Clinical Validation of a live-cell phenotypic biomarker - based diagnostic assay for the prediction of adverse pathology in Prostate Cancer

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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.

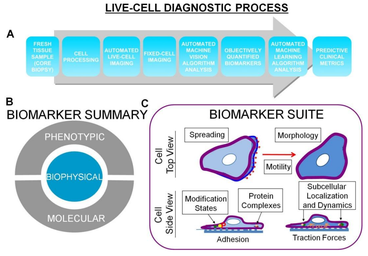
Results: The developed predictive dynamic biomarker metrics of adverse pathology: “Local Adverse Pathology Potential (LAPP)” & “Metastatic Adverse Pathology Potential (MAPP),” report on the local aggressiveness and invasiveness, respectively, are able to distinguish benign from malignant cells, risk 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.

Conclusion: 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.

INTRODUCTION:

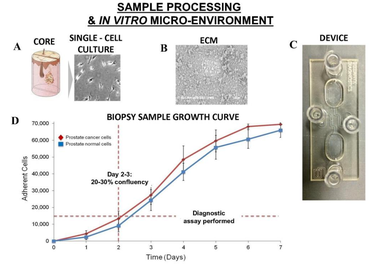
- Solid tumor cancers such as prostate, kidney, and bladder cancers are generally diagnosed through a combination of blood tests and macroscopic imaging including x-ray, computer tomography (CT), magnetic resonance imaging (MRI), and ultrasound. These tools do not provide a clear cellular diagnostic or prognostic analysis of the disease.
- Only when biopsy samples are imaged, is a microscopic cellular analysis possible. However, the current state-of-the-art does not consider the underlying biology of live biopsy cells, leaving an unclear diagnostic analysis.
- Due to the lack of clear diagnostic tools, cancers of the prostate, kidney, and bladder are over diagnosed and over treated (1-3).
- There is an urgent need for quantifiable & actionable risk-stratification biomarkers for prostate, kidney, and bladder cancer.

LIVE-CELL DIAGNOSTIC PROCESS

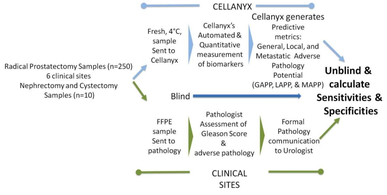


Novel diagnostic platform measures phenotypic (biophysical and molecular) biomarkers on live cells harvested from patient tumor samples. A) Flow diagram outlining the diagnostic process of fresh sample procurement, sample processing, biomarker measurement, algorithmic analysis and generation of predictive measurements. B) 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 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.

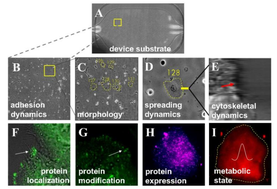


The goal of this blinded study was to demonstrate proof of principle and complete analytical validation of a diagnostic platform in prostate cancer. Further, an exploratory study was performed in kidney and bladder samples to test the platform as a diagnostic in these indications.

To accomplish these goals, Cellanyx:

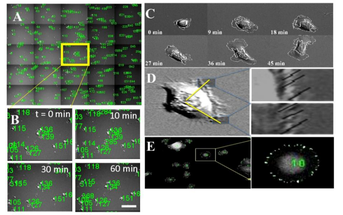
1. Collaborated with six clinical sites across the United States.
2. Obtained patient informed consent and institutional review board approval.
3. Procured samples from excised radical prostatectomy (n=250), nephrectomy (n=10) and cystectomy (n=10) specimens.
4. Received fresh/live samples shipped overnight at 4°C
5. Cultured the live tissue-derived cells on a microfluidic device.
6. Analyzed the cells for biomarkers within 72 h of sample collection.
7. Computed predictive metrics characterizing each patient's sample.
8. Unblinded the data and calculated sensitivity and specificity of diagnostic's predictive power.

AUTOMATED BIOMARKER IMAGING



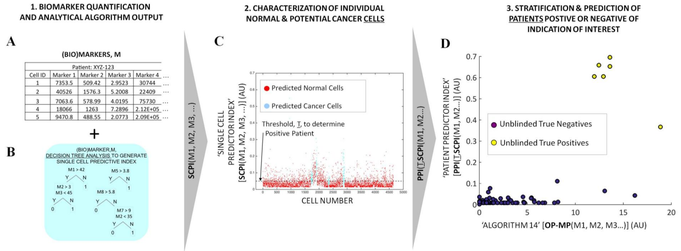
Phenotypic, biophysical, and molecular biomarkers are measured in a standardized microfluidic environment. A) Cell growth chamber coated with ECM. Biomarkers measured include B) cell adhesion rate to device substrate, C) cellular morphology, D) rate of cell spreading on substrate, E) rapid dynamics of 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



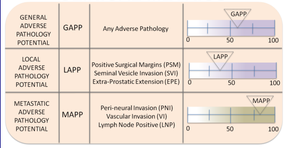
Machine vision algorithms are used to identify and track individual cell's biomarkers. A) Cells are identified and tagged with unique IDs B) Cell location is tracked over time. C) Cells' spreading dynamics are tracked automatically. D) Membrane fluctuations are measured to capture cytoskeletal dynamics. 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.

CELLANYX'S CLINICAL METRICS



PREDICTING PATIENTS WITH ANY ADVERSE PATHOLOGY AFTER RADICAL PROSTATECTOMY

Clinical Highlights

1. Sensitivity and specificity numbers describe the capability of proprietary* prostate cancer diagnostic test to predict adverse pathologic findings.
2. The Local Adverse Pathology Potential (LAPP) describes the extension of tumor in the prostate capsule and seminal vesicles, and the Metastatic Adverse Pathology Potential (MAPP) describes invasion into peripheral systems such as blood, lymph and/or bone. The LAPP & MAPP calculation is made with a proprietary* algorithm.
3. GAPP, LAPP, and MAPP values in the adjacent table represent predictive thresholds of disease status.

Note that not every sample tested had the same pathology data.

CLINICAL RESULTS

PREDICTIVE PERFORMANCE FROM PROSTATE TISSUE SAMPLES

Metric	Sensitivity	Specificity	AUC	N=
Gleason Score	0.89286	0.82609	0.87647	235
Lymphnode Positive	0.91667	0.97175	0.94492	189
Seminal Vesicle Invasion	0.89655	0.78894	0.86796	228
Positive Surgical Margin	0.81159	0.83234	0.86518	236
Extra Prostatic Extension	0.83333	0.8626	0.88961	221
Lymph-vascular Invasion	1	0.79703	0.9024	223
Perineural Invasion	0.87117	0.89744	0.9414	202
BCR	1	1	1	61
ANY2	0.8359	0.88095	0.87729	237
LAPP	0.79661	0.78992	0.84845	237
MAPP	0.76923	0.89706	0.88649	237

PROOF OF PRINCIPLE: IN KIDNEY CANCER

Kidney							
Metric	Sensitivity	Specificity	AUC	Threshold	N	Num Positive	Num Negative
Grade	1	1	1	0.97772	12	4	8
Tumorsize	1	1	1	0.82828	12	3	9
Lymph small vessel invasion	1	1	1	0.94949	12	1	11
ANY2	1	1	1	0.93018	12	5	7
ANY LAPP	1	1	1	0.95455	12	4	8
ANY MAPP	1	1	1	0.94949	12	1	11

PROOF OF PRINCIPLE: IN BLADDER CANCER

Bladder							
Metric	Sensitivity	Specificity	AUC	Threshold	N	Num Positive	Num Negative
Grade	1	1	1	0.67113	10	4	6
Lympho-vascular invasion	1	1	1	0.51324	10	3	7
Lymph node positive	1	1	1	0.67407	10	4	6
Margins	1	1	1	0.96392	10	1	9
ANY2	1	1	1	0.75135	10	4	6
ANY LAPP	1	1	1	0.75335	10	4	6
ANY MAPP	1	1	1	0.76697	10	4	6

Conclusion

- Proprietary* phenotypic (biophysical and molecular) biomarker panel in living cells obtained from fresh tumor tissue is strongly predictive of adverse pathology after radical prostatectomy (RP) specimens.
- Proprietary* predictive metrics, LAPP and MAPP, differentiate prostate cancer patients with low and intermediate grade disease based on tumor behavior.
- Proprietary* biomarkers were predictive of adverse pathologic findings in RP specimens. LAPP was predictive of tumor burden and MAPP of metastatic potential.
- This novel quantitative and actionable phenotypic biomarker panel has potential utility in risk stratification and predicts adverse pathology from biopsies that do not contain tumor..
- This extended proof of concept clinical 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 applicable to predicting adverse pathologies in bladder and kidney samples.

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