Studies in metastases: Or how chickens help fight prostate cancer.

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Disclosures

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Disclosure:

Financial Co-founder and employee of Nanostics Inc.

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Out of every 100 men...

16 will be diagnosed with prostate cancer in their lifetime.

In reality, up to 80 will have prostate cancer by age 70.

And 3 will die from it.

But which 3?
The deadliest aspect of prostate cancer is its spread, or metastasis.

In North America, the average 5 year survival rate for localized prostate cancer is 100%.

For metastatic cancer, it is less than 30%.

Current diagnosis tools do not predict whether metastasis will occur.

Current treatments do not prevent or cure metastasis.
Metastasis is a complex, multi-step process!
Snapshots provide limited information...
Let’s all be scientists for a moment…

1. Good Samaritan helps fallen woman during riot
2. Opportunistic rioter steals a kiss from an injured woman
3. Riot police defend couple’s right to Public Display of Affection (PDA)
4. “At least someone from Vancouver can score on the road”
1. Man consoles distraught girlfriend after she was violently knocked down by riot police
Modeling cancer dynamics in chicken embryos

Immediately after injection

24 hours later
Modeling cancer dynamics in ex ovo avian embryos
Intravital imaging of tumour growth and metastasis

4 mm tumour growing over 4 days

Small metastasis of 200-300 cells

Individual cancer cells
When studying metastasis context is crucial to data analysis.
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When studying metastasis context is crucial to data analysis.

3D reconstruction of single cancer cells (green) and their residence near a blood vessel. This type of image allows us to visualize cells moving toward a blood vessel in great detail.
Imaging the CAM in 3D

**TOP**

1. Endoderm
2. Stroma
3. Capillary bed
4. Ectoderm
5. Eggshell/membrane

**BOTTOM**

- **Rhodamine-Lectin** (*Lens culinaris* agglutinin)
- **2MDa FITC-Dextran**

- **Lectin-Rhodamine**
  - CAM plexus
  - Lectin-Rhodamine Junctions
  - Bottom of CAM

Leong et al., *Cell Reports*, 2014
What is the difference between cells that spread and cells that don’t?

Key:
- Epithelial
- Tumor
- Fibroblast
- RBC
- WBC
- Platelet

"Bummer of a birthmark, Hal."
What is the difference between cells that spread and cells that don’t?

**M- cell line**

**M+++ cell line**

**Key**
- Epithelial
- Tumor
- Fibroblast
- RBC
- WBC
- Platelet
Antibody 1A5 targets tetraspanin CD151

Immunize mouse with low metastatic cancer variant

Cyclophosphamide – “tolerizes” mouse immune system

Immunize mouse with high metastatic cancer variant

Isolate antibodies against targets in M+++ but not M-
Anti-CD151 antibody blocks spontaneous metastasis

Zijlstra et al., Cancer Cell, 2008
Dramatic differences in cell motility phenotype \textit{in vivo}
Anti-CD151 antibody inhibits cell migration \textit{in vivo}

Zijlstra et al., Cancer Cell, 2008

Control (IgG)

Average:
(velocity: 24.6 µm/hr)
(total distance: 271 µm)
(productive distance: 101 µm)

Anti-CD151 (1A5)

Average:
(velocity: 5.9 µm/hr)
(total distance: 85 µm)
(productive distance: 23 µm)

Productive Migration
6.6 fold inhibition

control

mAb 1A5 treated

Zijlstra et al., Cancer Cell, 2008
1A5 antibody binds CD151 that is “free” from integrins

If CD151\textsuperscript{free} marks cancer cells that have undergone a cell motility switch, perhaps we can use it as a test to detect or predict metastasis…

Palmer et al., Cancer Research, 2014
138 prostate cancer surgery patients

Follow up: 12.1 years

Recurrence: 34 cases
Metastasis: 38 cases

1. Does CD151\textsuperscript{free} predict recurrence after surgery?
2. Does CD151\textsuperscript{free} predict metastasis?

CD151\textsuperscript{free} is distinct from CD151\textsuperscript{all} in prostate cancer

Palmer et al., Cancer Research, 2014
CD151\textsuperscript{free} predicts prostate cancer recurrence and metastasis

Palmer et al., Cancer Research 2014
Summary: Tetraspanin CD151\text{free}

- Tetraspanin CD151 and $\alpha3$ integrin interactions comprise a cell migration “switch” between maintenance of epithelial structure and invasive cell migration.

- Cross-linking CD151\text{free} with 1A5 antibody blocks metastasis by stabilizing cell-cell adhesion.

- mAb 1A5 detects a pool of CD151 (CD151\text{free}) that is distinct from that detected by other antibodies.

- CD151\text{free} is associated with earlier biochemical recurrence and earlier onset of metastasis, independent predictor of outcome.
Intravital imaging – metastasis screening platform

- **Expansion of compact colonies**
- **Deep sequencing to identify shRNAs**
- **Phenotype validation (re-injection)**
- **Compactness Index (C.I.) determination**
  - **Clone prioritization**
- **Metastasis phenotype assessment using in vitro and in vivo assays**

**Summary**

- **Excised clones (67)**
  - **Clones**
  - **Scramble**
  - **Partially compact**
  - **Highly compact**

**Counts**

<table>
<thead>
<tr>
<th>Clones</th>
<th>Genes</th>
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<tr>
<td>67</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>209</td>
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<tr>
<td>27</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
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</table>
Whole genome *in vivo* screen for mediators of metastasis

Identified 27 clones with significant reduction in *in vivo* motility

Identified 11 single shRNAs required for *in vivo* cell motility

Stoletov et al., *Nature Communications, 2018*
Screen hits are required for invasive cell migration *in vivo*

*In vivo* cell migration assays (spontaneous and experimental metastasis)

Stoletov et al., *Nature Communications*, 2018
Inhibition of screen hits blocks metastasis in vivo

Spontaneous metastasis to lung

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative metastatic burden</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Scramble</td>
<td>1.00</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Kif3Bsh</td>
<td>0.40 (n=23)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Srpk1sh</td>
<td>0.22 (n=10)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Nrf2f1sh</td>
<td>0.19 (n=10)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Stoletov et al., Nature Communications, 2018
Screen hits associated with metastatic cancers and poor prognosis

Expression in matched primary tumour/metastasis

**Skin (melanoma)**
- SRPK1
  - Primary site: n=16, Fold change: 8.7E-4, P-value: 0.007, n=40
- KIF3B
  - Primary site: n=16, Fold change: 1.32, P-value: 0.007, n=40

**Prostate**
- SRPK1
  - Primary site: n=10, Fold change: 1.13E-9, P-value: 0.004, n=21
- KIF3B
  - Primary site: n=10, Fold change: 2.75, P-value: 0.004, n=21

**Head and neck**
- KIF3B
  - Primary site: n=22, Fold change: 1.35, P-value: 0.016, n=5

**Lung**
- SRPK1
  - Primary site: n=101, Fold change: 1.32, P-value: 0.042, n=8
- TMEM229b
  - Primary site: n=101, Fold change: 1.78, P-value: 0.016, n=8

**Ovary**
- NR2F1
  - Primary site: n=166, Fold change: 1.41, P-value: 0.006, n=75

**Colon**
- NR2F1
  - Primary site: n=52, Fold change: 1.34, P-value: 0.002, n=28

Staining of 98 patient prostate cancer TMA with SRPK1 and Kif3b
If “cell motility switch” genes are required for prostate cancer spread, perhaps we can incorporate them into a test to detect aggressive prostate cancer.
Screening for prostate cancer causes unnecessary harm

**Screening**
- Symptoms/risk factors (Family doctor)
- PSA Blood test (20M per year)
- DRE
  - Invasive
  - Uncomfortable

Only 15-25% specific for prostate cancer, resulting in many unnecessary biopsies

**Diagnosis**
- Biopsy (1.3M per year)
  - 12 needles = Pain, Discomfort, Infection
  - 1.5% chance of life-threatening sepsis

More than 3/4 of men diagnosed with prostate cancer have indolent, non-aggressive disease
Serious Adverse Events (SAE) from biopsies

- >1M biopsies are done per year in the US\textsuperscript{1,2}

- Incidence of sepsis following transrectal ultrasound guided prostate biopsy ranges from 2-4% in developed countries and can go as high as >9% in developing countries\textsuperscript{3,4}

- Antibiotic resistance and sepsis are on the rise\textsuperscript{1}

Careful patient selection for prostate biopsy is essential to minimize the potential harms

Prostate cancer is a **heterogeneous disease**: some forms are **lethal**, others are not.

Men with Gleason Grade Group 3-5 prostate cancers have significantly worse outcomes.

Epstein et al. 2015, European Urology, 69, 428-435.
Living cancer cells detached from the primary tumor and circulating through bloodstream

Very rare (1 in a billion!)

CTC counting has prognostic value for OS (>4 CTCs/7.5mL)
Extracellular vesicles (EVs) provide much greater dynamic range than CTCs.
Extracellular vesicles (EVs) are released by all cells in the body

- Exomeres (< 50nm)
- Small Exosomes (60-80nm)
- Large Exosomes (80-120nm)
- Microvesicles (50nm-1µm)
- Oncosomes (1-10µm)
- Apoptotic bodies (800nm-10µm)
- As yet undescribed?
**The challenge:** single particle detection of prostate EVs

PSMA receptors on vesicles

- **10’s to 100’s of receptors**

![Graph showing PSMA receptors on vesicles with different diameters and counts.]

EVs x 10^6/mL plasma (mean of 5 patients)

- LNCaP cells
- CTC AR+
- CTC AR−
- EVs x 10^6/mL

How small can we go?
MicroFlow Cytometry can detect and characterize a wide range of EVs

Leong et al., J of Thrombosis and Haemostasis, 2011
MicroFlow Cytometry resolves small biological particles
Detection of **prostate-derived EVs** in complex biofluids

Localized PCa

- CD151+ PSMA+
- PSMA-405

Metastatic PCa

- CD151+ PSMA+
- PSMA-405

66 patient cohort
University Health Network - Toronto

Age, PSA matched
Biofluid: plasma
Microflow cytometry of plasma EVs is highly sensitive and reproducible

Reliable detection of 6 positive EVs (0.0003%) against a highly enriched blood EV background (2.5M)

Excellent %CV for clinical testing at a wide range of biomarker concentrations
Sample stability of EVs in human plasma and serum

Sample stability matrix

<table>
<thead>
<tr>
<th>Biomarker-1</th>
<th>Biomarker-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Ice</td>
<td>Wet Ice</td>
</tr>
<tr>
<td>Dry Ice</td>
<td>Dry Ice</td>
</tr>
<tr>
<td>Ambient</td>
<td>Ambient</td>
</tr>
</tbody>
</table>

Total Particles
PSMA
Biomarker 2
Biomarker 3
Biomarker 4
Biomarker 5
Combining size and surface biomarkers: disease prediction?

PSMA+

Ghrelin (Lu et al, Prostate 2012)

Metastasis-associated biomarkers

P = 0.5337
P = 0.0054
P = 0.0144
P < 0.0001

Analyzed using one-tailed t-test with Welch’s corrections
Machine learning approach to generate classifiers from multi-dimensional microflow cytometry data

3D plot of ROC area under the curve
XGBoost provides highest AUCs for predicting clinically significant prostate cancer

Ensembled: Results of multiple decisions trees averaged into 1 result

Boosted: Each additional decision tree is designed to correct misclassified observations

XGBoost is an ensembled, boosted, decision tree-based model.
Nanostics’ platform technology generates EV fingerprints to predict disease with a liquid biopsy.
Generating the **ClarityDX Prostate** Risk Score

**Clinical trial Training Phase data**
- EV Concentration
- EV Size
- EV Biomarker +/-

**Microflow cytometry data**

**XGBoost**
- Powerful decision tree-based algorithm
- Creates non-linear models
- State-of-the-art for high accuracy predictive models

**Flow score**

**Clinical data**

**XGBoost**

**Clinical score**

**Logistic Regression**

**Risk score**
(Probability of clinically significant prostate cancer)

**Clinical trial Training Phase data + APCaRI data of >4,000 men**
- PSA
- Age
- Digital rectal exam
- Previous negative biopsy
- Ethnicity
- Family history of PCa
Prospective pre-diagnosis cohort in Alberta, Canada

Male patient with abnormal PSA and/or DRE referred for prostate biopsy

- Identified by urologist - refer to CRC at clinical site
- Informed Consent
- Intake Survey/QOL
- Biospecimens are collected
- Demographic and clinical data

Biopsy is performed

PCa is detected

**Samples Collected:**
Once a year/5 years
At time of changes of cancer behaviour

**Data Collected:**
- QoL from patients once a year/5 years
- Database: 10-25 years

PCa is not detected

- Usual care by family doctor
- Follow up and PSA tracking

Patient is re-referred for biopsy

PCa is detected

PCa is not detected
Validation of ClarityDX Prostate in a 377 patient prospective cohort

Average AUC = 0.83

39% higher specificity for clinically significant prostate cancer than PSA alone
**Proposed prostate cancer diagnosis model**

1. **Patient at risk 40-75 yrs**
2. **PSA**
   - **PSA >3 & ≥10 ng/ml**
   - **PSA <3ng/ml**
3. **Repeat PSA at 1-4y intervals**
4. **ClarityDX prostate**
5. **High Risk**
   - **TRUS Guided Biopsy**
6. **Low Risk**
   - **Continue Monitoring**
**Pivotal Clinical Validation Study**

**Participants enrolled**
- SST Serum
- Ambient

**Annual ~ #s**
- NAUC: 500
- PCC: 500
- YUK: 100
- US: 250

Interim analysis in 6m ~ 700 pts

**Shipping**
- NAUC: DynaLIFE
- PCC: Overnight Express door to door service
- YUK & US: TBD

**Processing**
- Within 48h
- Flow Score

**Quality & Regulatory Framework**
- FDA CFR 820.21
- HC SOR/98-282 CE Mark 98/79/EC Regulations
- ISO 13485:2016
- Standard / framework to address regulatory requirements
Acknowledgements