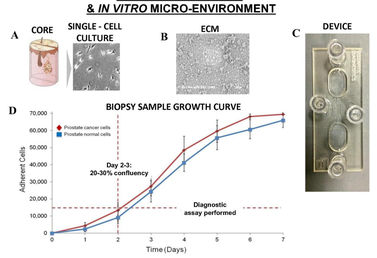
- Prostate specific antigen (PSA) is a non-specific biomarker for prostate cancer (PCa).
- Widespread use of PSA screening has led to significant over diagnosis and over-treatment of non-aggressive/indolent PCa (Gleason 6 and Gleason 7 (3+4).
- The lack of reliable risk-stratification biomarkers has resulted in approximately 80% of low risk patients receiving unnecessarily aggressive treatment.
- There is a clear need for quantifiable and actionable risk-stratification biomarkers for PCa.

LIVE-CELL DIAGNOSTIC PROCESS



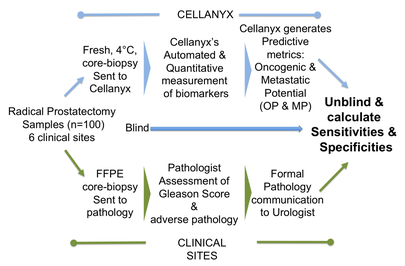
Novel diagnostic platform measures phenotypic, biophysical, and molecular biomarkers on live cells harvested from patient tumor samples. A) Flow diagram outlining the diagnostic process of fresh sample procurement, sample processing, biomarker measurement, algorithmic analysis and generation of predictive measurements. B) Phenotypic, biophysical, and molecular biomarkers are measured on live and subsequently fixed samples. C) Diagram of example biomarkers measured with single cell resolution.

SAMPLE PROCESSING & *IN VITRO* MICRO-ENVIRONMENT



Live cells are harvested from fresh radical prostatectomy samples. A) Biopsy/surgical samples are collected and processed into single cell cultures. B) Extracellular matrix (ECM) formulations are used to produce a permissive environment for cell survival. C) Microfluidic device, used in conjunction with ECM to promote cell survival, automates and standardizes biomarker measurement. D) Growth curve of cells derived from patient sample shows cells are available for analysis on day 2.

BLINDED, MULTI-CENTERED CLINICAL STUDY DESIGN

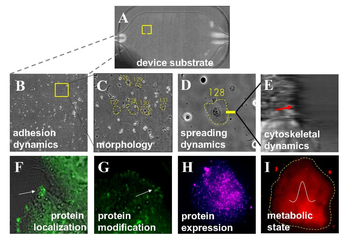


The goal of this blinded study was to demonstrate proof of principle and complete analytical validation of a platform diagnostic in prostate cancer.

To accomplish this goal, Cellanyx:

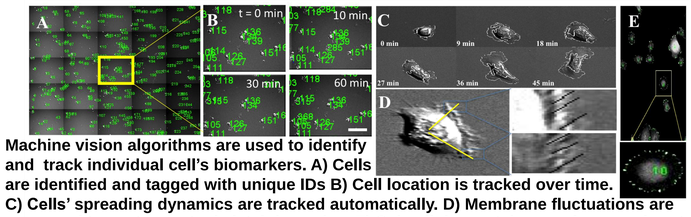
1. Collaborated with six clinical sites from across the United States
2. Recruited prostate cancer patients scheduled for radical prostatectomy
3. Obtained patient informed consent and institutional review board approval
4. Procured samples (n = 104) from excised radical prostatectomy specimens
5. Received fresh/live samples shipped overnight at 4°C
6. Cultured the live prostate cells on a microfluidic device for up to 48 hrs
7. Analyzed the population of cells' biomarkers within 72 hrs of sample collection
8. Computed predictive metrics characterizing each patient's sample
9. Unblinded data and calculated sensitivity and specificity of diagnostic's predictive power

AUTOMATED BIOMARKER IMAGING



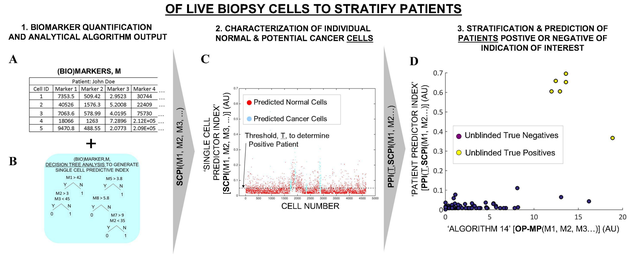
Phenotypic, biophysical, and molecular biomarkers are measured in a standardized microfluidic environment. A) Cell growth chamber coated with ECM. Biomarkers measured include B) cell adhesion rate to device substrate, C) cellular morphology, D) rate of cell spreading on substrate, E) rapid dynamics of the membrane surface, F,G,H) expression, localization, and phosphorylation state of subcellular protein complexes and individual proteins, I) and metabolic activity. 20x DIC and 40x fluorescence images were measured via a standard automated fluorescent microscope.

AUTOMATED SINGLE CELL IDENTIFICATION, TRACKING, & BIOMARKER QUANTIFICATION



Machine vision algorithms are used to identify and track individual cell's biomarkers. A) Cells are identified and tagged with unique IDs B) Cell location is tracked over time. C) Cells' spreading dynamics are tracked automatically. D) Membrane fluctuations are measured to capture cytoskeletal dynamics. E) Subcellular protein complexes and protein activation states are automatically identified and measured on fixed, fluorescently stained cells.

MACHINE LEARNING ALGORITHMIC ANALYSIS OF LIVE BIOPSY CELLS TO STRATIFY PATIENTS



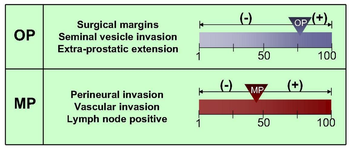
Cellanyx's Machine Learning algorithm has the ability to process multiple biomarkers and accurately predict various pathological outcomes. (A) A set of biomarkers measured for each cell in a patient are input to (B) Cellanyx's machine learning algorithm that generates multiple decision trees stratifying cells of a negative patient from cells of a positive patient for a given pathological outcome. The decision trees are weighted to optimize algorithm accuracy. (C) A representative plot demonstrating stratification among negative and positive cells utilizing combinations of biomarkers as described by the decision trees. Patient level results are obtained by summarizing cell level results into (D) A (representative) plot demonstrating stratification of patients for a given predicted pathology finding.

Conclusion

- Proprietary* phenotypic, molecular and biophysical biomarker panel in living cells obtained from fresh tumor tissue is strongly predictive of Gleason grade in radical prostatectomy (RP) specimens.
- Proprietary* predictive metrics, Oncogenic Potential (OP) and Metastatic Potential (MP), differentiate prostate cancer patients with low and intermediate grade disease and tumor behavior.
- Proprietary* biomarkers were predictive of adverse pathologic findings in RP specimens. OP was predictive of tumor burden and MP of metastatic potential.
- This novel quantitative and actionable phenotypic biomarker panel has potential utility in risk stratification in men with Gleason 6 and Gleason 7 (3+4, 4+3) prostate cancer.
- This initial proof of concept study in prostate cancer strongly supports future risk stratification validation studies in prostate cancer as well as other tumors (genito-urinary and other).
- Biomarker platform is currently being applied to bladder, kidney, and lung tumors.

CLINICAL RESULTS

OP & MP Utility



DIAGNOSTIC TEST PREDICTION STATISTICS

Pathology Finding (n= 104)	OP	MP	Total Positive	True Positive	False Negative	Total Negative	True Negative	False Positive
Gleason 6 vs. Gleason 7 (n=89)	>82		73	64	9	16	13	3
Gleason 3 + 4 vs. Gleason 4 + 3 (n = 73)		>32	27	23	4	46	37	9
Gleason 6 vs. Gleason ≥8 (n = 29)	>51		13	12	1	16	16	0
Seminal vesicle invasion (+) (n = 95)	>51		10	8	2	85	72	13
Extra-prostatic extension (+) (n = 95)	>51		33	27	6	62	51	11
Vascular invasion (+) (n = 92)		>73	8	7	1	84	73	11
Lymph node (+) (n = 78)		>41	7	7	0	71	61	10

DIAGNOSTIC TEST PERFORMANCE STATISTICS

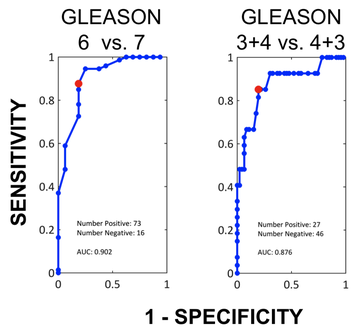
Predicted Pathology (red) (n= 104)	Sensitivity	Specificity	AUC
Gleason 6 vs. Gleason 7 (n=89)	0.88	0.81	0.90
Gleason 3 + 4 vs. Gleason 4 + 3 (n = 73)	0.85	0.80	0.88
Gleason 6 vs. Gleason ≥8 (n = 29)	0.92	1.00	0.97
Seminal vesicle invasion (+) (n = 95)	0.80	0.85	0.91
Extra-prostatic extension (+) (n = 95)	0.82	0.82	0.85
Vascular invasion (+) (n = 92)	0.88	0.87	0.91
Lymph node (+) (n = 78)	1.00	0.86	0.95

Clinical Highlights

1. Sensitivity and specificity numbers describe the capability of proprietary* prostate cancer diagnostic test to predict pathologic (Gleason and other) findings.
2. The Oncogenic Potential (OP) describes the extension of tumor in the prostate capsule and seminal vesicles, and the Metastatic Potential (MP) describes invasion into peripheral systems such as blood, lymph and/or bone. The OP & MP calculation is made with a proprietary* algorithm.
3. OP and MP values in the adjacent table represent predictive thresholds of disease status.
4. Gleason 6 vs. Gleason 7 denotes predicting Gleason 7 patients from a set of Gleason 6 & Gleason 7 patients.
5. Gleason 3+4 vs. 4+3 denotes predicting Gleason 4+3 patients from the set of all Gleason 7 patients.

$$\text{sensitivity} = \frac{\text{true positives}}{\text{(true positives + false negatives)}}$$

$$\text{specificity} = \frac{\text{true negatives}}{\text{(true negatives + false positives)}}$$



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