A NOVEL LIVE CELL MICROFLUIDIC DIAGNOSTIC USING PHENOTYPIC BIOMARKERS WITH OBJECTIVE ALGORITHMIC ANALYSIS FOR PROSTATE, KIDNEY, AND BLADDER CANCER RISK STRATIFICATION.

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Poster # 156

Introduction and Objectives: Prostate, kidney and bladder cancer diagnostics currently lack sufficient biomarkers to effectively assess disease aggressiveness and metastatic potential. Utilizing a new biomarker diagnostic platform incorporating functional molecular and cellular information may allow a better understanding of local growth and metastatic potential of the cancer. A novel and proprietary diagnostic test was developed based on advancements in four areas – matrix biology and cell culturing, molecular & cellular biomarkers, microfluidics, and machine vision. We evaluate our ability to culture patient cell samples, assess and automate biomarker measurement from live and fixed cells, and objectively analyze the cancer's disease stage and potential for progression through a proprietary machine vision−based algorithmic analysis.

Methods: Conditions were optimized for reliably culturing primary cancer cells in vitro by simulating in vivo conditions on a specialized and proprietary extra−cellular matrix (ECM) formulation. We developed a novel and proprietary microfluidic device that was used to culture live tumor biopsy samples ex vivo, thus enabling automated imaging of label−free and label−based, molecular and biophysical biomarkers.

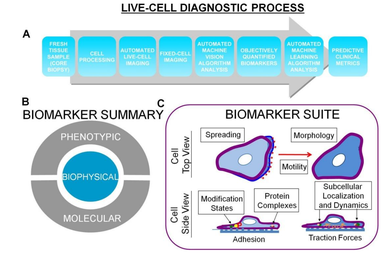
Results: Following the results of a study in prostate cancer (n=178) that showed AUCs > 0.85 in predicting adverse pathologies, an exploratory study in kidney and bladder cancer demonstrated a novel set of phenotypic (molecular and biophysical) biomarkers produce secondary biomarker metrics termed General Adverse Pathology Potential (GAPP), Local Adverse Pathology Potential (LAPP), & Metastatic Adverse Pathology Potential (MAPP) using unbiased machine learning algorithms. Concordance analysis supports that GAPP, LAPP, & MAPP, derived from primary biomarkers, are useful for distinguishing between normal and malignant cells, predicting stage, and predicting adverse pathology states such as lympho−vascular invasion in kidney (n=20) and bladder (n=10) cancer samples. Importantly the results of the exploratory study yield specificities and sensitivities greater than 90% when predicting stage and lympho−vascular invasion.

Conclusion: These results constitute the first step towards clinical validation of a novel diagnostic test and demonstrate the capability of a live−cell in vitro tumor biopsy diagnostic test to be useful in predicting adverse pathologies for kidney and bladder cancer samples. Ultimately this diagnostic platform will be used to better stage & risk−stratify cancer patients towards optimized treatment.

INTRODUCTION:

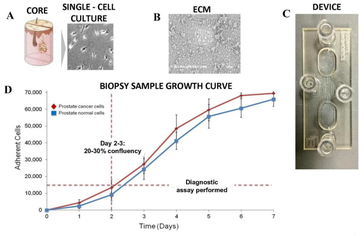
- Solid tumor cancers such as prostate, kidney, and bladder cancers are generally diagnosed through a combination of blood tests and macroscopic imaging including x-ray, computer tomography (CT), magnetic resonance imaging (MRI), and ultrasound. These tools do not provide a clear cellular diagnostic or prognostic analysis of the disease.
- Only when biopsy samples are imaged, 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

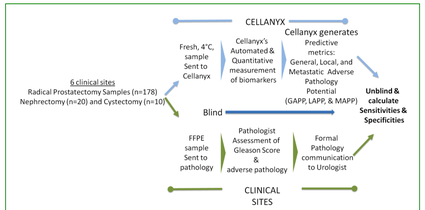


Novel diagnostic platform measures phenotypic (biophysical and molecular) biomarkers on live cells harvested from patient tumor samples. A) Flow diagram outlining the diagnostic process of fresh sample procurement, sample processing, biomarker measurement, algorithmic analysis and generation of predictive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

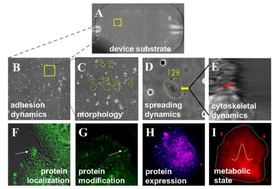


The goal of this blinded study was to demonstrate proof of principle and complete analytical validation of a diagnostic platform in prostate cancer. Further, an exploratory study was performed in kidney and bladder samples to test the platform as a diagnostic in these indications.

To accomplish these goals, Cellanyx:

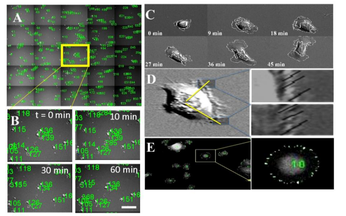
1. Collaborated with six clinical sites across the United States.
2. Obtained patient informed consent and institutional review board approval.
3. Procured samples from excised radical prostatectomy (n=178), nephrectomy (n=20) and cystectomy (n=10) specimens.
4. Received fresh/live samples shipped overnight 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



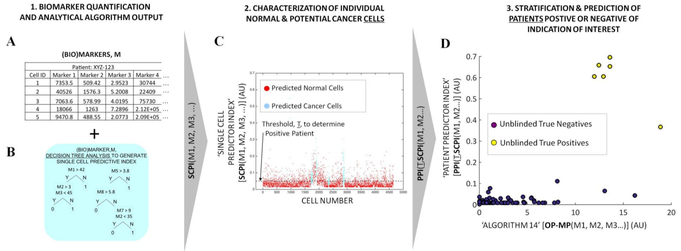
Phenotypic, biophysical, and molecular biomarkers are measured in a standardized microfluidic environment. A) Cell growth chamber coated with ECM. Biomarkers measured include B) cell adhesion rate to device substrate, C) cellular morphology, D) rate of cell spreading on substrate, E) rapid dynamics of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



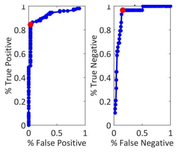
Machine vision algorithms are used to identify and track individual cell's biomarkers. A) Cells are identified and tagged with unique IDs B) Cell location is tracked over time. C) Cells' spreading dynamics are tracked automatically. D) Membrane fluctuations are measured to capture cytoskeletal dynamics.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

CLINICAL RESULTS

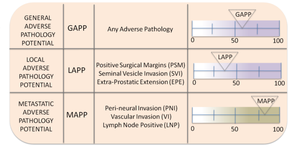


Number Positive: 148
Number Negative: 29

AUC: 0.923

GAPP = 33

Sensitivity: 0.84
Specificity: 0.97



Predicted Adverse Pathology	Number of patients (of 49)	LAPP	MAPP	Sensitivity	Specificity	AUC
ANY	44	77 (GAPP)	-	0.98	1.00	0.99
ANY LAP	29	84		0.93	0.90	0.93
ANY MAP	28	-	85	.93	1.00	0.97
PSM	20	80	-	0.90	0.93	0.93
SVI	3	53	-	1.0	1.0	1.0
EPE	21	49	-	0.95	0.86	0.92
PNI	40	-	79	1.0	1.0	1.0
VI	7	-	95	1.0	0.98	0.99
LNI	2	-	98	1.0	0.92	0.98

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 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.
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.

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

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

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

Kidney

Metric	Sensitivity	Specificity	AUC	Threshold	N	Num Positive	Num Negative	TP	FP	TN	FN
grade	1	1	1	0.98186	18	15	3	15	0	3	0
lymphsmallvesselinvasion	1	1	1	0.9899	17	1	16	1	0	16	0
GAPP	1	1	1	0.96186	18	15	3	15	0	3	0
LAPP	1	1	1	0.91239	18	15	3	15	0	3	0
MAPP	1	1	1	0.96707	18	1	17	1	0	17	0

Bladder

Metric	Sensitivity	Specificity	AUC	Threshold	N	Num (+)	Num (-)	TP	FP	TN	FN
squamous differentiation	1	1	1	0.92929	9	1	8	1	0	8	0
glandular differentiation	1	1	1	0.33333	9	1	8	1	0	8	0
grade	1	1	1	0.80303	10	7	3	7	0	3	0
lymphovascular invasion	1	1	1	0.86869	9	5	4	5	0	4	0
lymph node positive	1	1	1	0.73737	10	5	5	5	0	5	0
margins	1	1	1	0.83838	10	1	9	1	0	9	0
GAPP	1	1	1	0.74074	10	8	2	8	0	2	0
LAPP	1	1	1	0.86869	10	7	3	7	0	3	0
MAPP	1	1	1	0.66667	10	6	4	6	0	4	0

Conclusion

- Proprietary* phenotypic (biophysical and molecular) biomarker panel in living cells obtained from fresh tumor tissue is strongly predictive of Gleason grade in radical prostatectomy (RP) specimens.
- Proprietary* predictive metrics, GAPP, 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 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 applicable to predicting adverse pathologies in bladder and kidney samples.

References:

1. Moyer, V.A. (2012) Preventive Services Task Force. Screening for prostate caner: U.S.Preventive Services Task Force recommendation statement. *Ann Intern Med*, 157: 120-134.

2. Carter, H.B., Albertsen, P.C., Barry, M.J., Etzioni, R., Freeland, S.J., Greene, K.L., Holmberg, L., Kantoff, P., Konety, B.R., Murad, M.H., Penson, D.F., and Zietman, A.L. (2013) Early Detection of Prostate Cancer: AUA Guideline. *American Urological Association (AUA) Guideline*. 1-28.

3. Amin, M.B., Lin, D.W., Gore, J.L., Srigley, J.R., Samaratunga, H., Egevad, L., Rubin, M., Nacey, J., Carter, B., Klotz, L., Snadler, H., Zietman, A.L., Holden, S., Montironi, R., Humphery, P.A., Evens, A.J., Epstein, J.I., Delahunt, B., McKenney, J.K., Berney, D., Wheeler, T.M., Chinnaiyan, A.M., True, L., Knudsen, B.,Hammond, E.H. (2014) Active Surveillance in Prostate Cancer Patients. *Arch Pathol Lab Med* 138: 1387-1405.

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