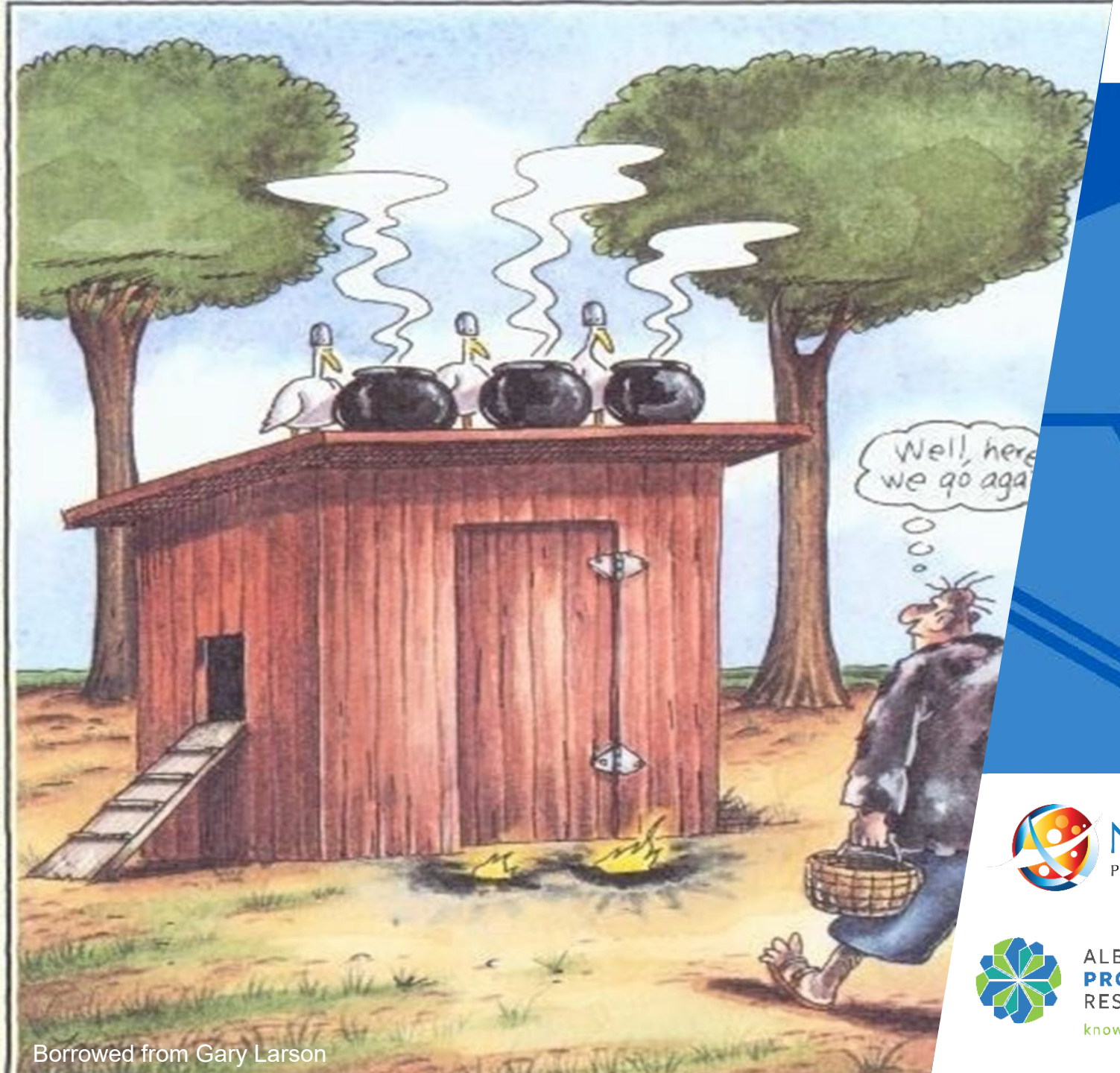

Borrowed from Gary Larson

Studies in metastases:

Or how chickens help fight prostate cancer.

Desmond Pink, Ph.D.
Chief Science Officer
Nanostics Inc

NANOSTICS PRECISION HEALTH

Bird Dogs For Prostate Cancer Research
Pointing to the cure

UNIVERSITY OF ALBERTA

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RIDE FOR DAD

DynaLIFE MEDICAL LABS

Disclosures

Dr. Desmond Pink Ph.D.

Disclosure:

Financial Co-founder and employee of Nanostics Inc.

NANOSTICS
PRECISION HEALTH

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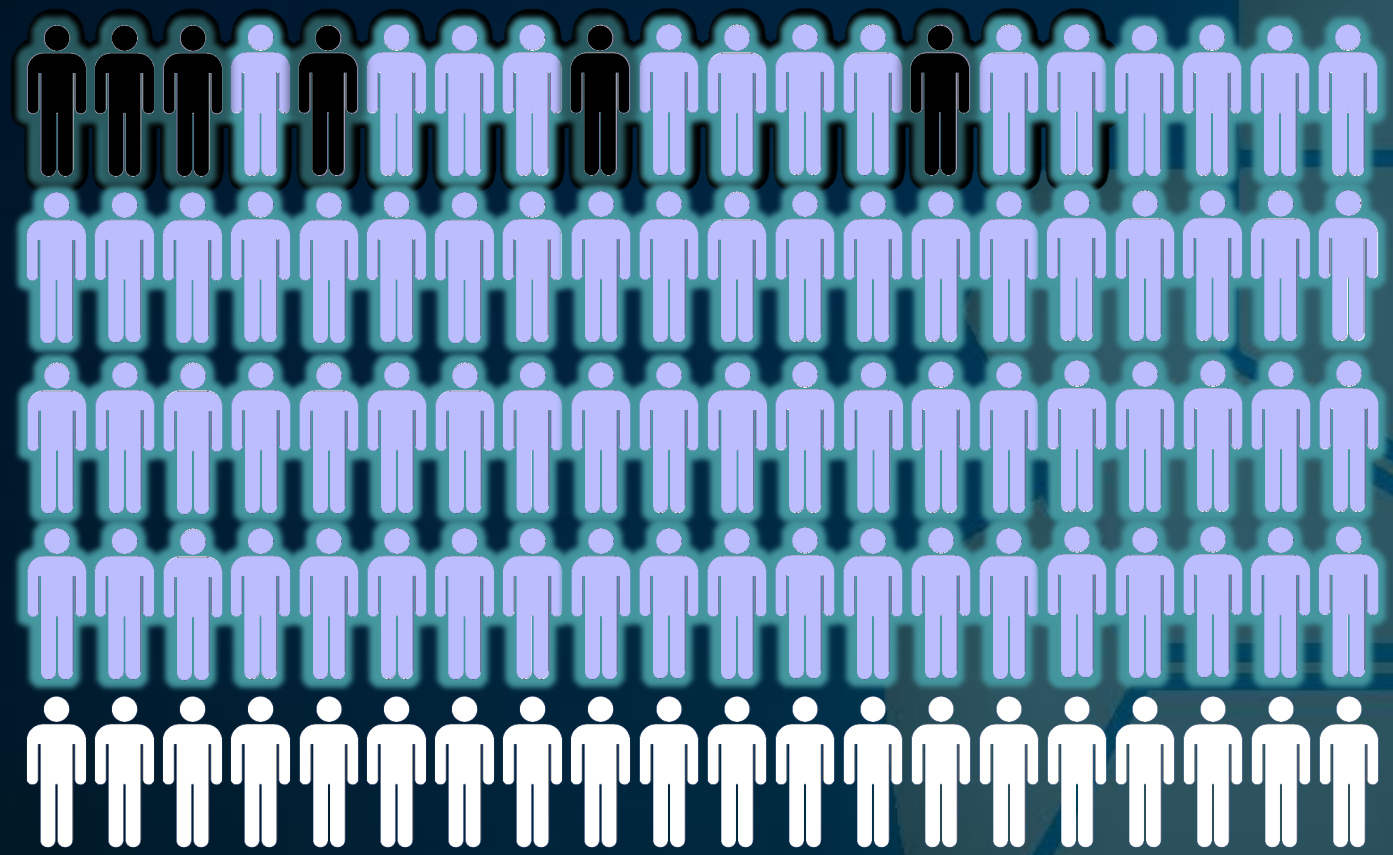
Prostate cancer is the most commonly diagnosed cancer in men

36% of newly diagnosed cancers, and 10% of all cancer deaths in men



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 ?

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The deadliest aspect of cancer

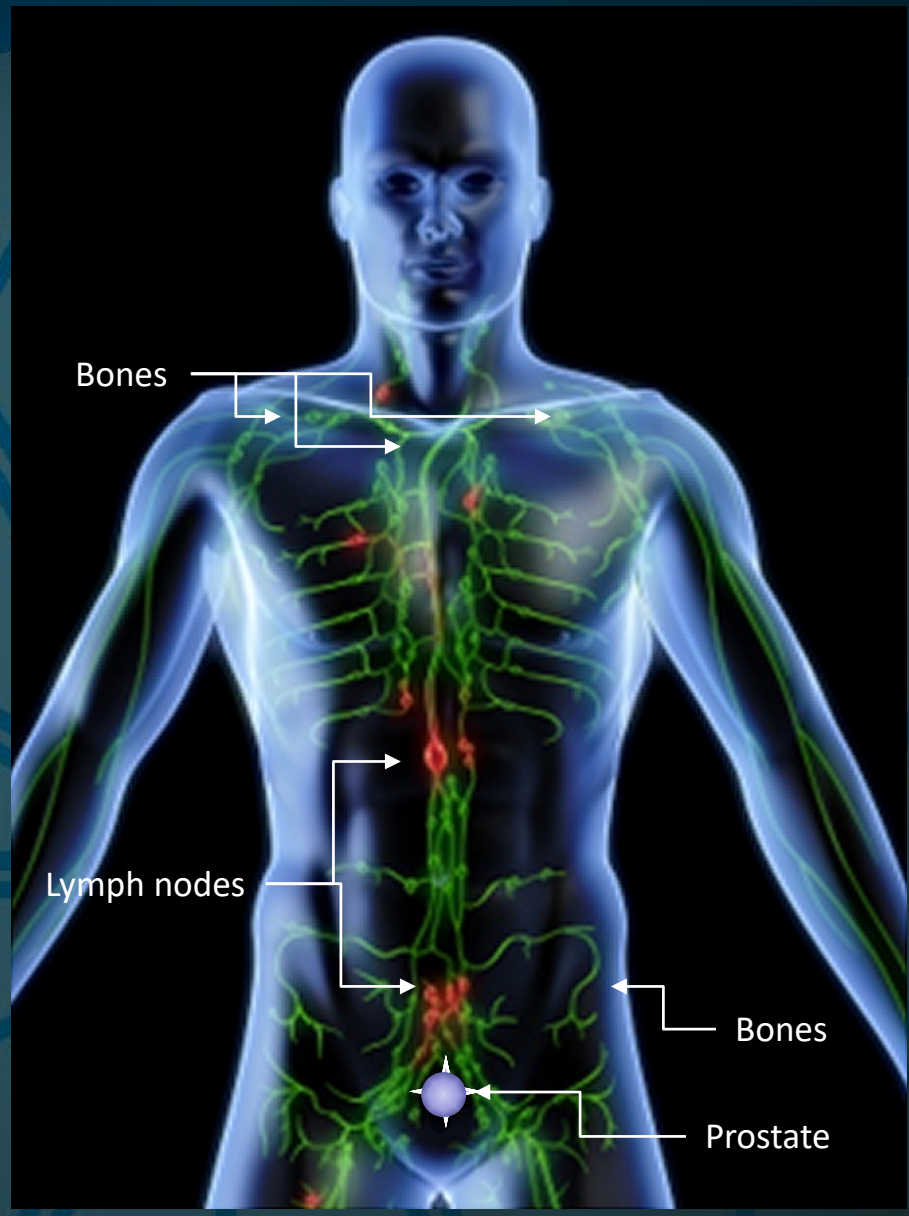
The deadliest aspect of prostate cancer is its spread, or metastasis

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



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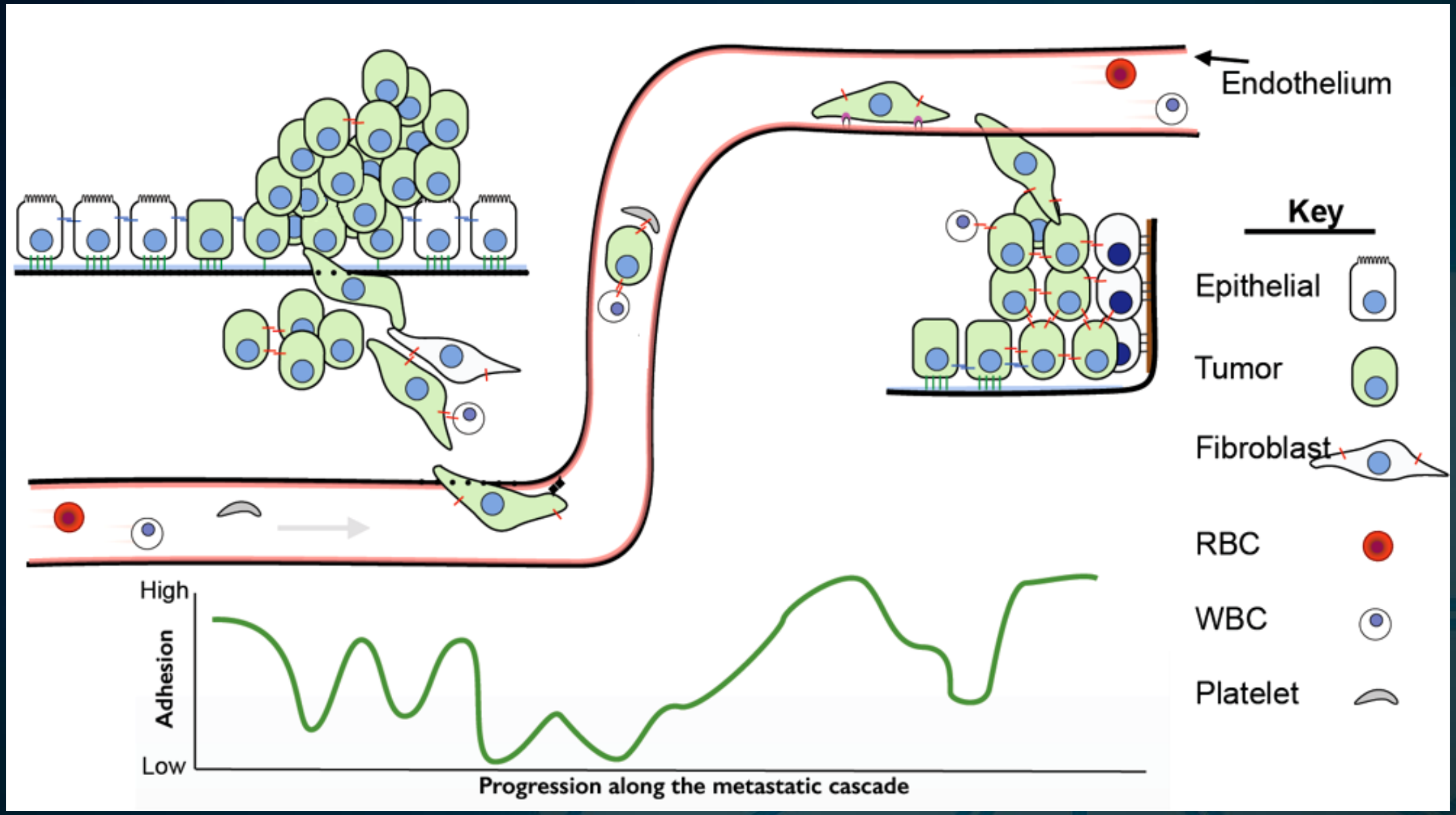
Metastasis is a complex, multi-step process!



Palmer et al., Advanced Drug Delivery Reviews 2011

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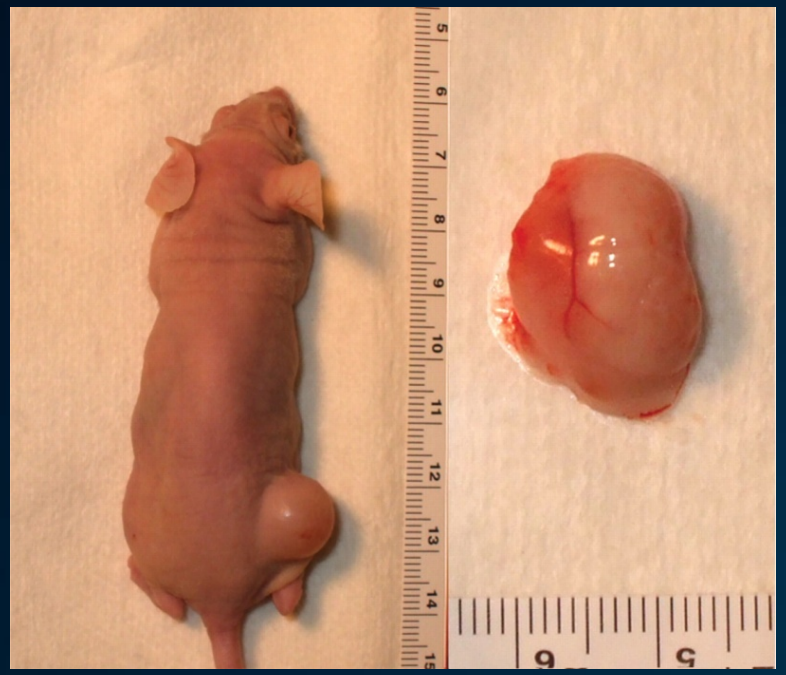
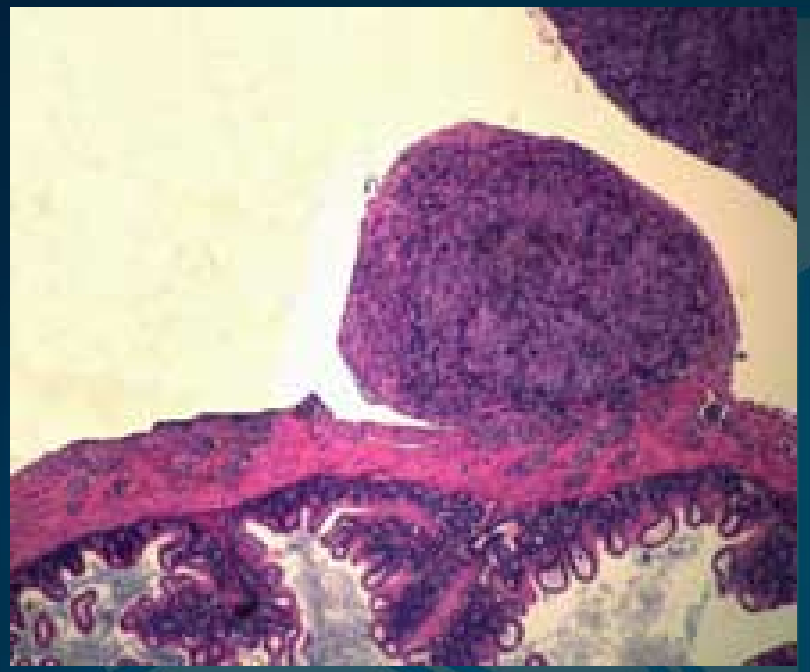
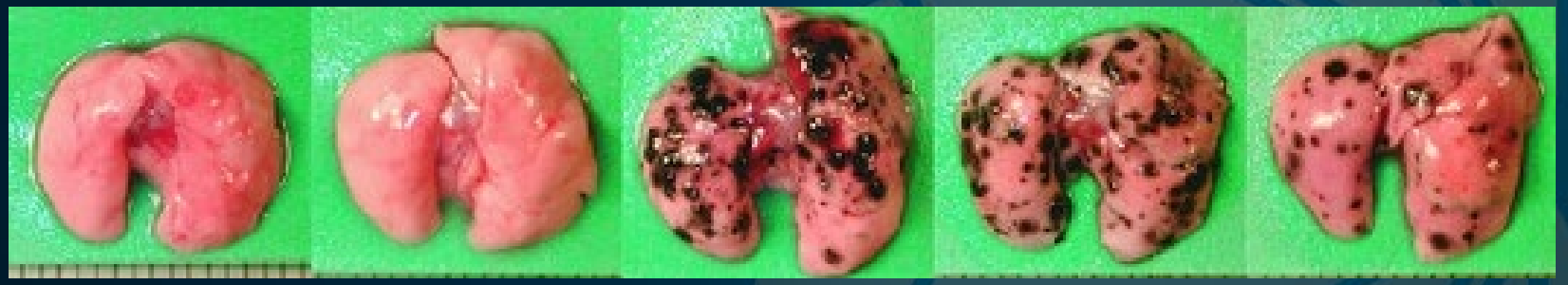
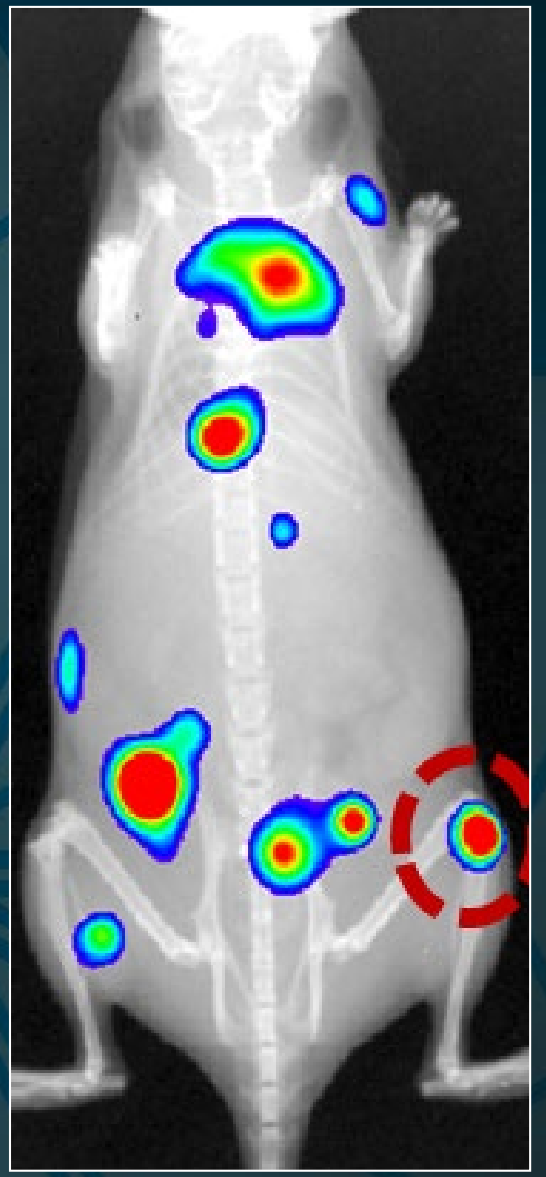
Animal models for metastasis









Snapshots provide limited information…

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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"

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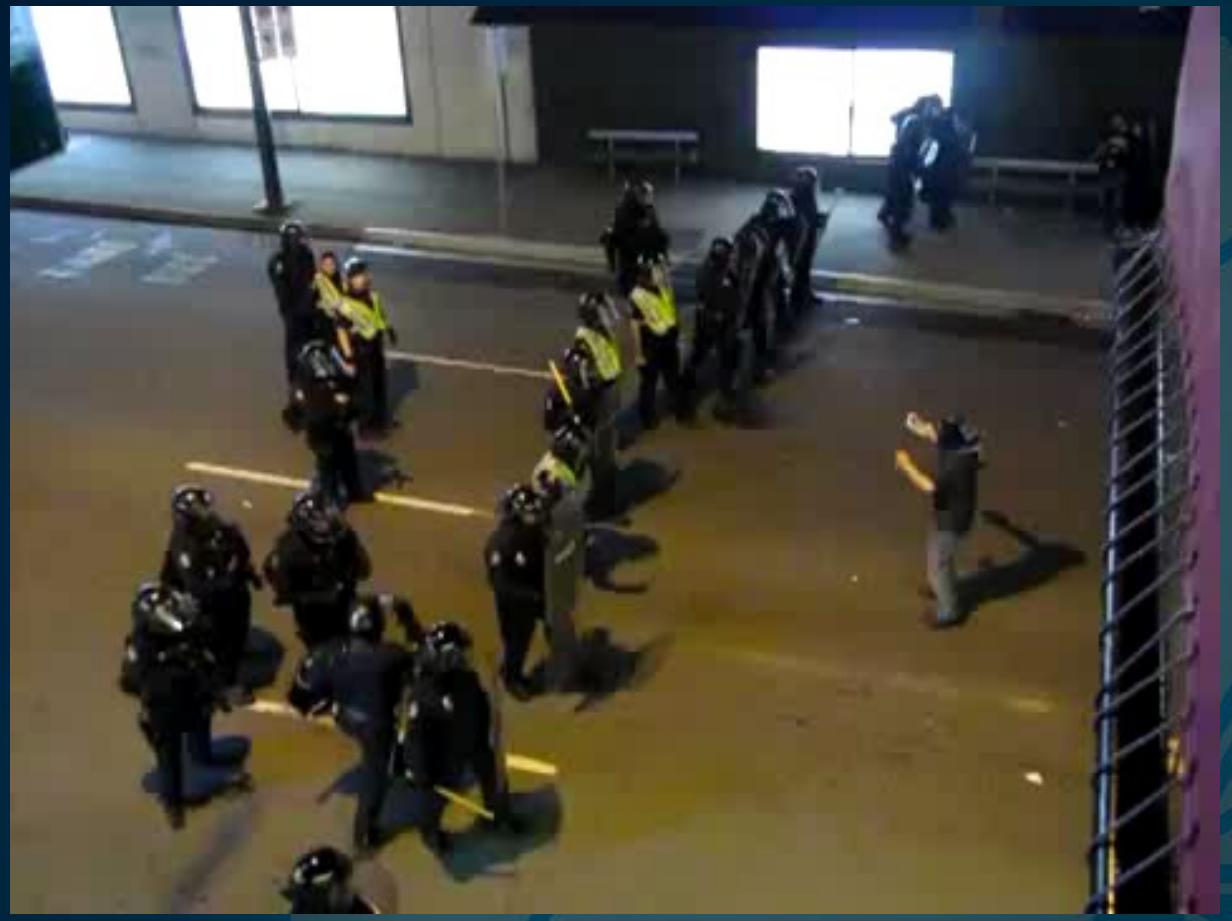
Let's all be scientists for a moment…



1. Man consoles distraught girlfriend after she was violently knocked down by riot police

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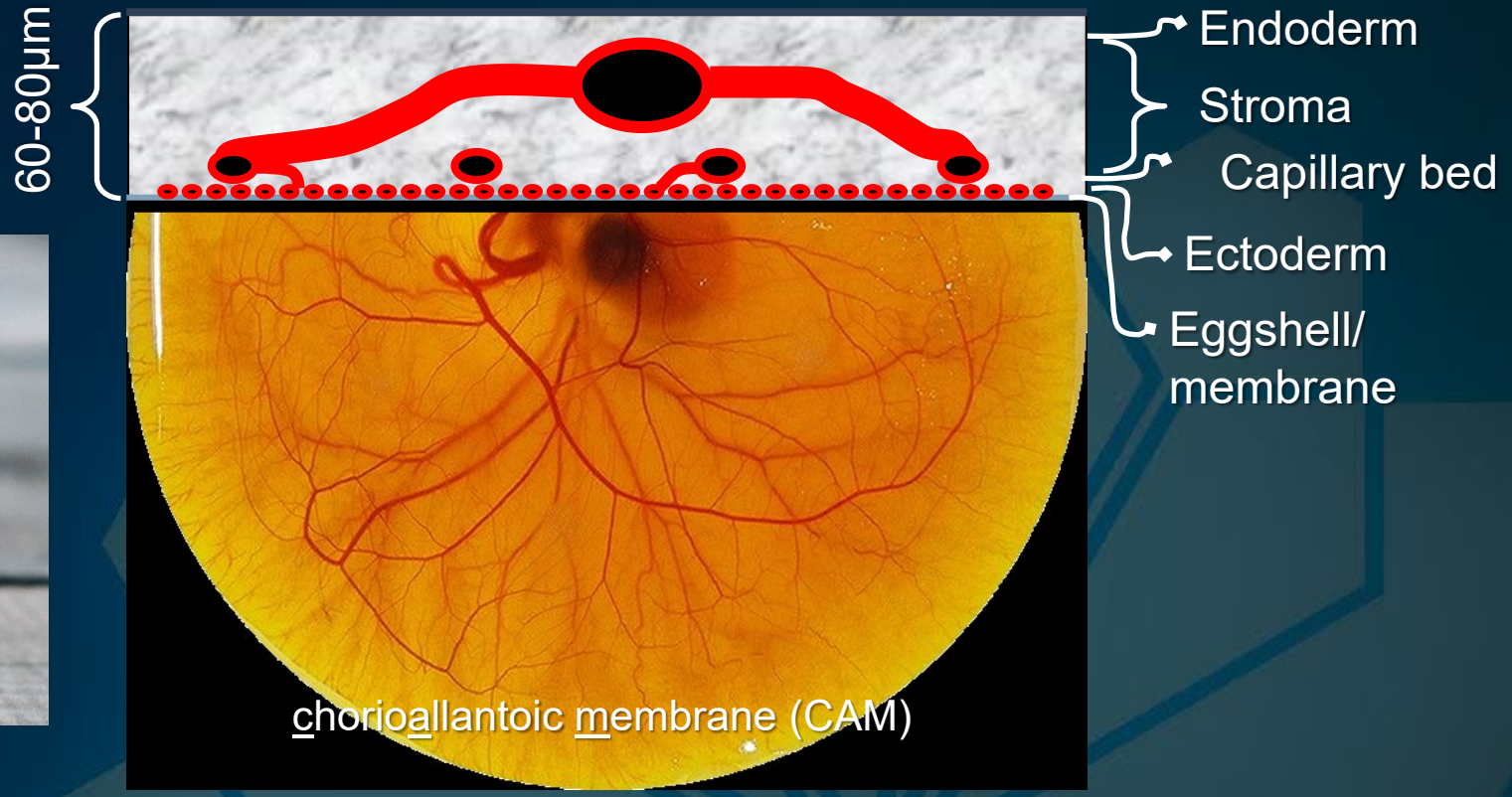
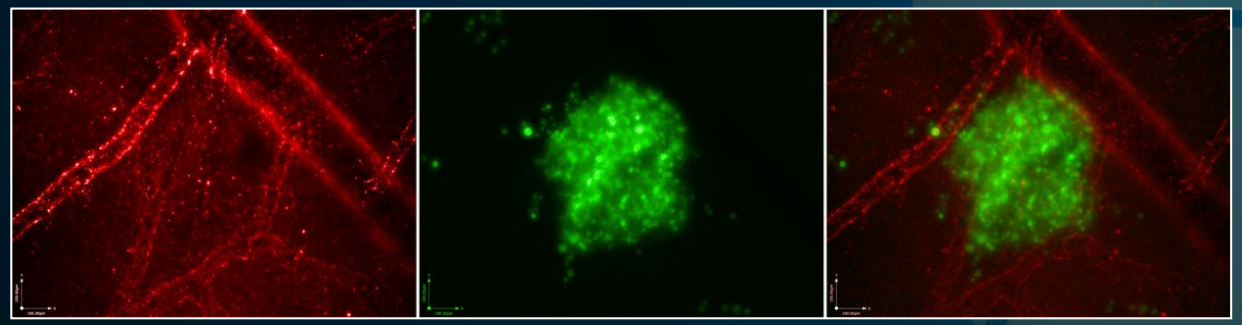
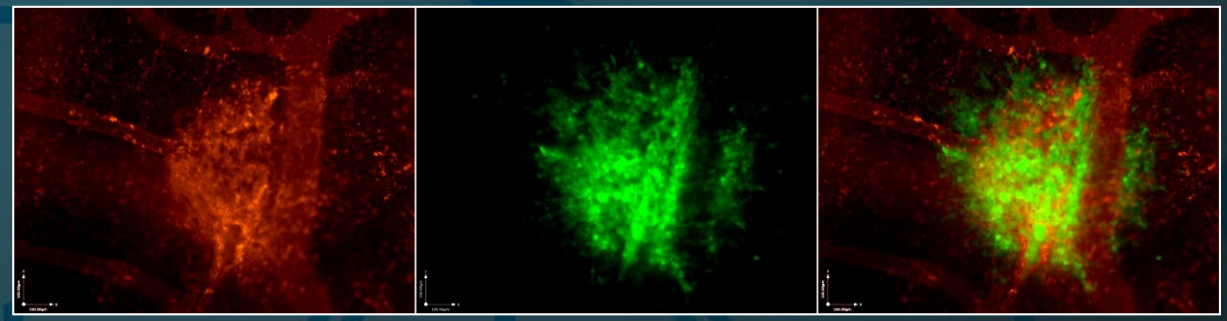
Modeling cancer dynamics in chicken embryos



Immediately after injection



24 hours later



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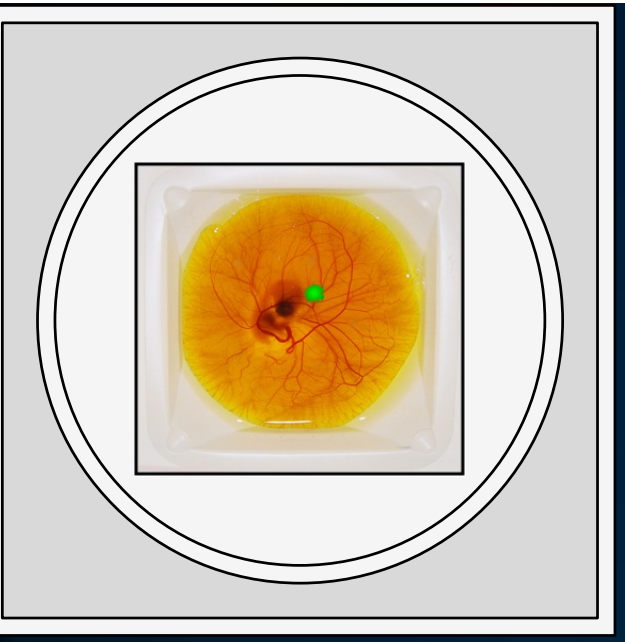
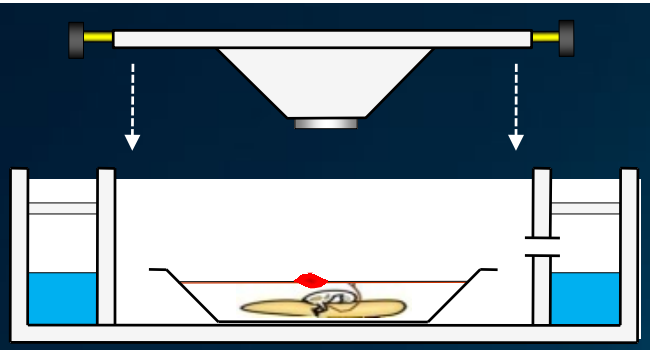
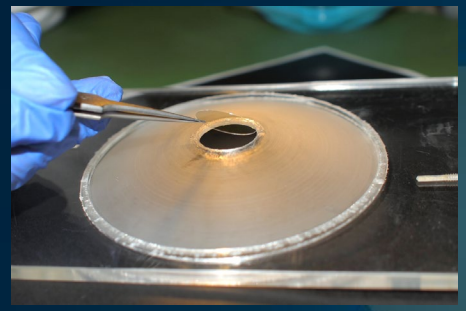
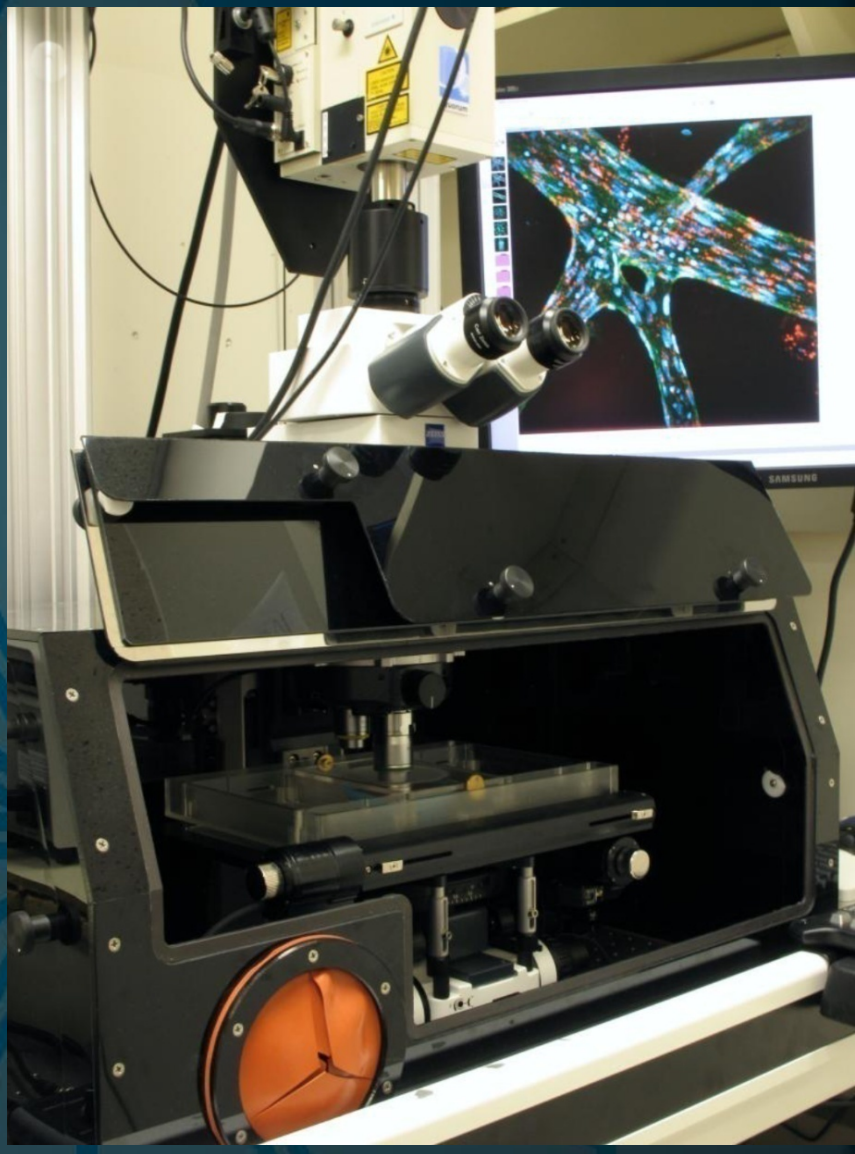
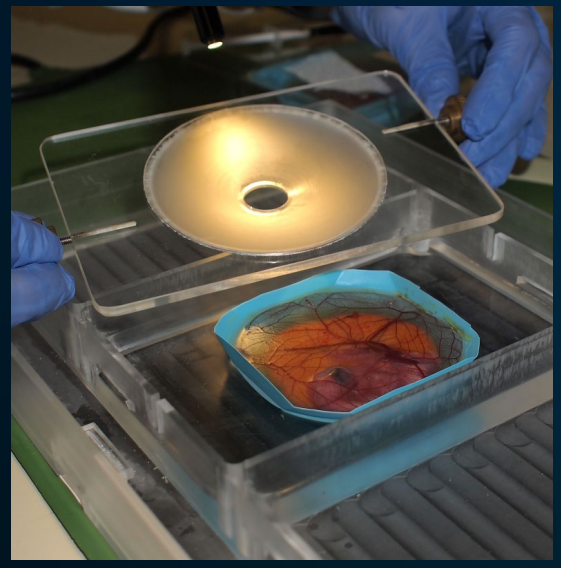
Modeling cancer dynamics in *ex ovo* avian embryos

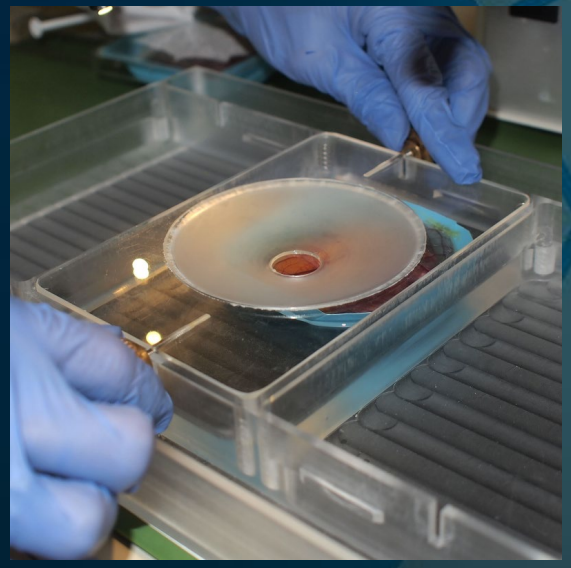


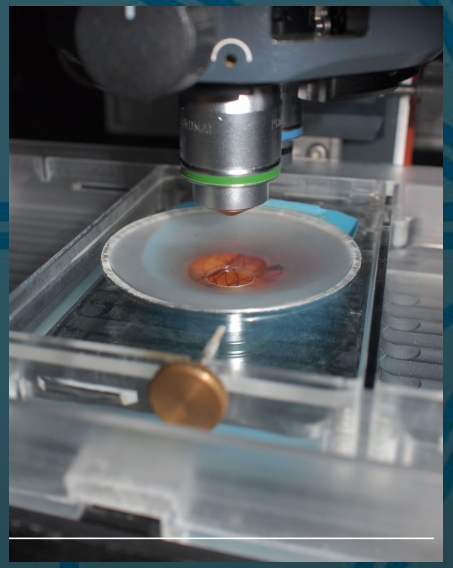












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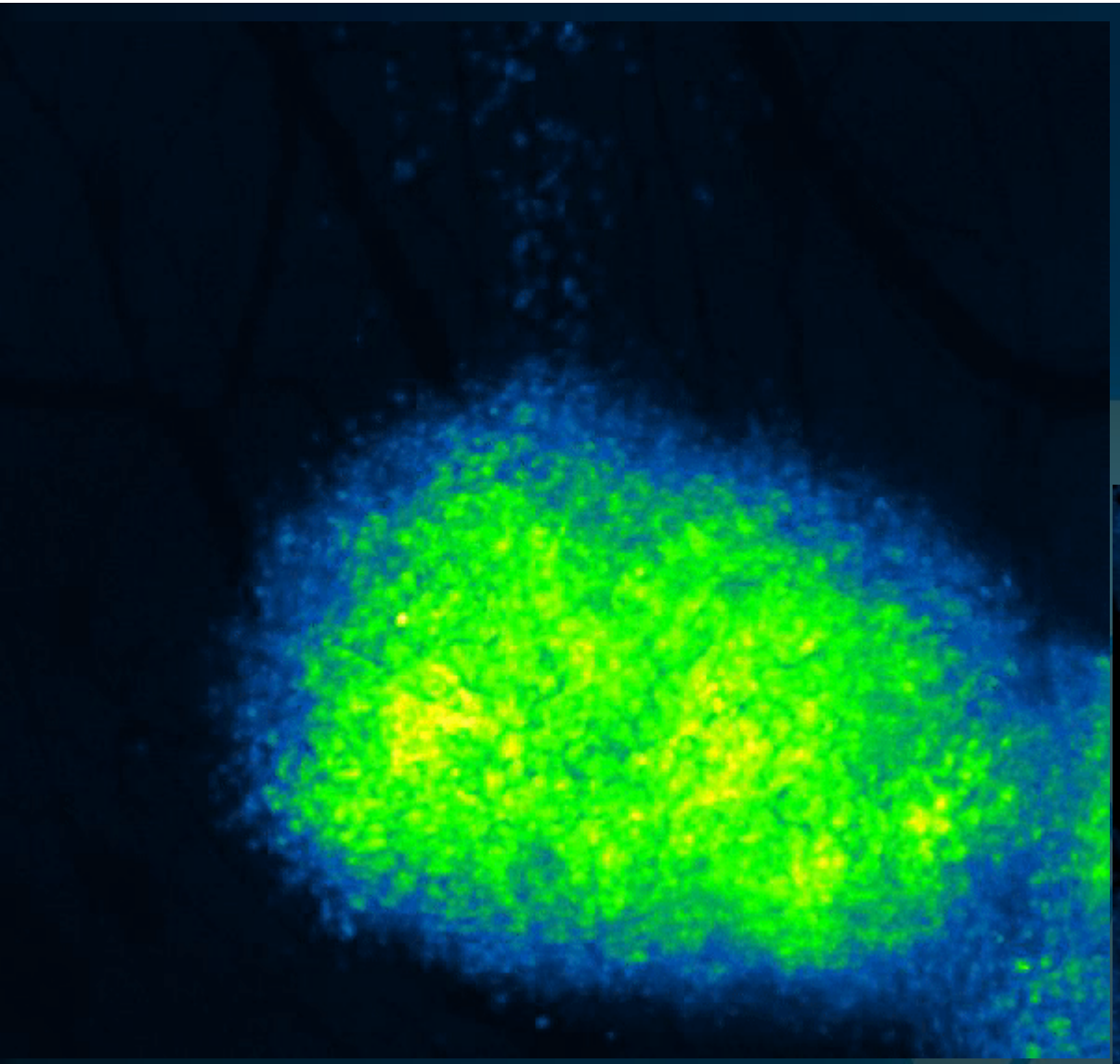
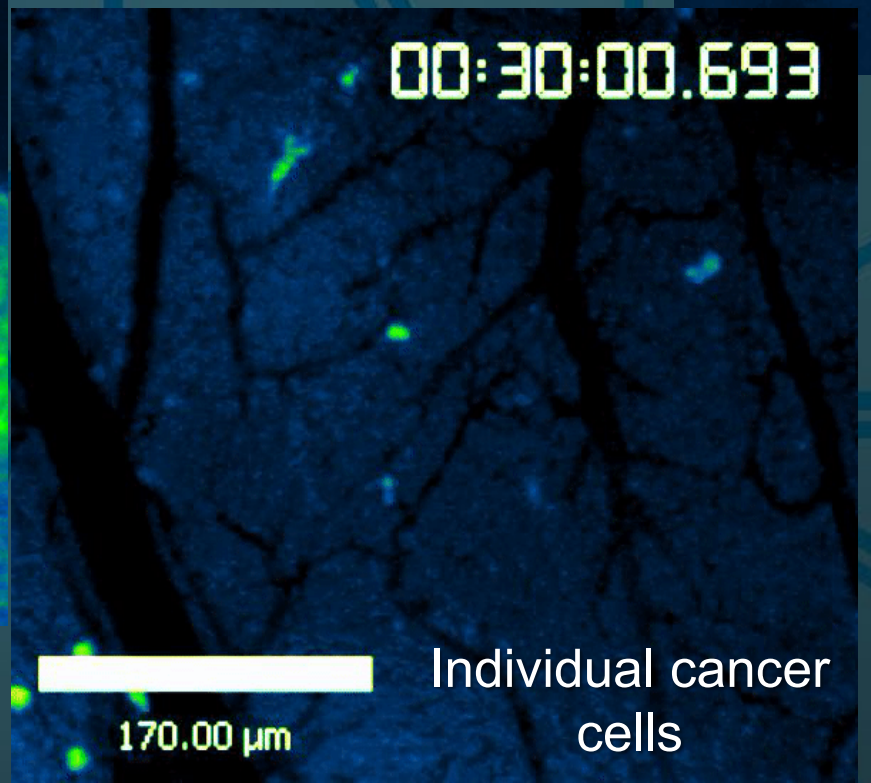
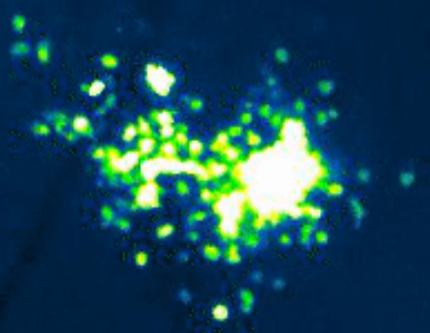
Intravital imaging of tumour growth and metastasis



4 mm tumour growing over 4 days



Individual cancer cells



Small metastasis of 200-300 cells

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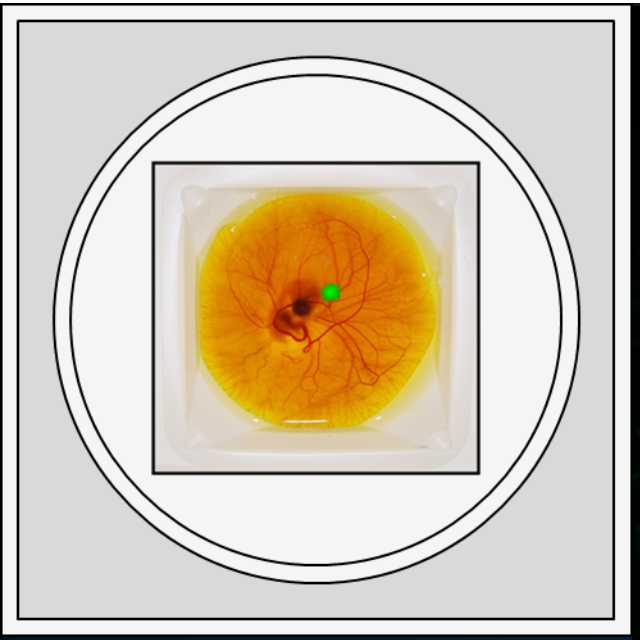
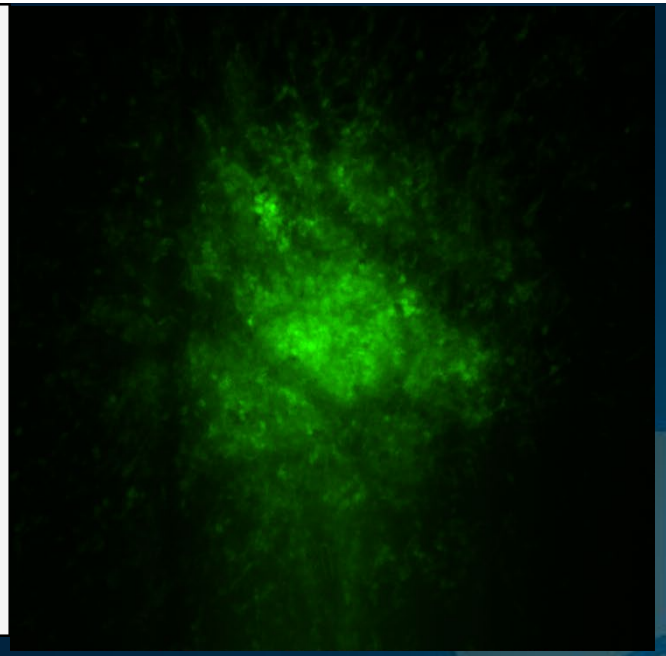
When studying metastasis context is crucial to data analysis





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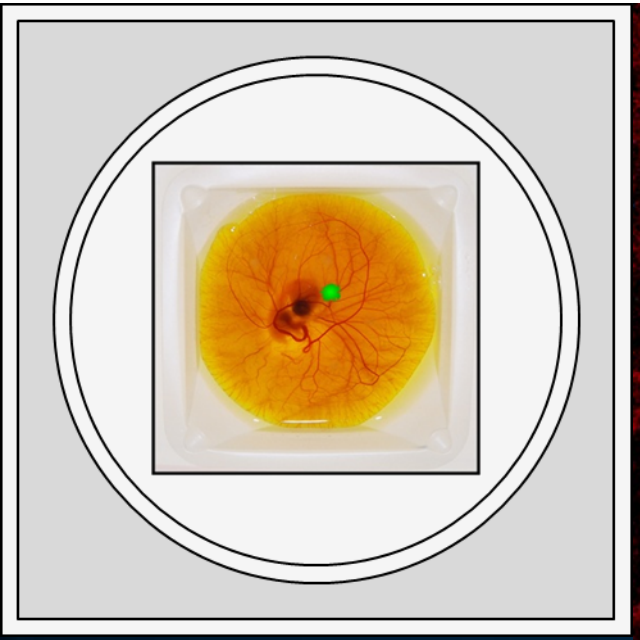
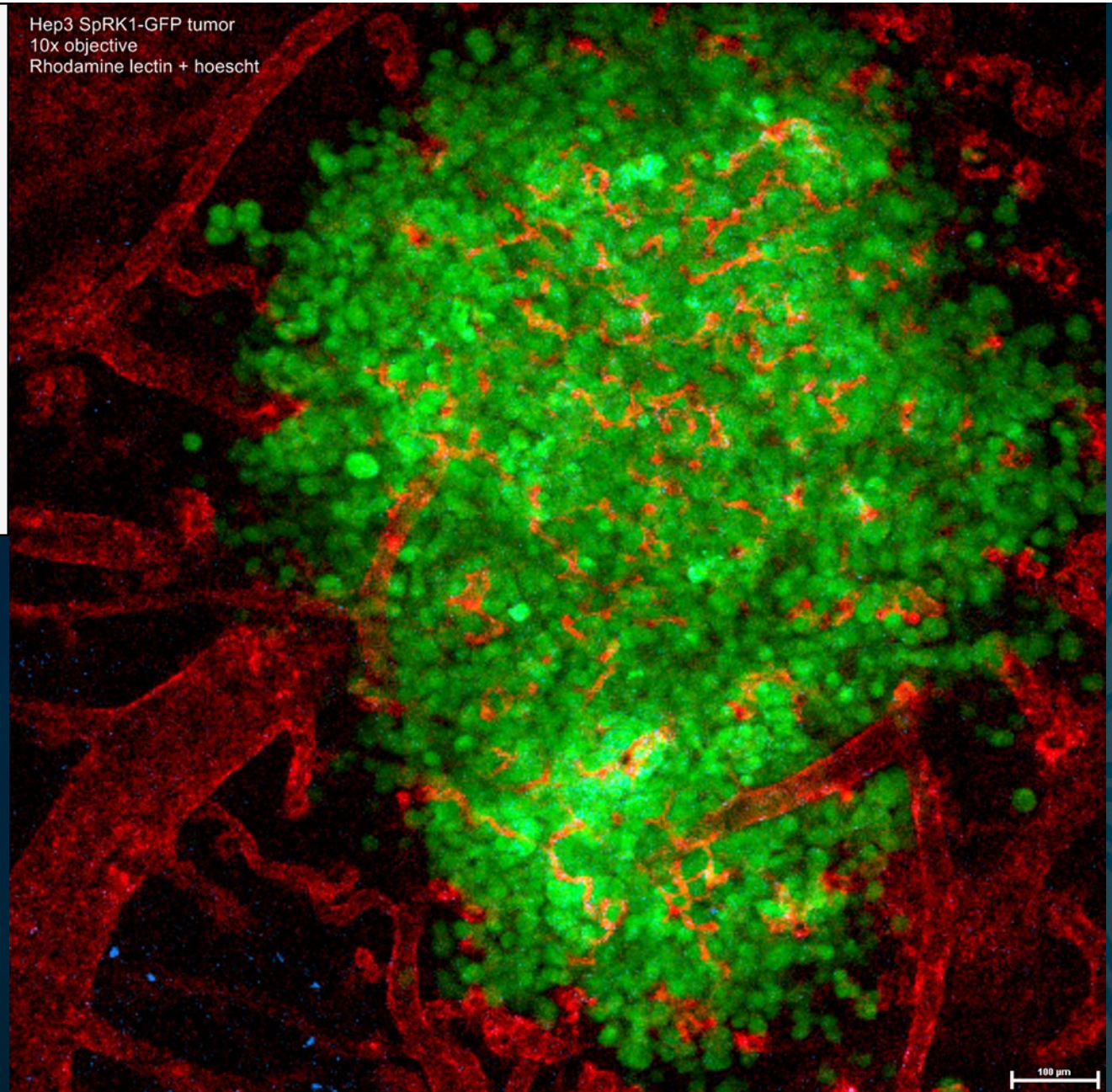
When studying metastasis context is crucial to data analysis





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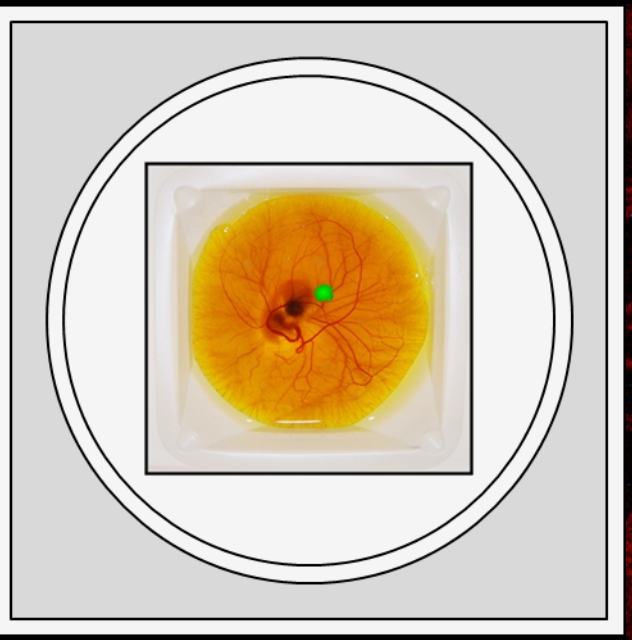
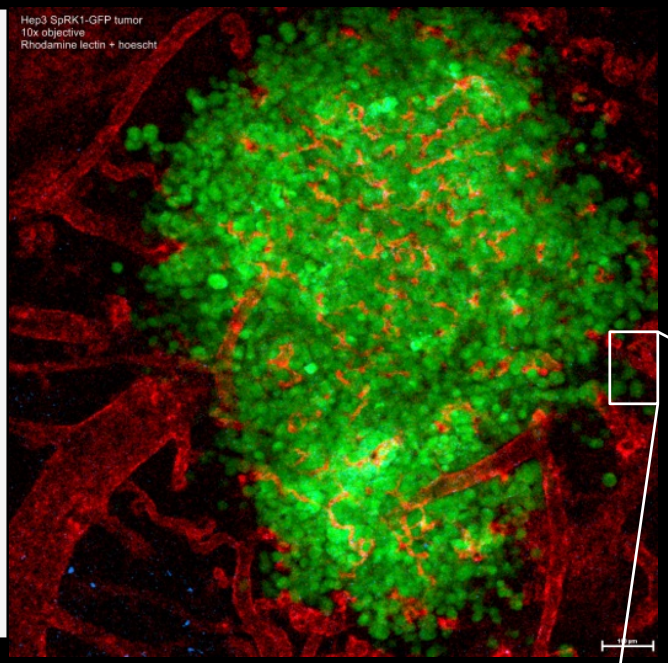
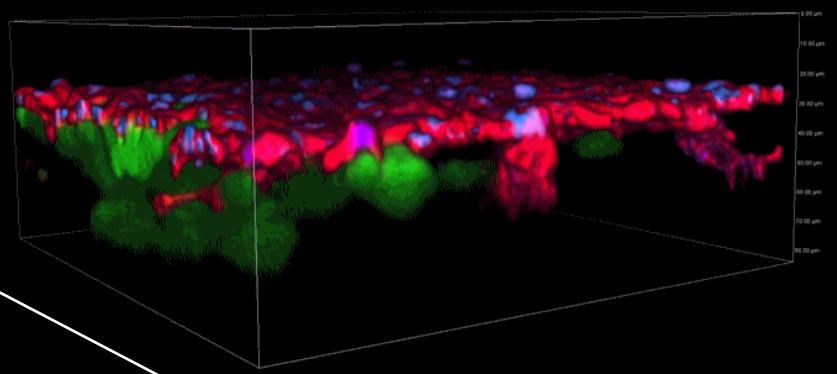
When studying metastasis context is crucial to data analysis







Microvasculature

Tumor cells

Blood vessel

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.

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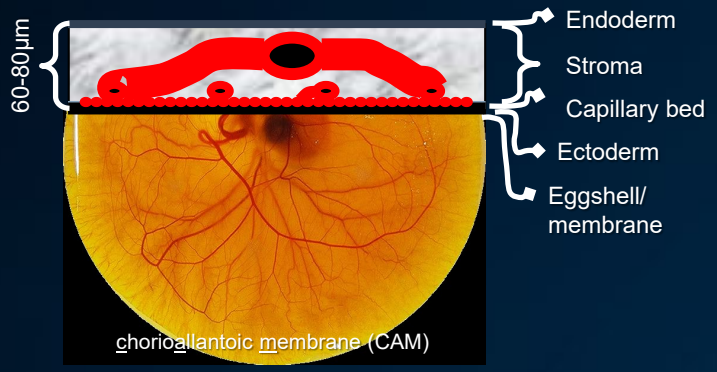
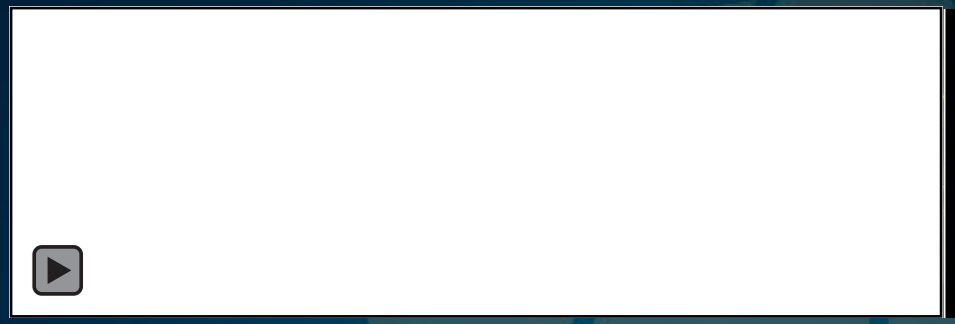
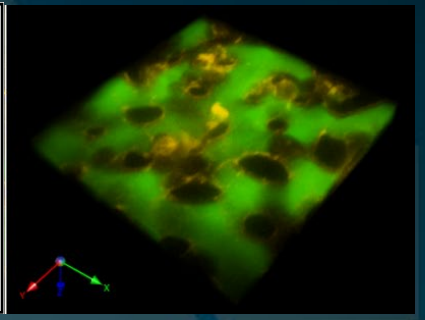
Imaging the CAM in 3D

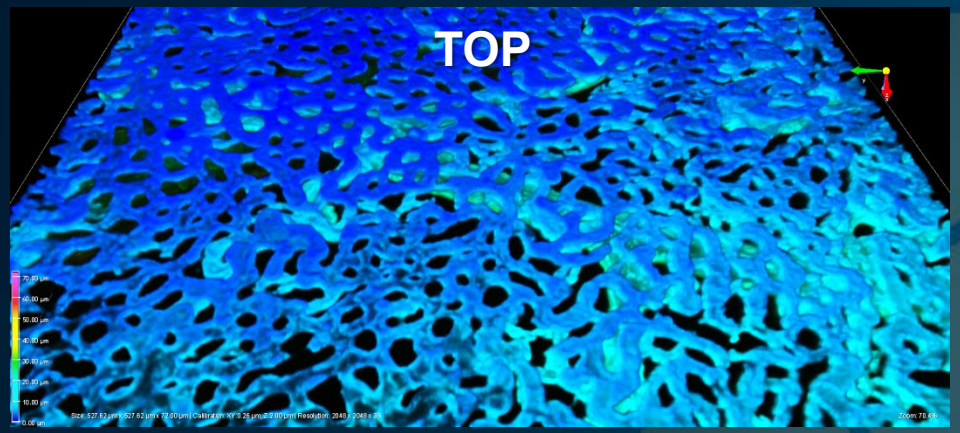


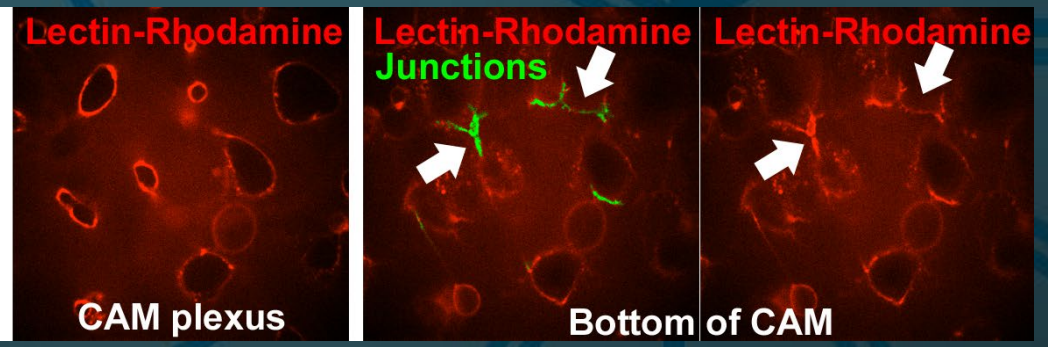
Rhodamine-Lectin (*Lens culinaris* agglutinin)



2MDa FITC-Dextran







Leong et al., *Cell Reports*, 2014

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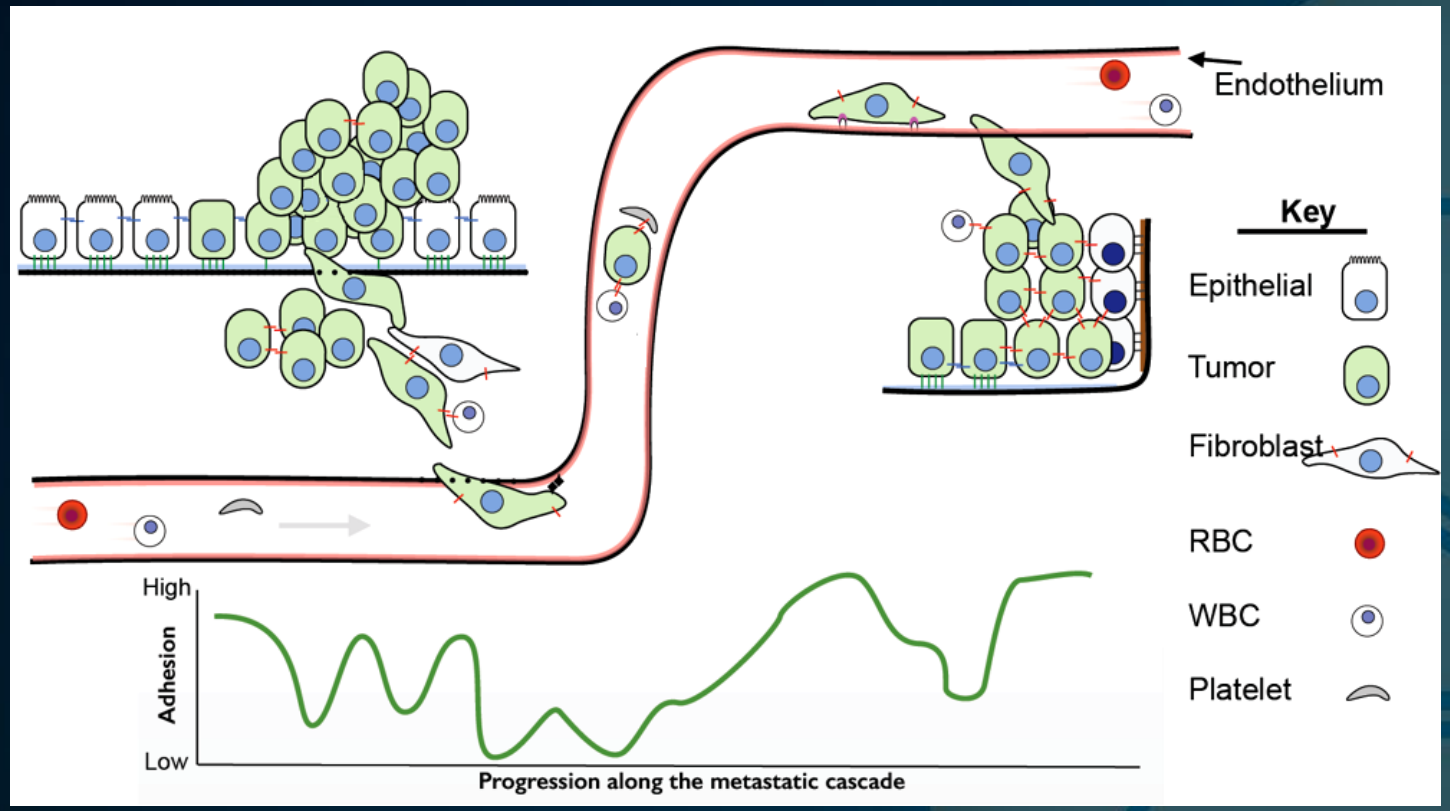
What is the difference between cells that spread and cells that don't?



"Bummer of a birthmark, Hal."

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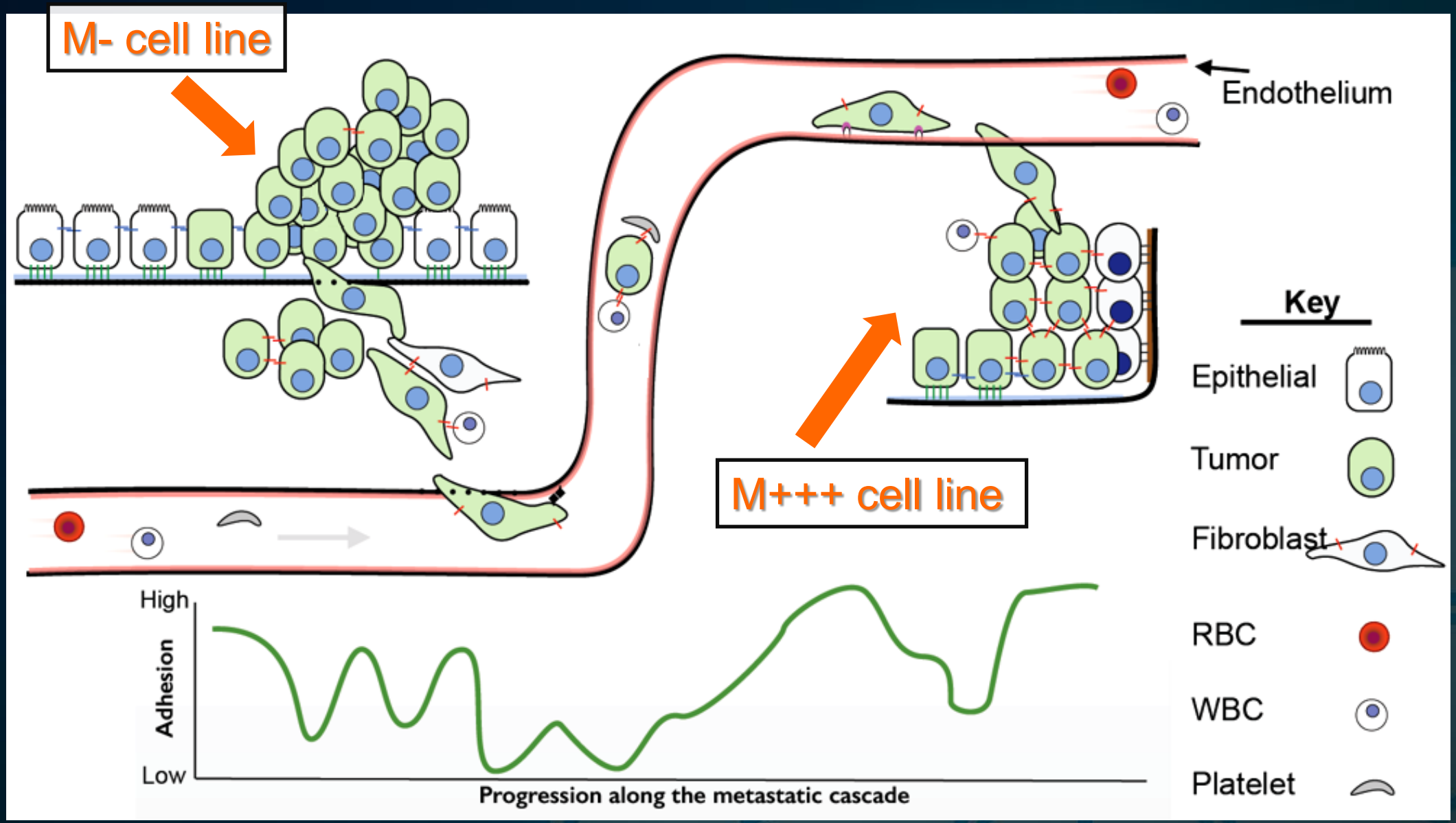
What is the difference between cells that spread and cells that don't?



Advanced Drug Delivery Reviews 2011

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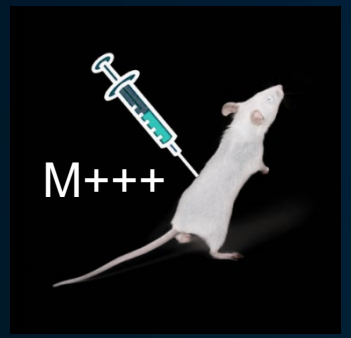
Antibody 1A5 targets tetraspanin CD151

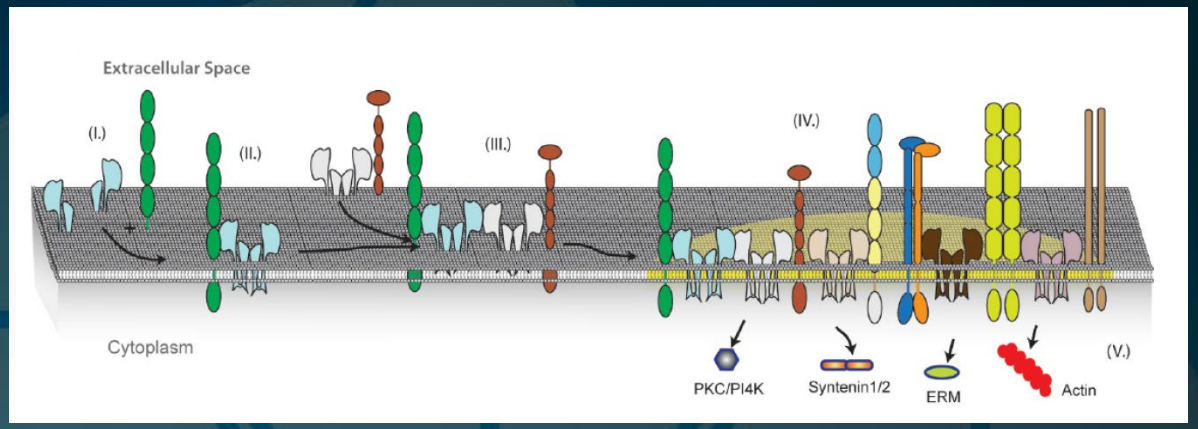
Immunize mouse with low metastatic cancer variant

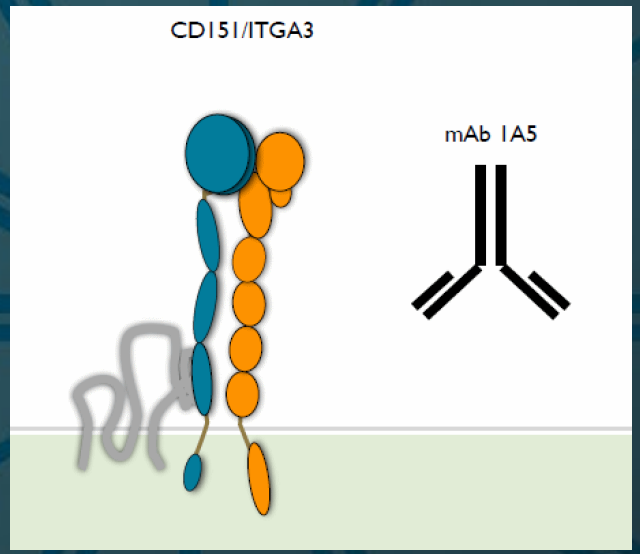
Cyclophosphamide – "tolerizes" mouse immune system



Immunize mouse with high metastatic cancer variant

Isolate antibodies against targets in M+++ but not M-





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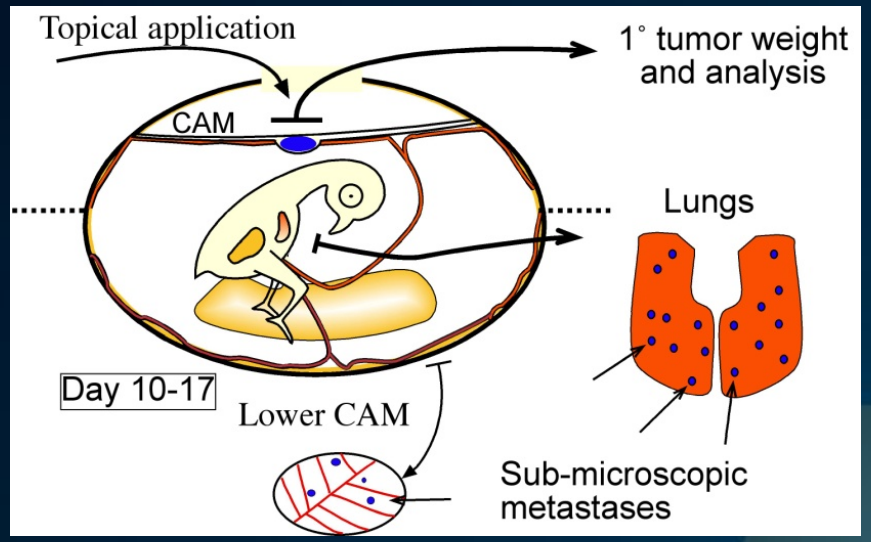
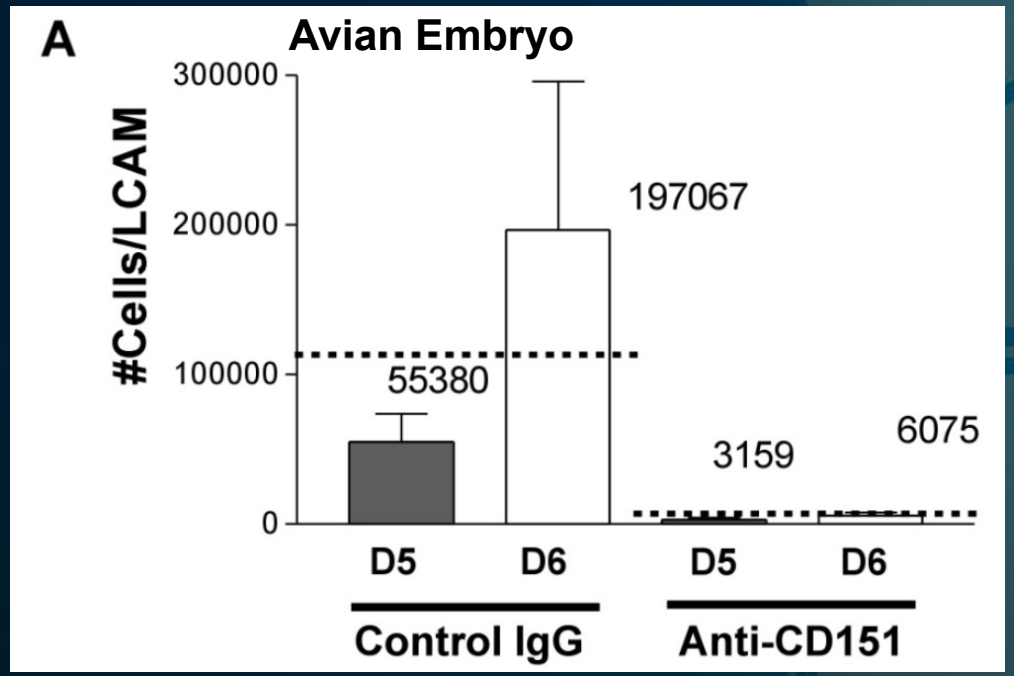
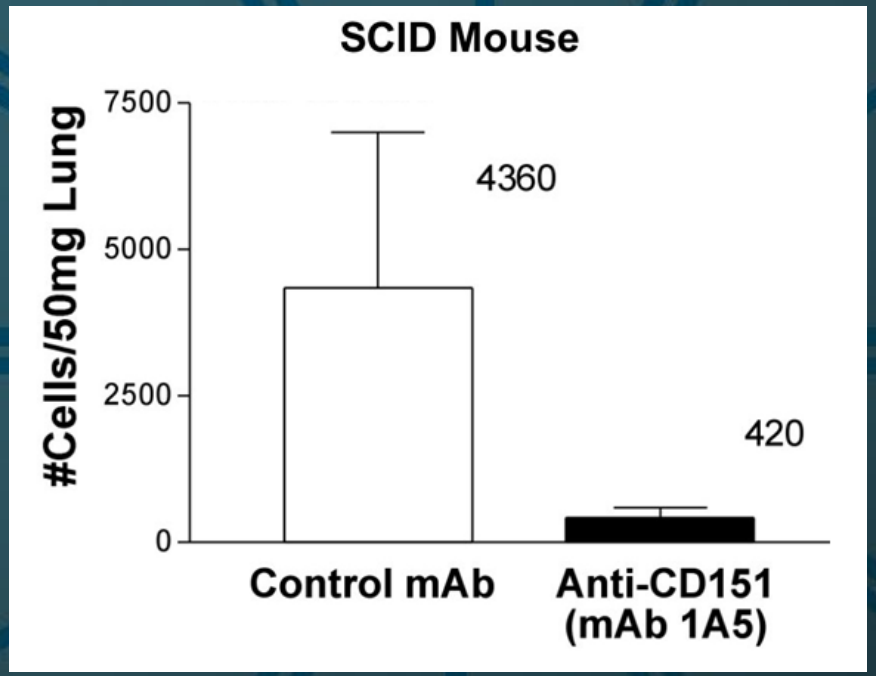
Anti-CD151 antibody blocks spontaneous metastasis







Zijlstra et al., *Cancer Cell*, 2008

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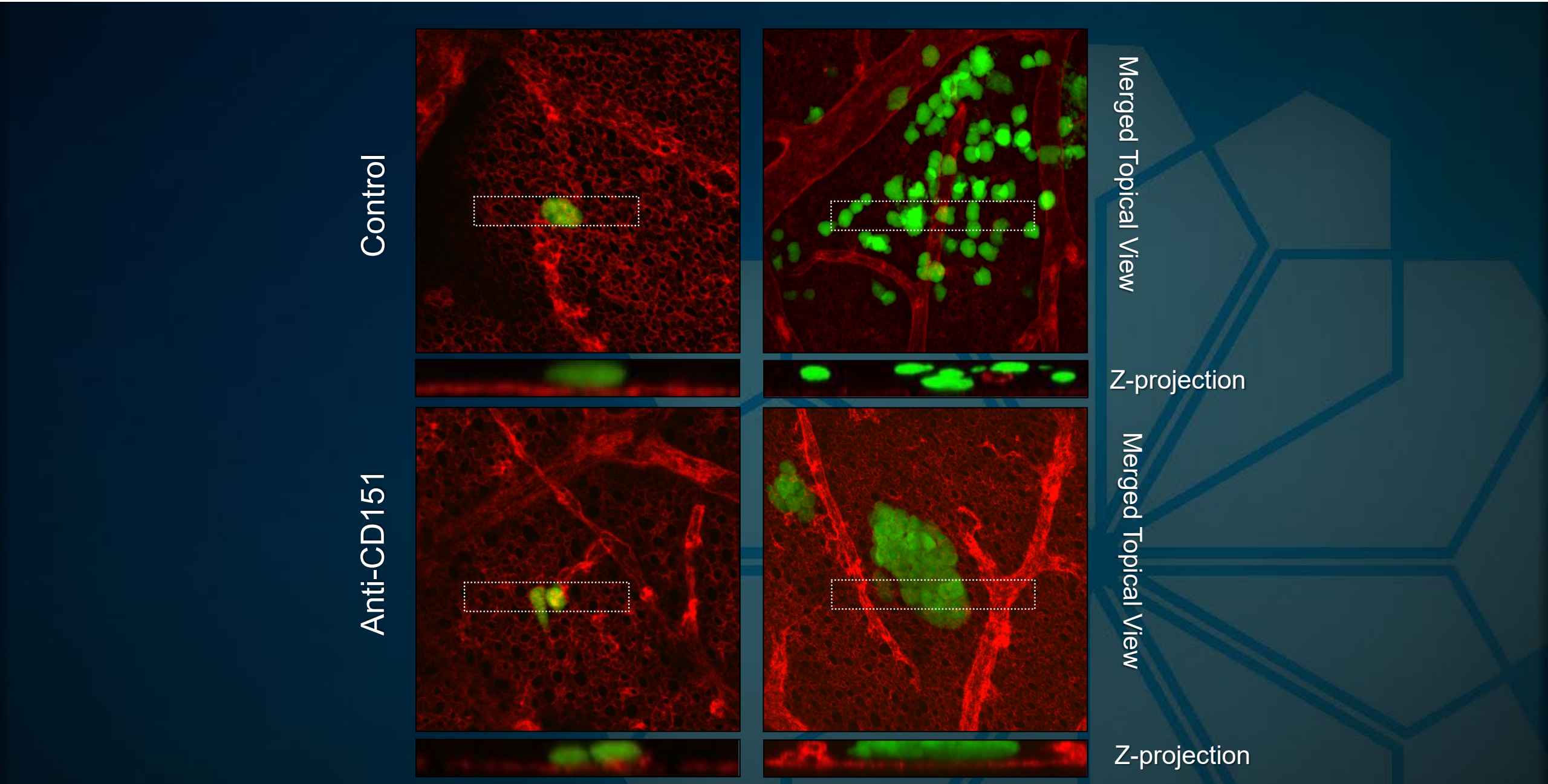
Dramatic differences in cell motility phenotype *in vivo*



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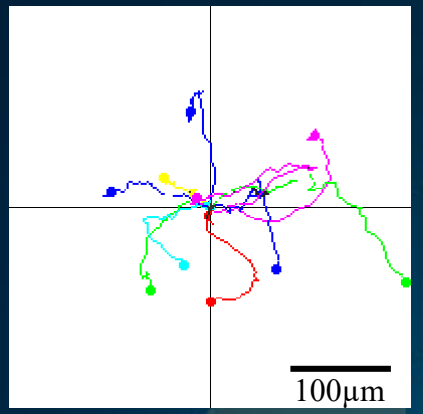
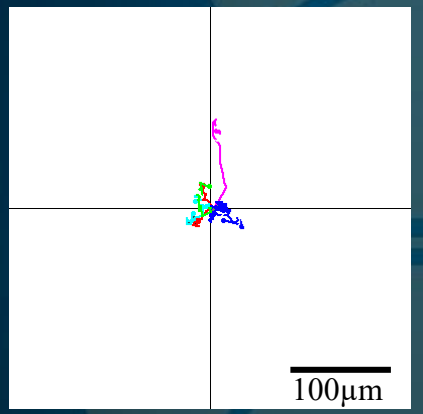
Anti-CD151 antibody inhibits cell migration *in vivo*

Control (IgG)



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

Anti-CD151 (1A5)



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

Productive Migration
6.6 fold inhibition

control

mAb 1A5 treated

Zijlstra et al., *Cancer Cell*, 2008

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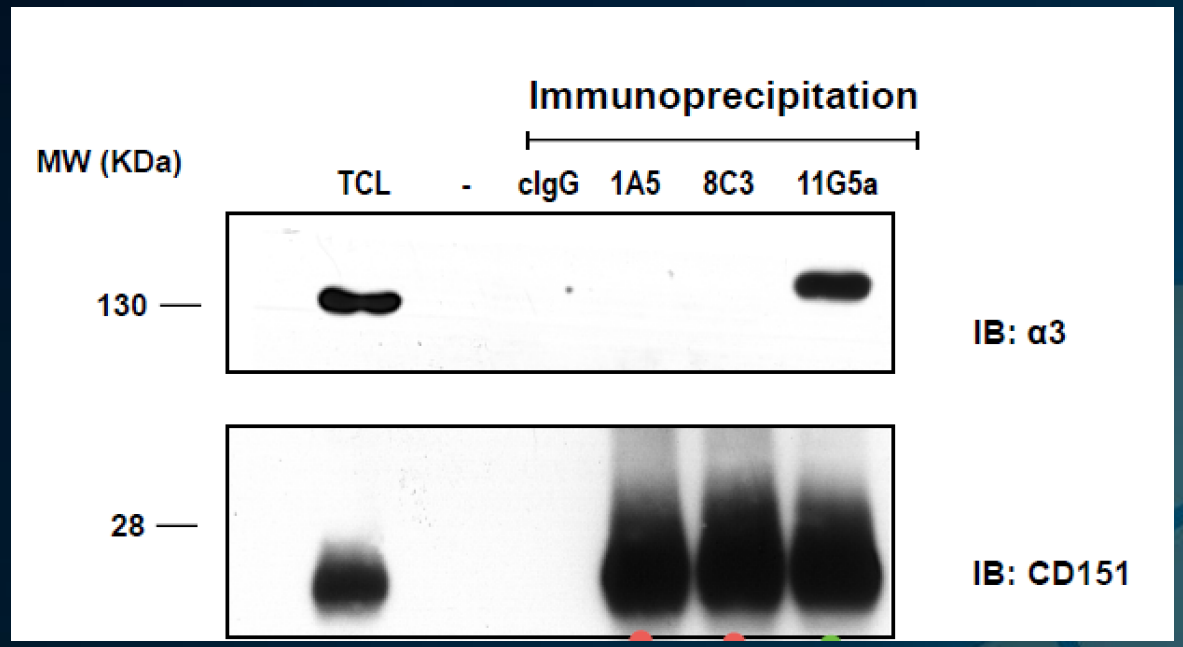
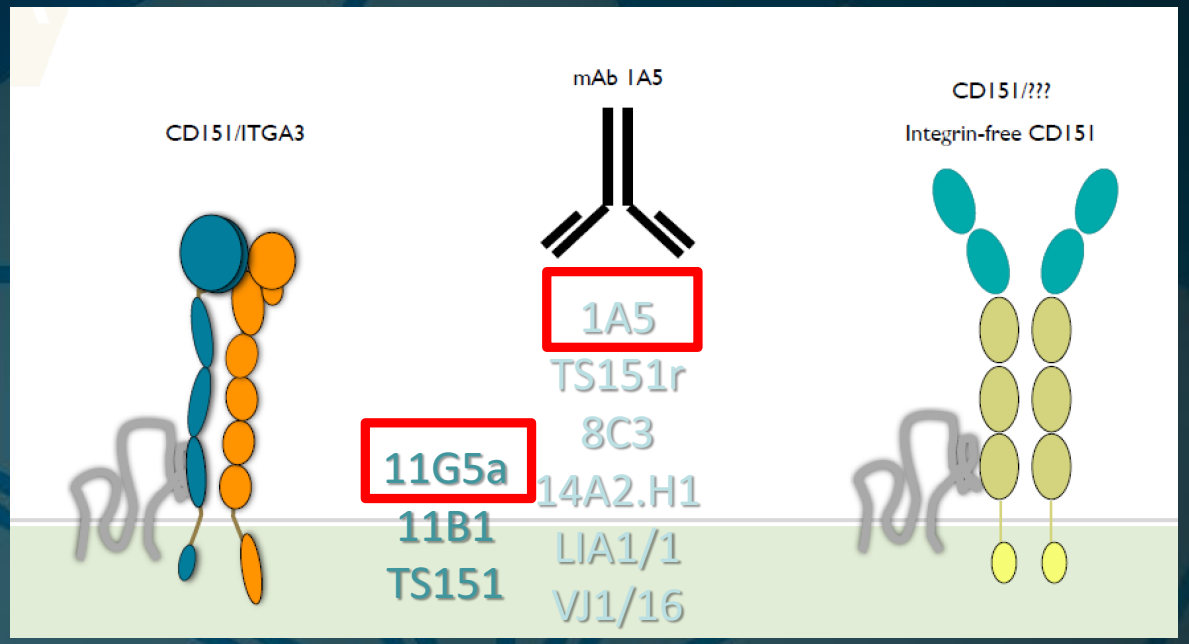
1A5 antibody binds CD151 that is "free" from integrins





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

Palmer et al., *Cancer Research*, 2014

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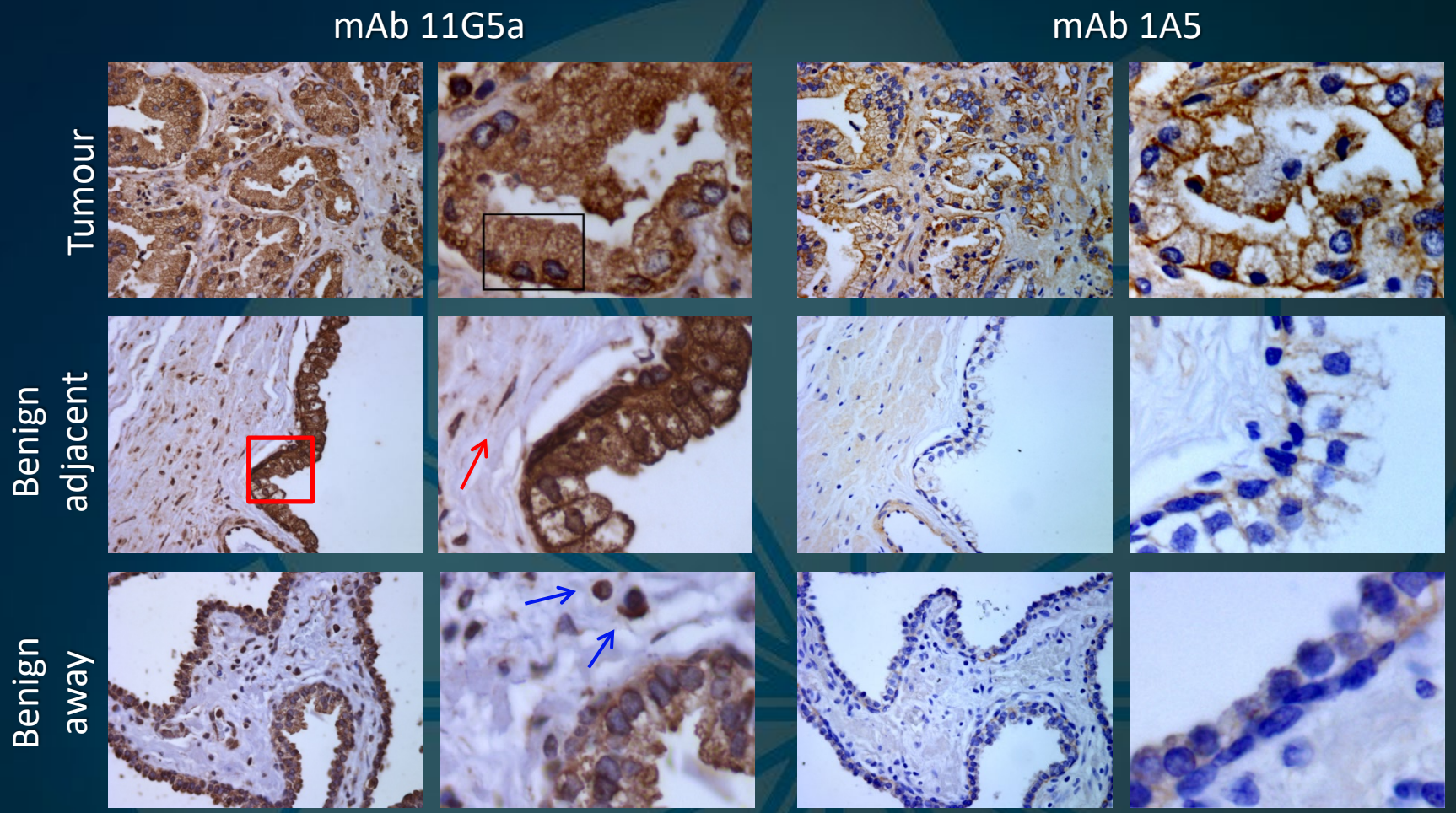
CD151^{free} is distinct from CD151^{all} in prostate cancer

138 prostate cancer surgery patients

Follow up: 12.1 years

Recurrence: 34 cases
Metastasis: 38 cases

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



Palmer et al., *Cancer Research*, 2014

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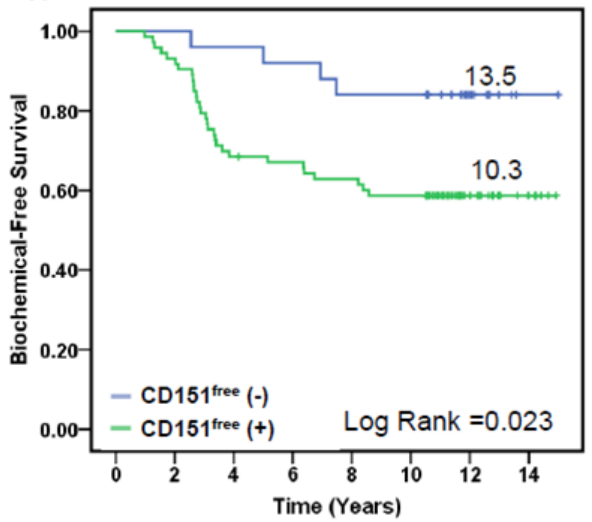
CD151^{free} predicts prostate cancer recurrence and metastasis

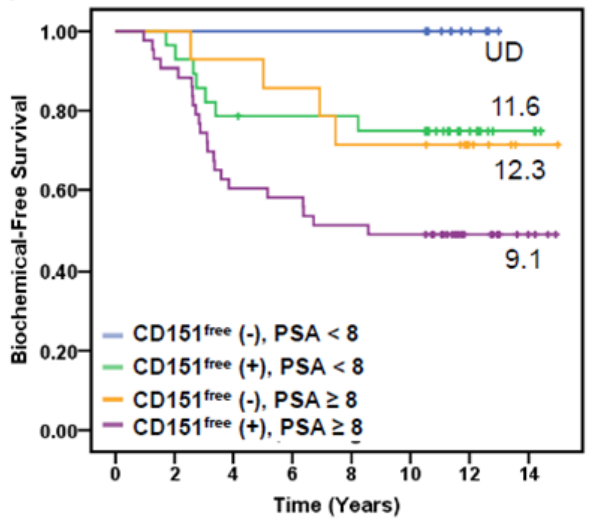
Biochemical Relapse

F.



# at Risk (Years)	0	2	4	6	8	10	12	14
CD151	25	25	24	23	21	19	6	1
CD151	73	68	50	48	45	38	15	4

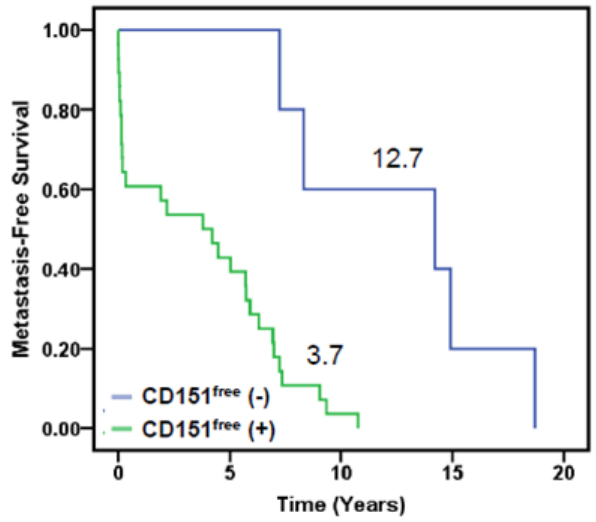
G.



# at Risk (Years)	0	2	4	6	8	10	12	14
CD151	11	11	11	11	11	10	2	0
CD151	28	27	22	21	21	18	7	2
CD151	14	14	13	12	10	10	4	1
CD151	43	39	26	25	22	20	8	2

Metastasis

H



# at Risk (Years)	0	2	4	6	8	10	12	14	16	18	20
CD151	5	5	5	5	4	3	3	3	1	1	0
CD151	28	16	14	8	3	1	0	0	0	0	0

Palmer et al., *Cancer Research* 2014

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Summary: Tetraspanin CD151^{free}

- Tetraspanin CD151 and α3 integrin interactions comprise a cell migration “switch” between maintenance of epithelial structure and invasive cell migration
- Cross-linking CD151^{free} with 1A5 antibody blocks metastasis by stabilizing cell-cell adhesion
- mAb 1A5 detects a pool of CD151 (CD151^{free}) that is distinct from that detected by other antibodies
- CD151^{free} is associated with earlier biochemical recurrence and earlier onset of metastasis, independent predictor of outcome

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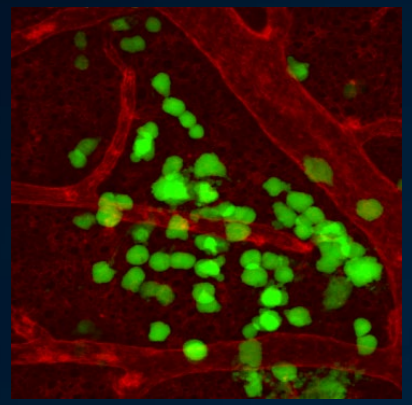
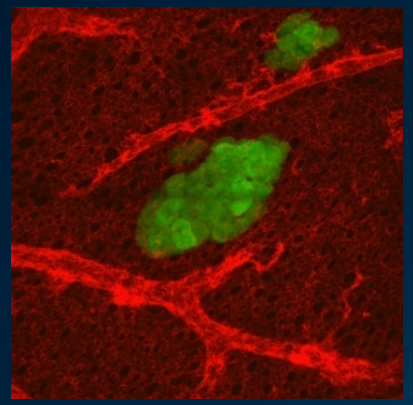
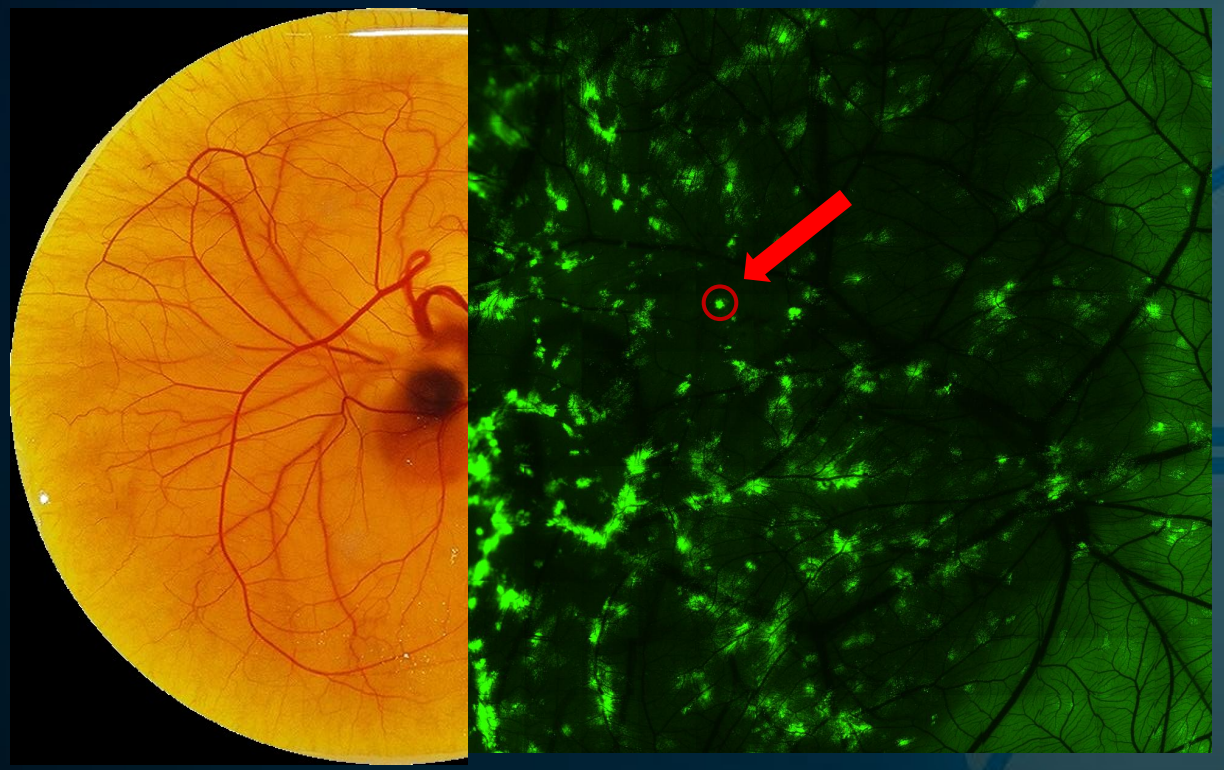
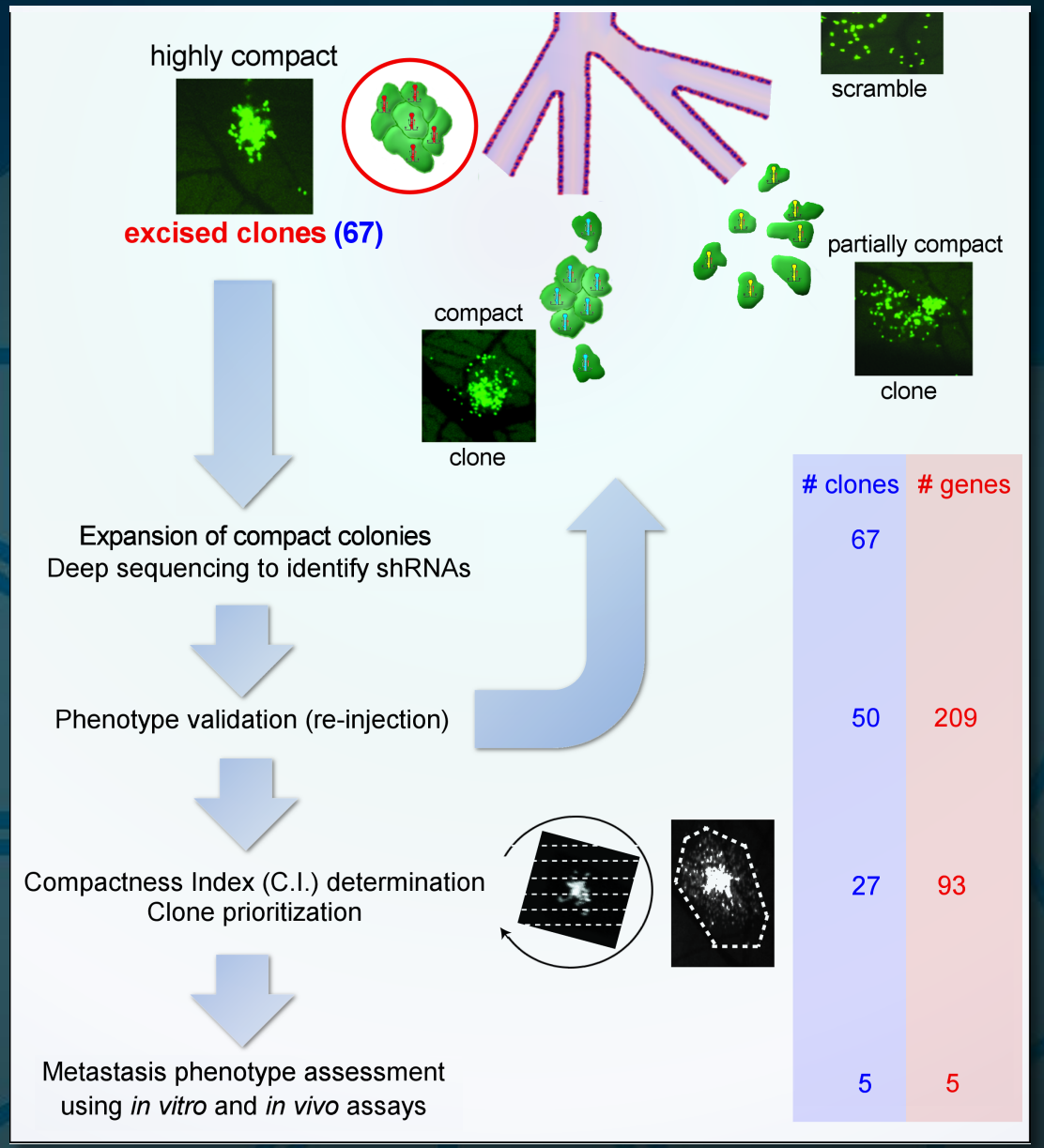
Intravital imaging – metastasis screening platform









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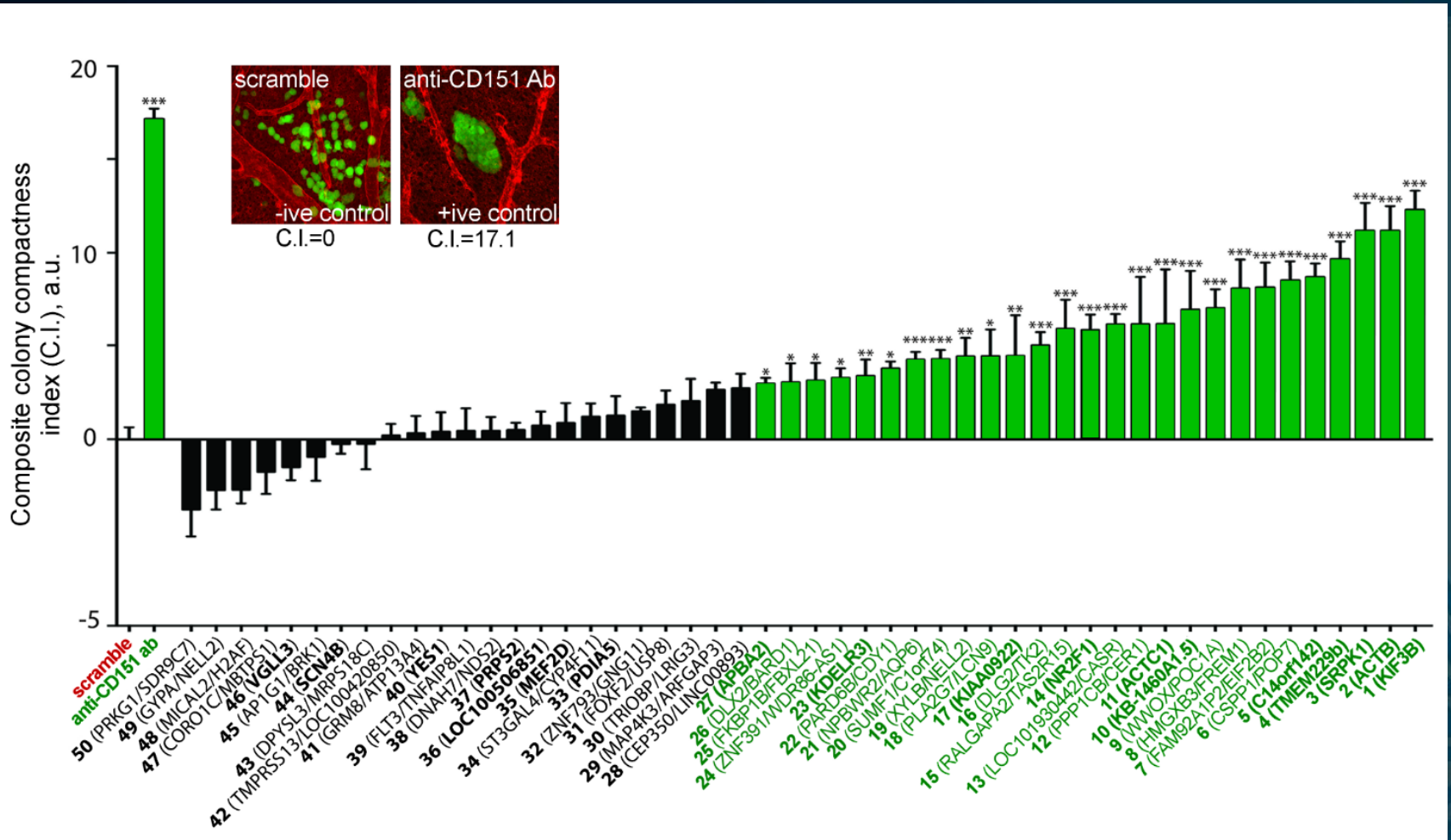
Whole genome *in vivo* screen for mediators of metastasis



Clone #	shRNA IDs	Function	C.I.
	anti-CD151 ab	positive control	17.1±0.5
1	KIF3B	Kinesin motor complex subunit, vesicle transfer	12.4±0.92
2	ACTB	Cell cytoskeleton protein, cytoskeleton organization	11.2±01.2
3	SRPK1	Protein kinase, splicing regulation	11.2±01.3
4	TMEM229B	Transmembrane protein, function unknown	9.7±0.8
5	C14orf142	Expressed at protein level, function unknown	8.8±0.6
10	KB-1460A1.5	Long non-coding RNA; Function unknown	6.9±2.0
11	ACTC1	Cell cytoskeleton protein; Cytoskeleton organization	6.1±2.9
14	NR2F1	Orphan nuclear receptor; Gene expression regulation	5.9±0.7
17	KIAA0922	Expressed at protein level; Function unknown	4.4±2.1
23	KDELR3	Endoplasmic Reticulum Receptor; Protein sorting	3.4±0.8
27	APBA2	Neuronal adapter protein; Vesicular trafficking	2.9±0.3
	Scramble	negative control	0.0±0.6

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*

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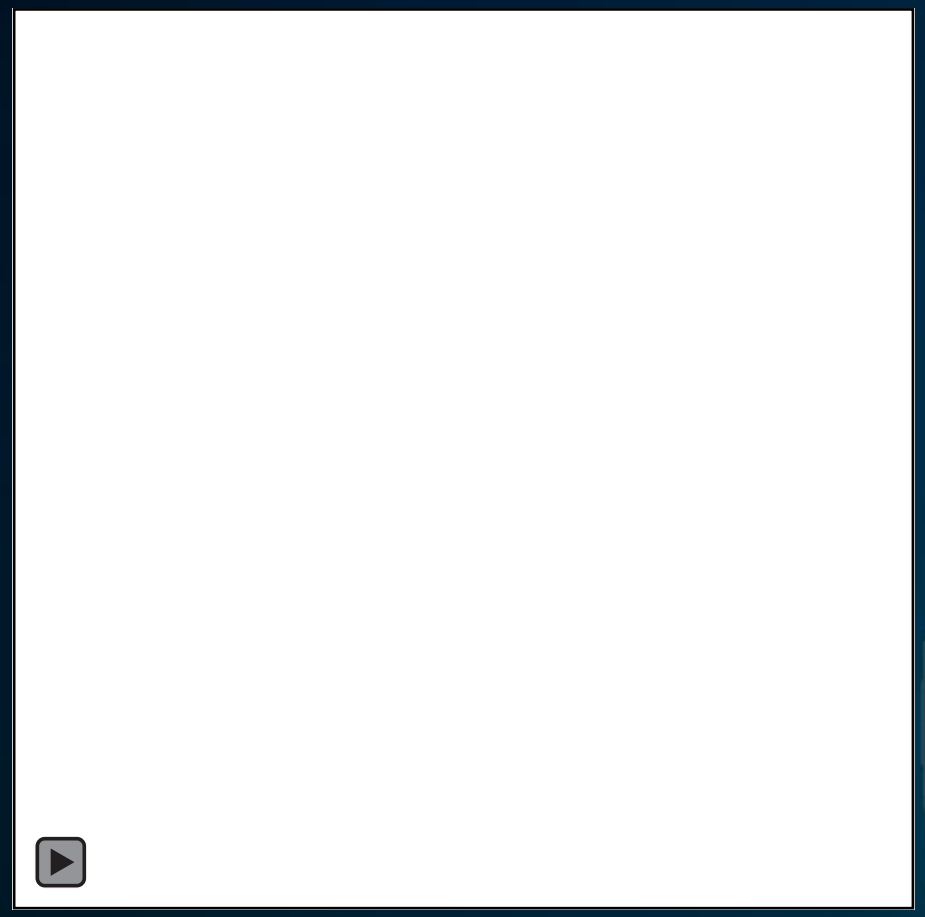
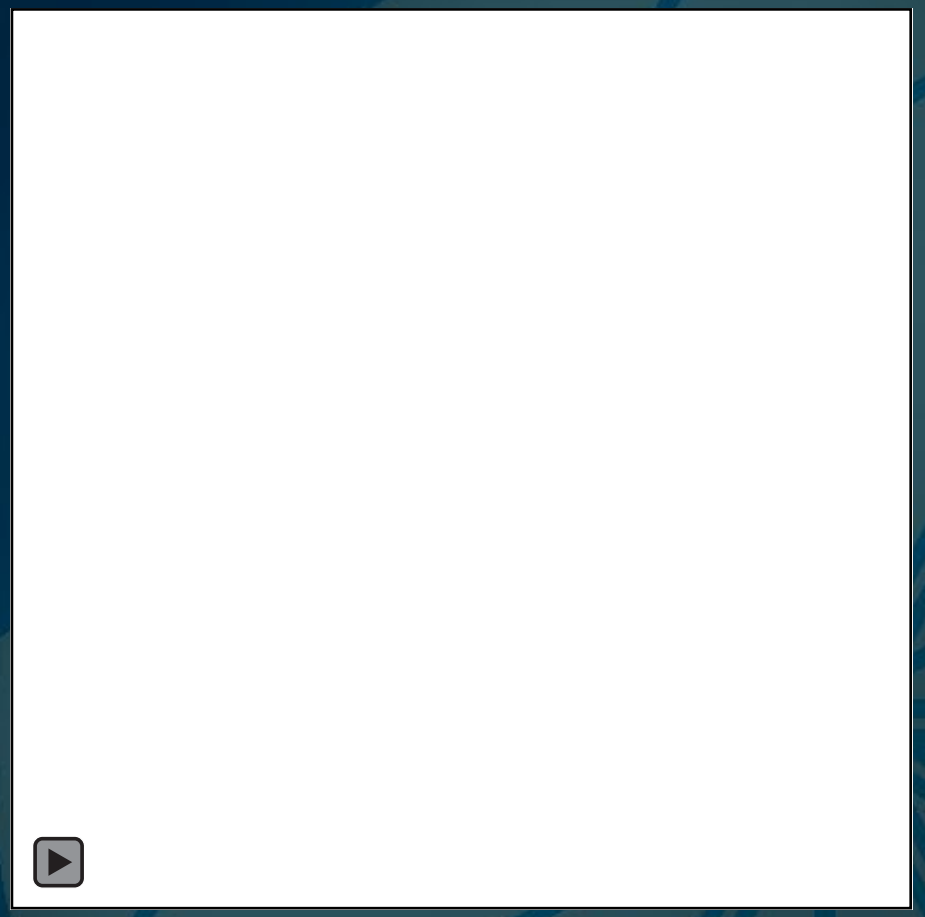
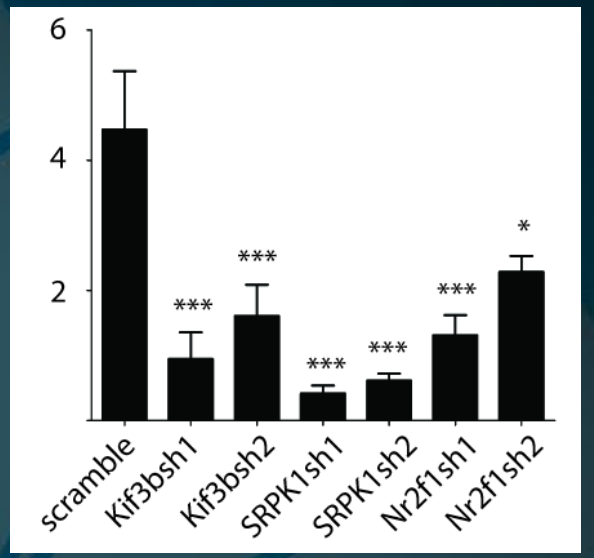
Screen hits are required for invasive cell migration *in vivo*

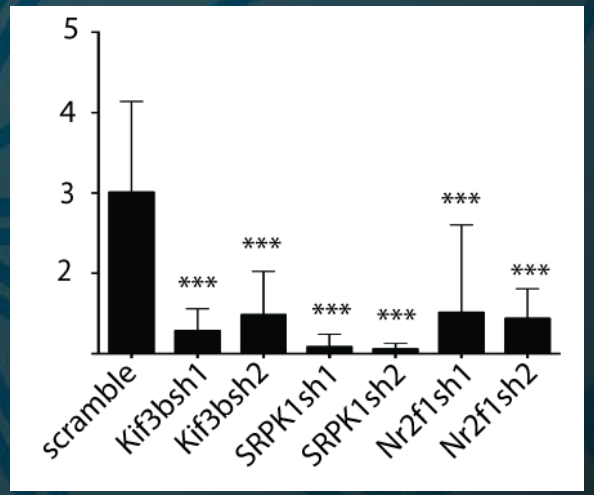
In vivo cell migration assays (spontaneous and experimental metastasis)





Displacement ($\mu m^{-2}/sec$)





Stoletov et al., *Nature Communications, 2018*

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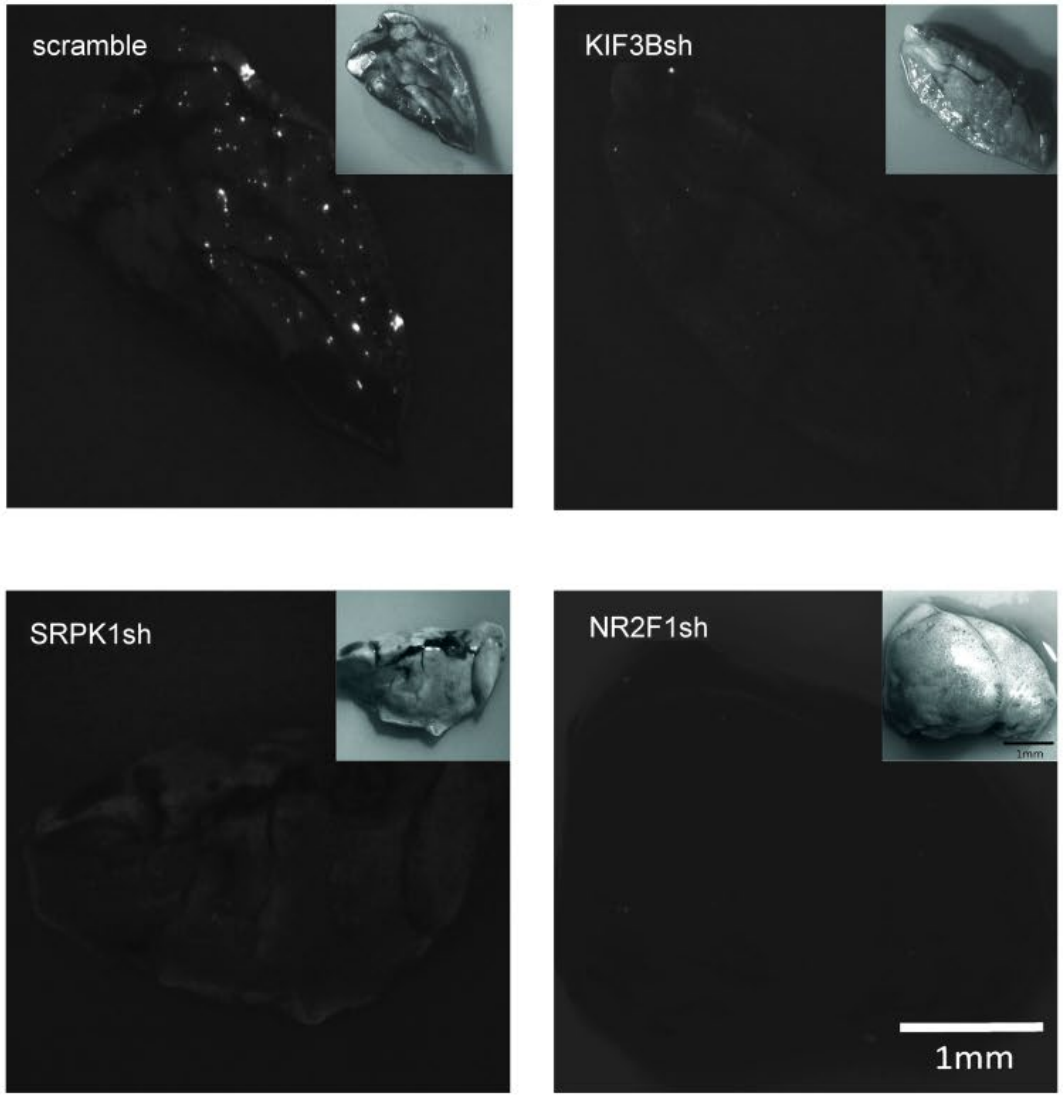
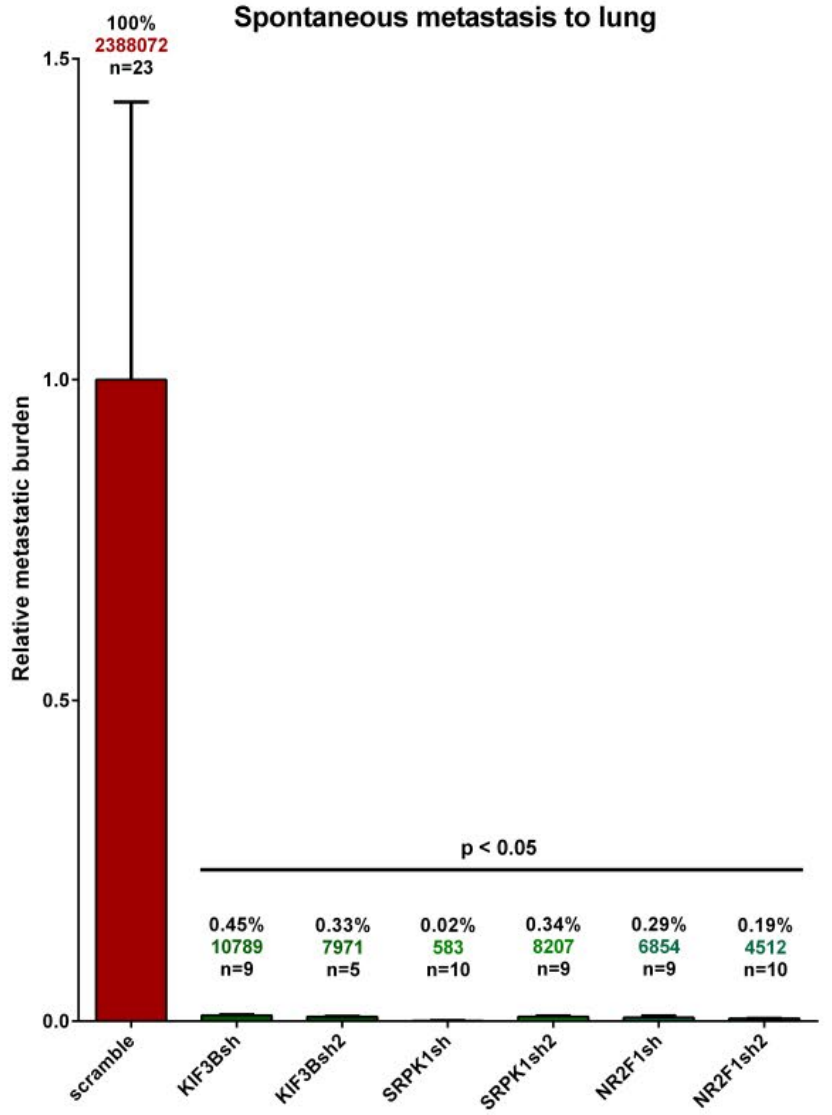
Inhibition of screen hits blocks metastasis *in vivo*





Stoletov et al., *Nature Communications, 2018*

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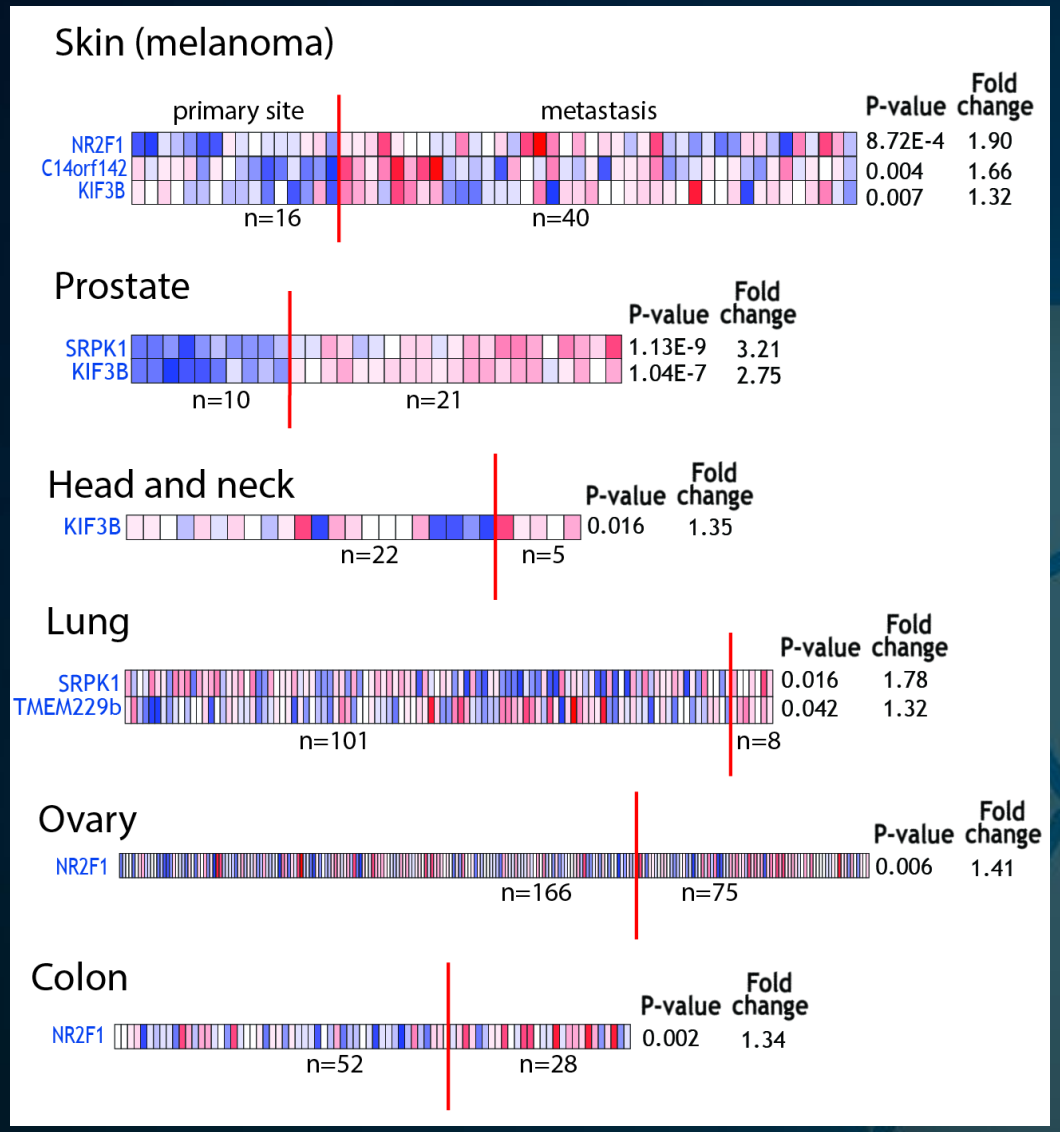
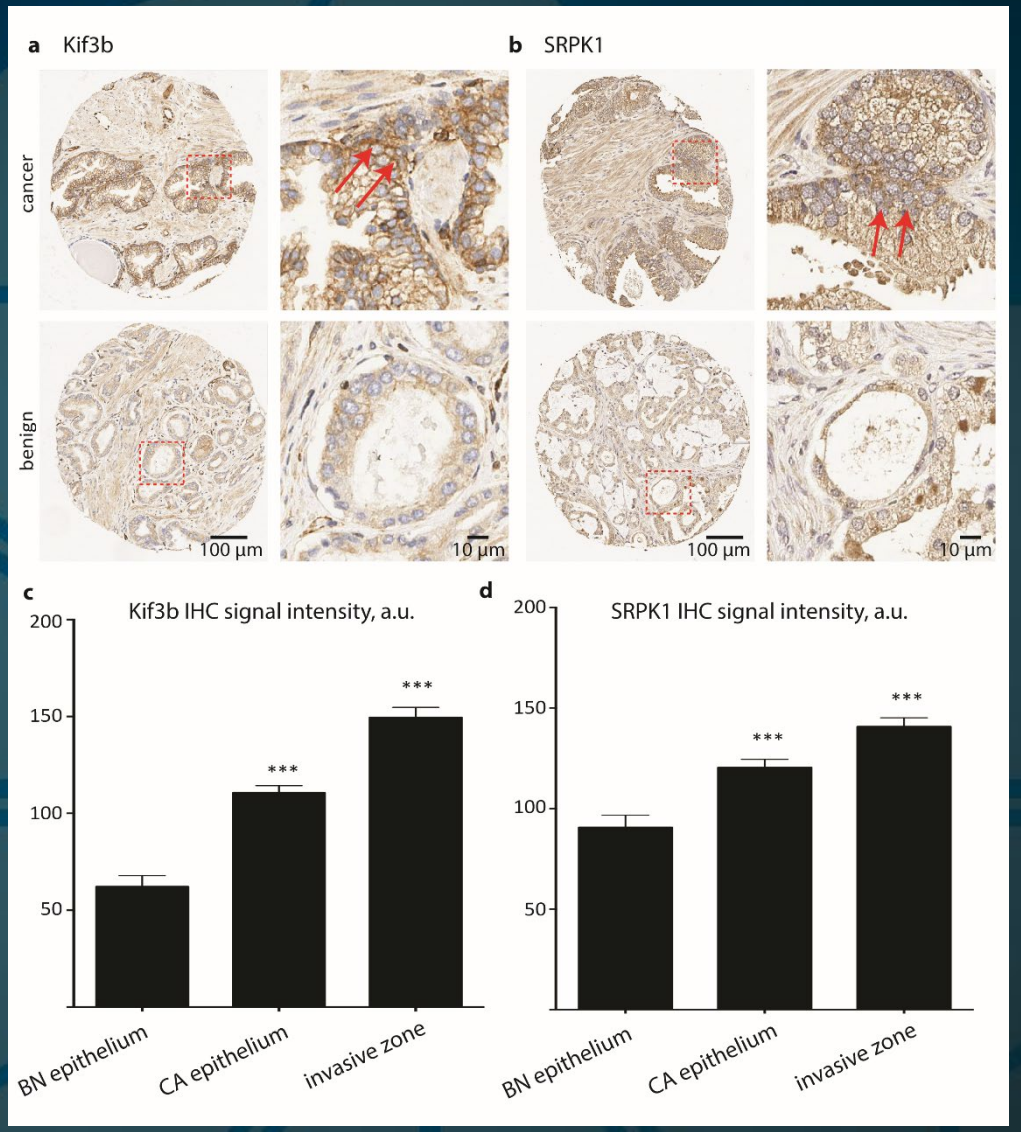
Screen hits associated with metastatic cancers and poor prognosis

Expression in matched primary tumour/metastasis



Staining of 98 patient prostate cancer TMA with SRPK1 and Kif3b



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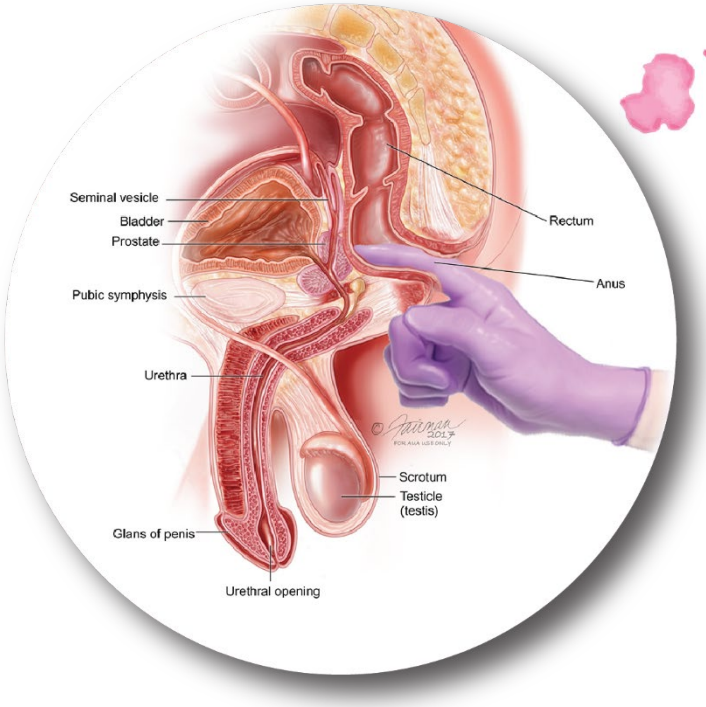
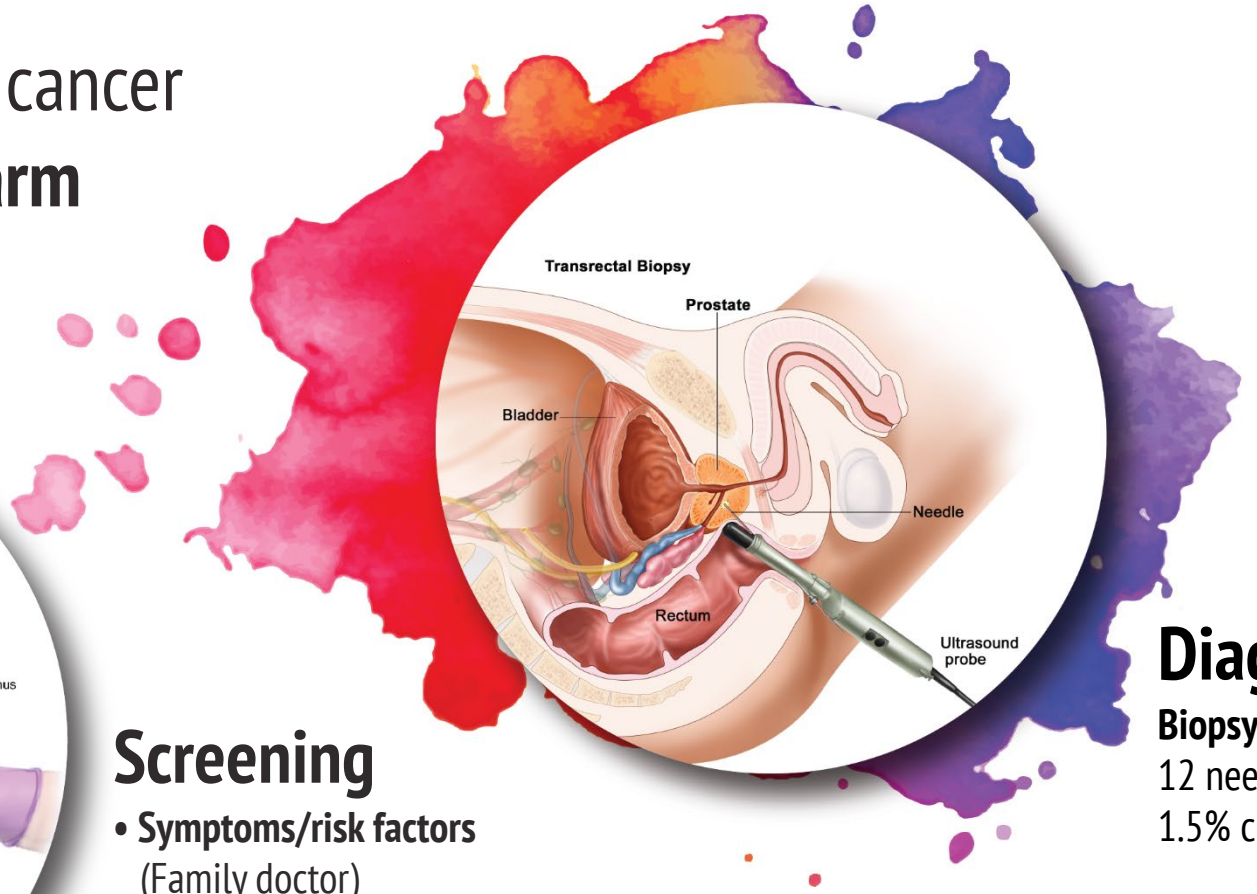
Can we use this information to improve screening?

If “cell motility switch” genes are required for prostate cancer spread, perhaps we can incorporate them into a test to <u>detect</u> 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

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Serious Adverse Events (SAE) from biopsies

- >1M biopsies are done per year in the US[1,2]
- Incidence of sepsis following transrectal ultrasound guided prostate biopsy ranges from 2-4% in developed countries and can go a high as >9% in developing countries[3,4]
- Antibiotic resistance and sepsis are on the rise[1]

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

1. Sanders A[1], Buchan N. ANZ J Surg. 2013, 2. Loeb S. J Urol. 2011, 3. Robert K.Nam. J Urol. 2013, 4. Mohammed Shahait. Int Braz J Urol. 2016

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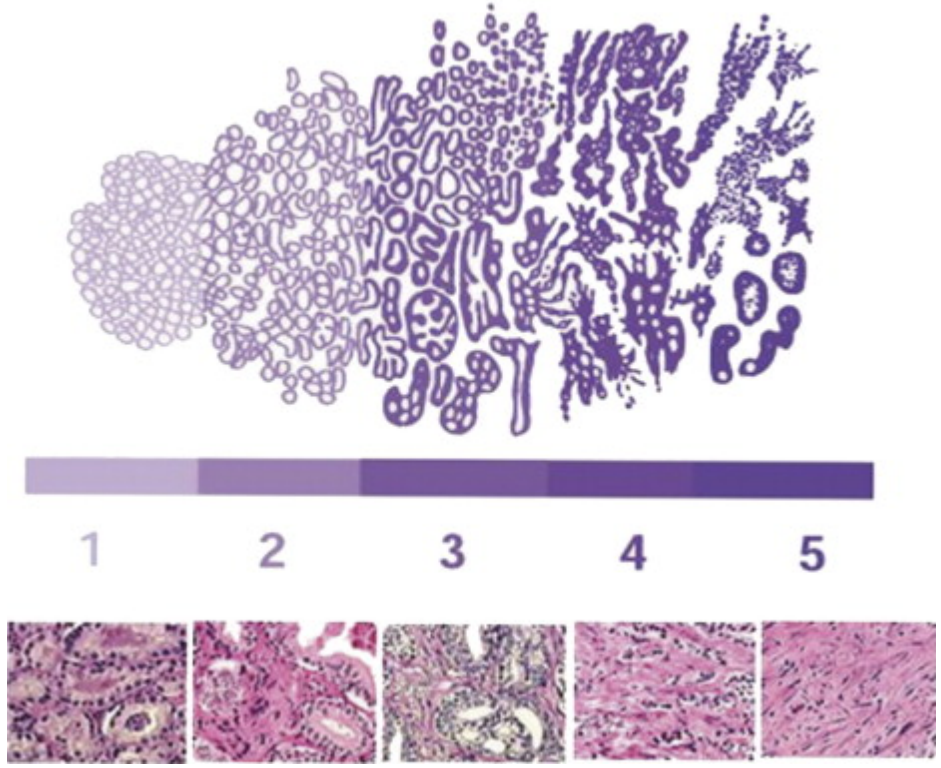
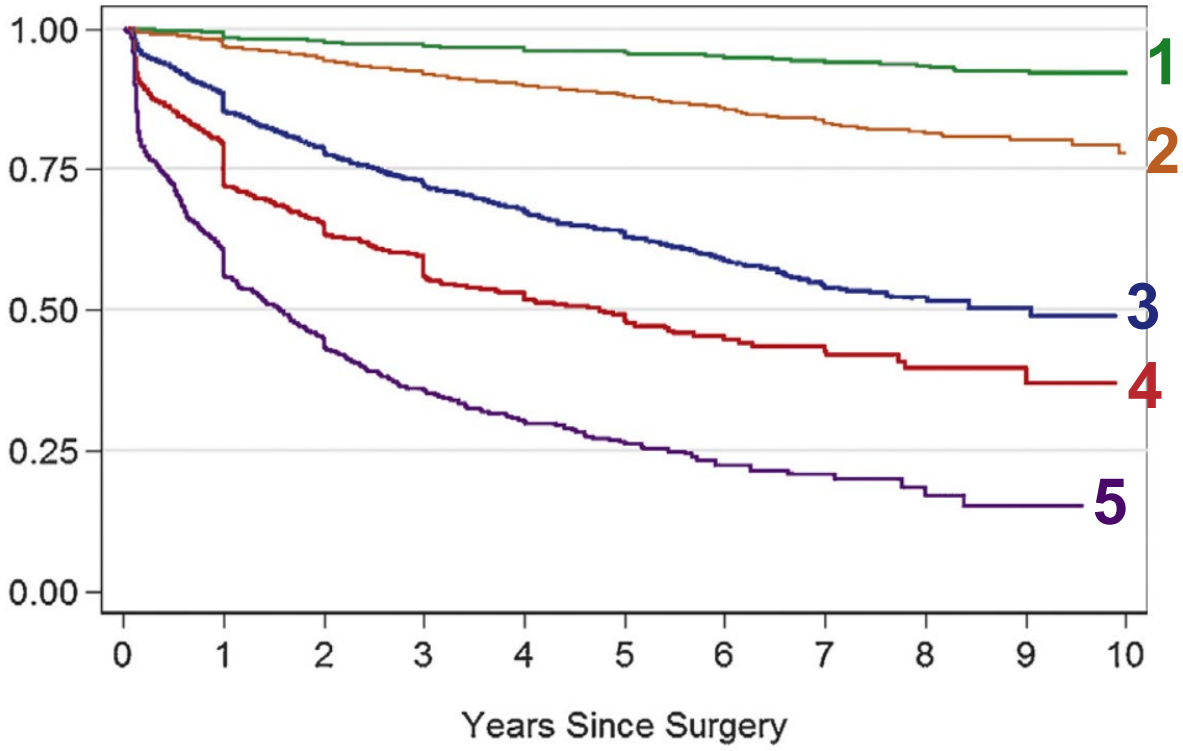
Prostate cancer is a **heterogeneous disease**: some forms are **lethal**, others are not



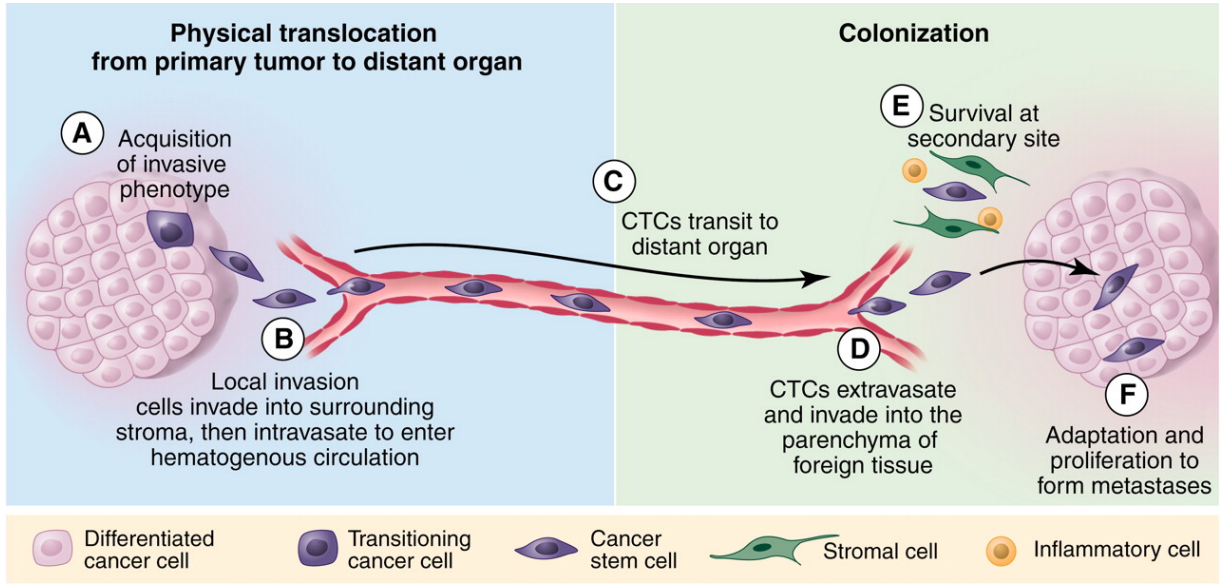


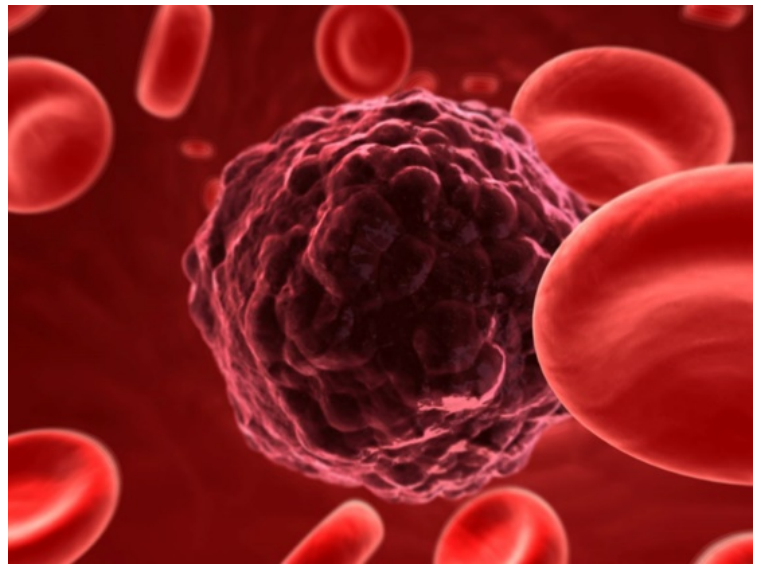
Men with Gleason Grade Group 3-5 prostate cancers have significantly worse outcomes

Epstein et al. 2015, European Urology, 69, 428-435

NANOSTICS

Circulating tumour cells (CTC) as biomarkers





- **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)**

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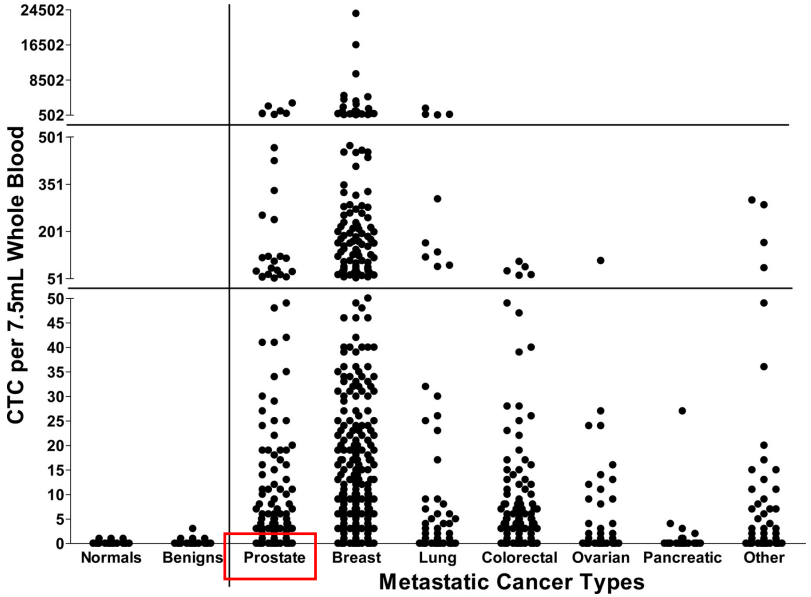
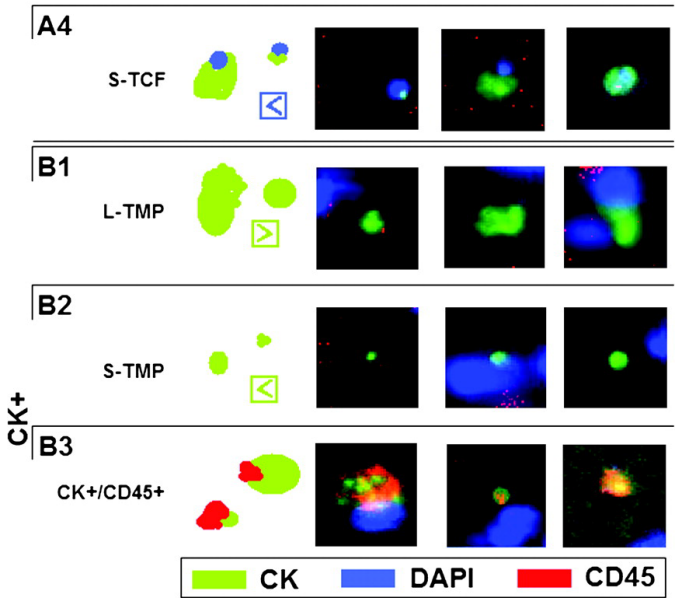
A problem of numbers…

CTC numbers too low even in patients with confirmed metastatic disease



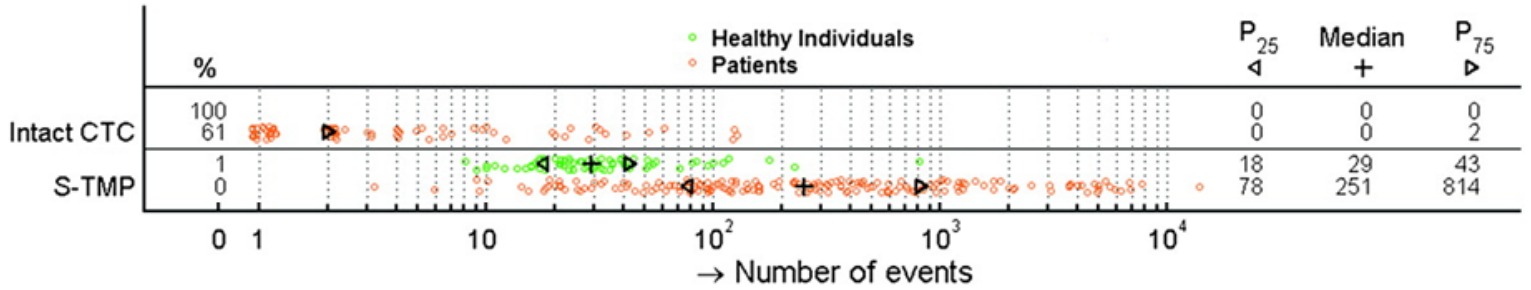
43% have <2
18% have 0

But many cancer cell "events" don't meet the criteria for CTC



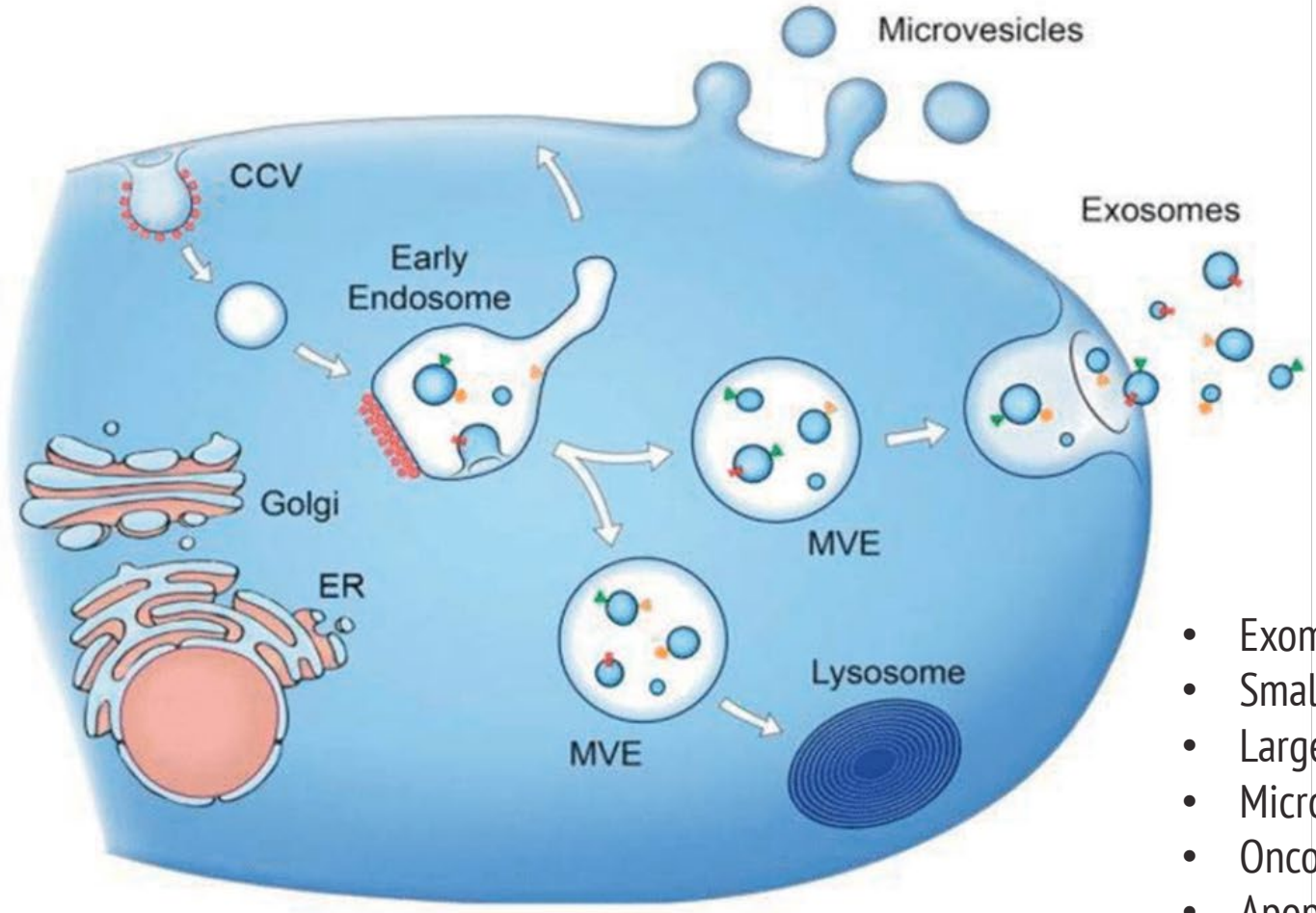
Allard WJ *Clin Cancer Res* 2004

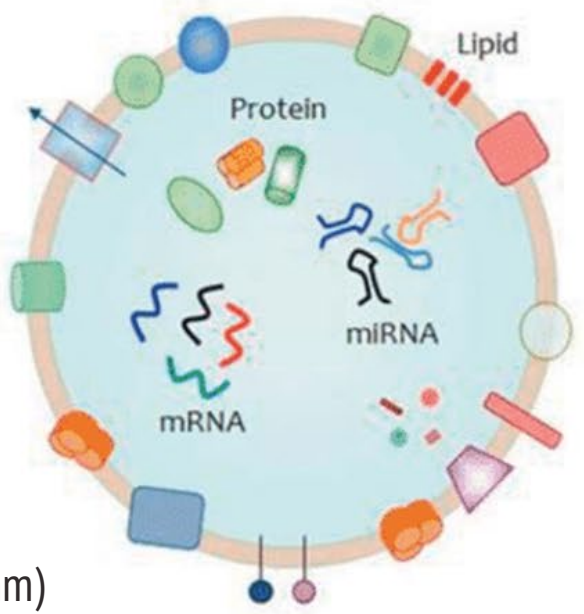
Extracellular vesicles (EVs) provide much greater dynamic range than CTCs



Coumans FAW *Annals of Oncology* 2010

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?**

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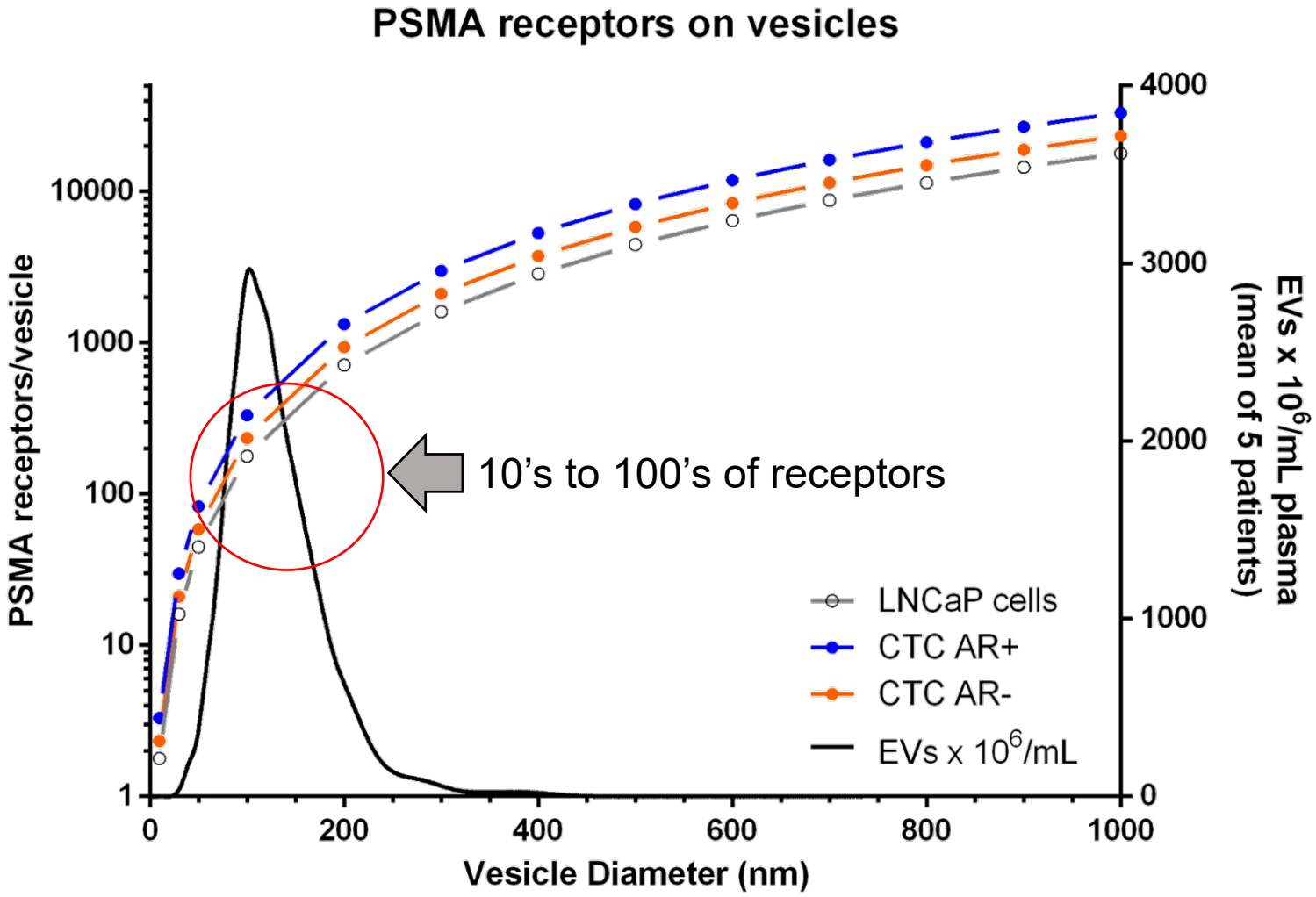
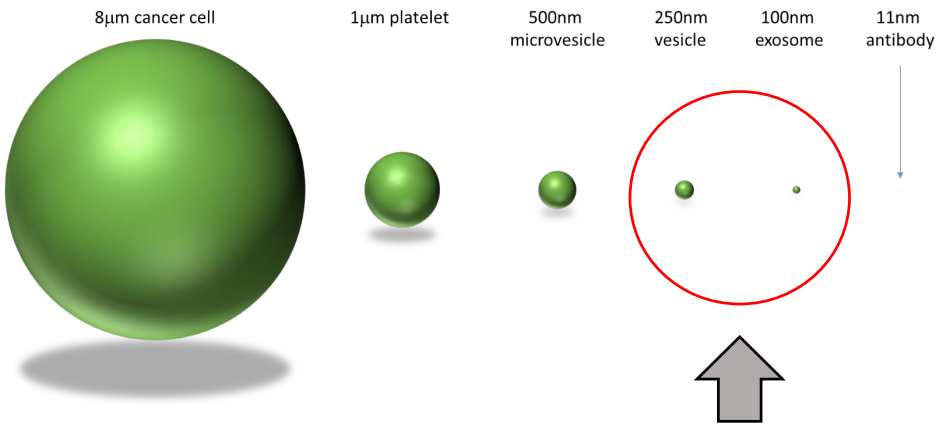
The challenge: single particle detection of prostate EVs





How small can we go?

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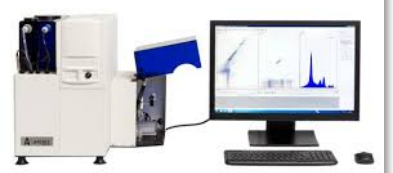
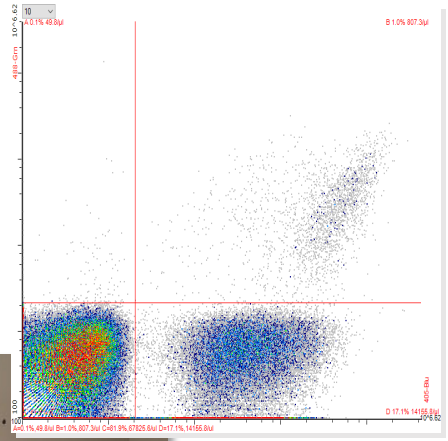
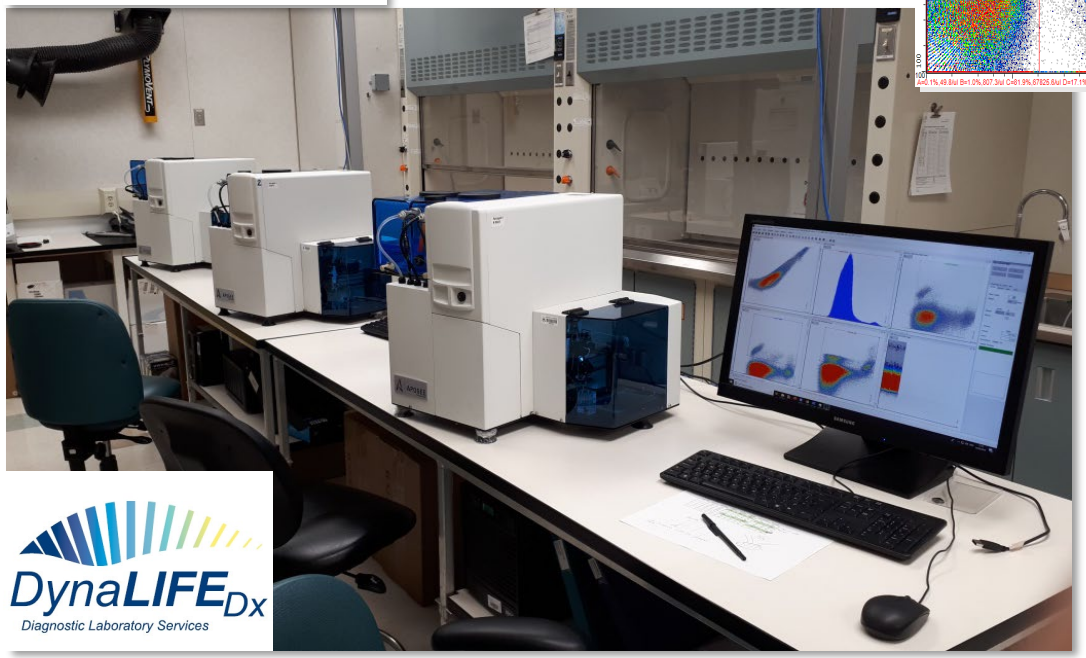
MicroFlow Cytometry can detect and characterize a wide range of EVs

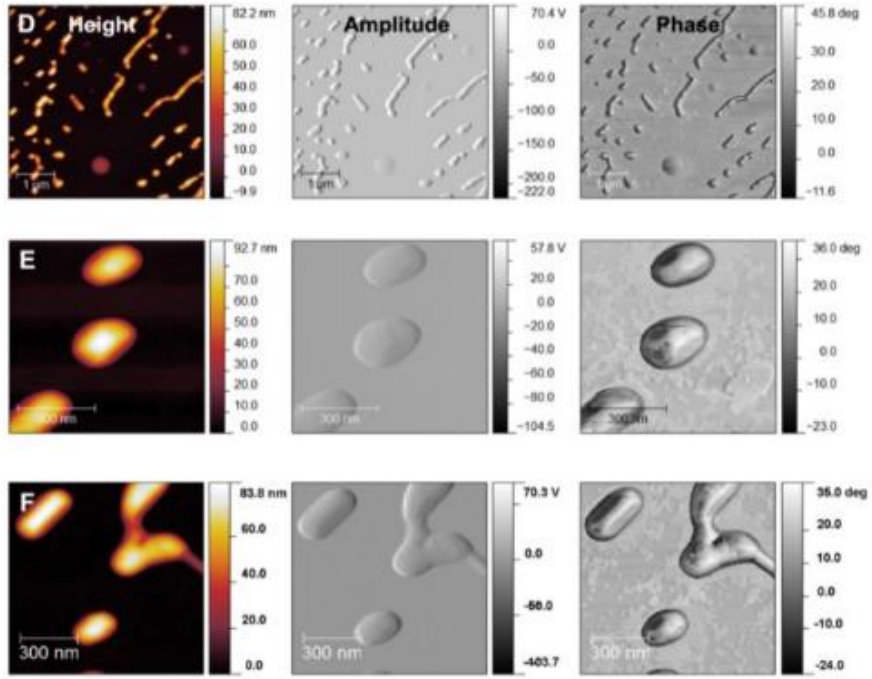
APOGEE FLOW SYSTEMS







DynaLIFE Dx
Diagnostic Laboratory Services



Leong et al., J of Thrombosis and Haemostasis, 2011

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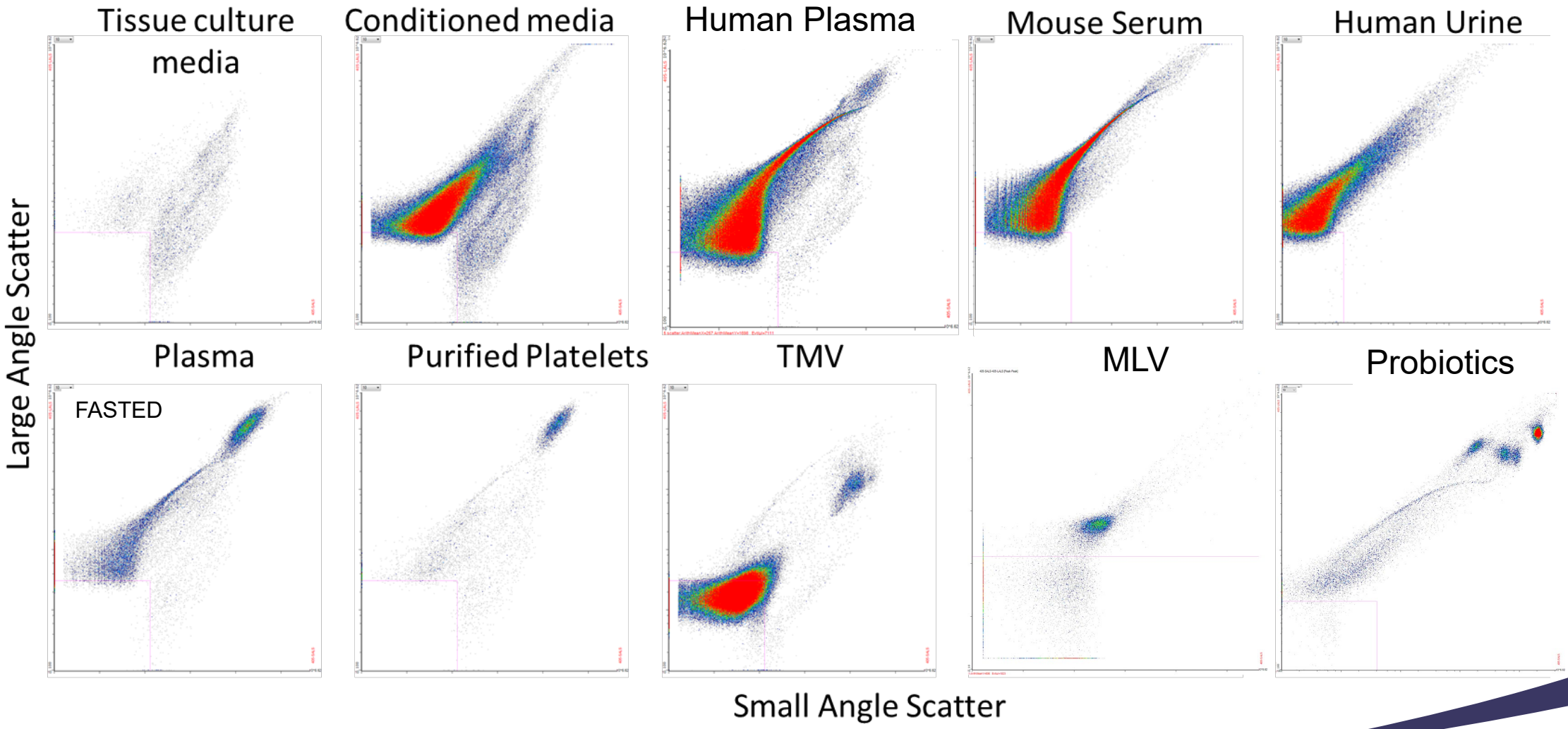
MicroFlow Cytometry resolves small biological particles



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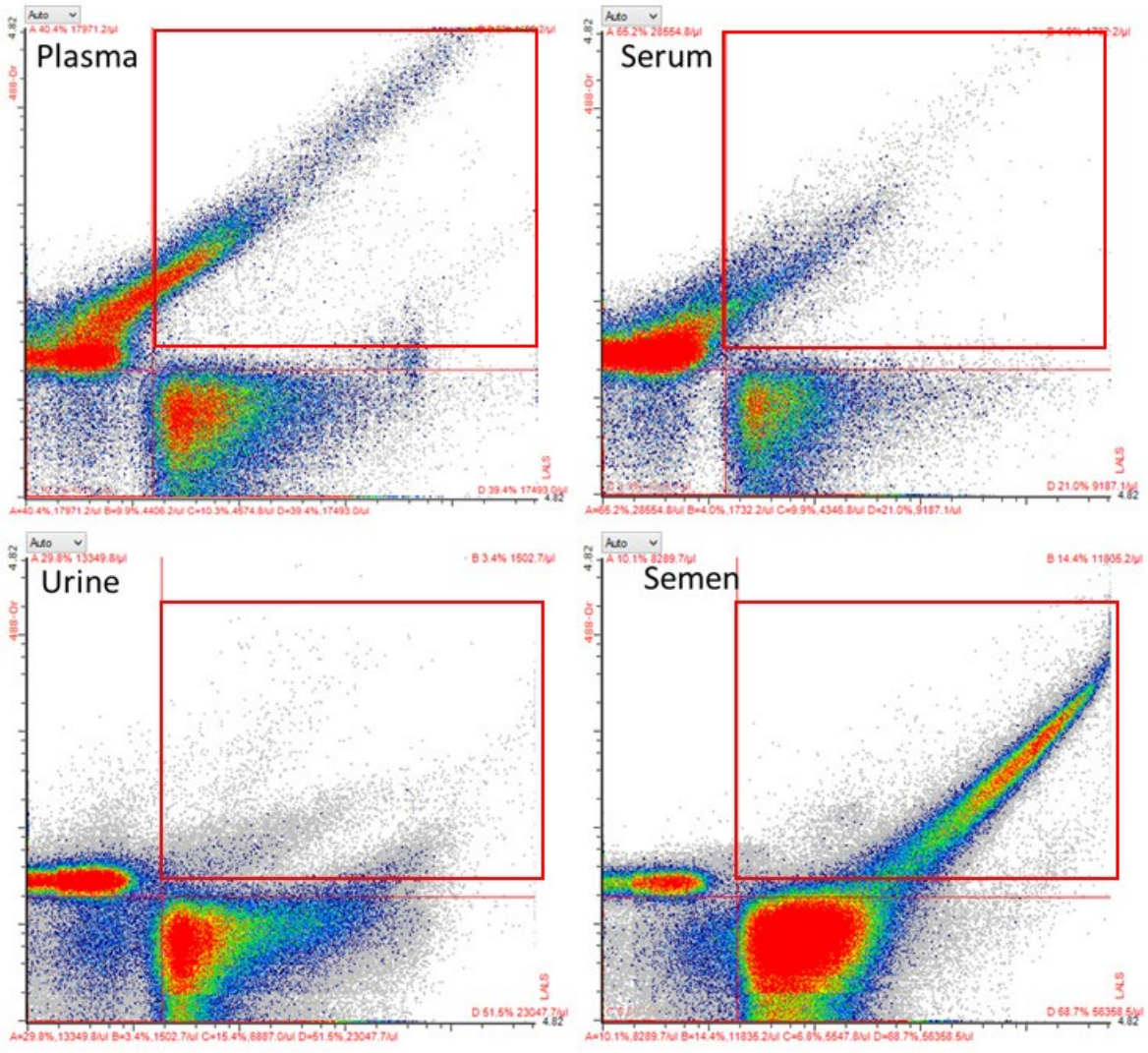
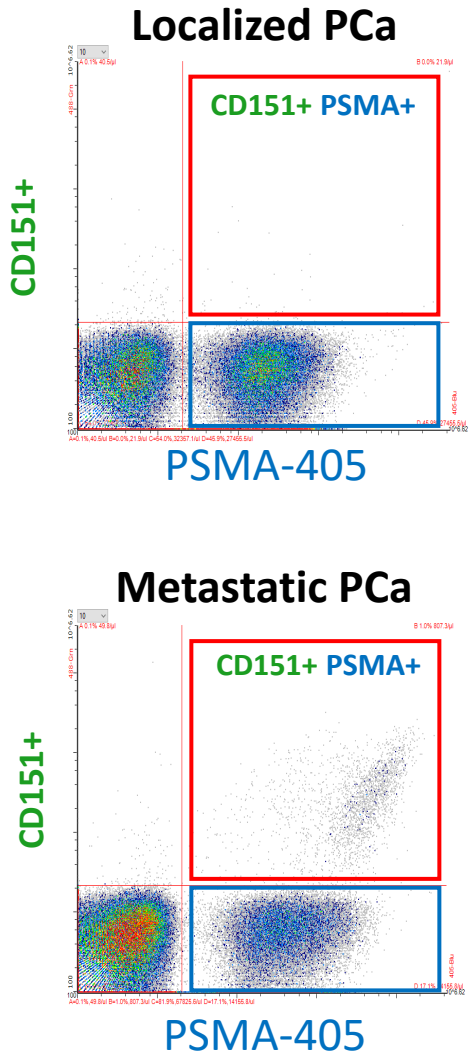
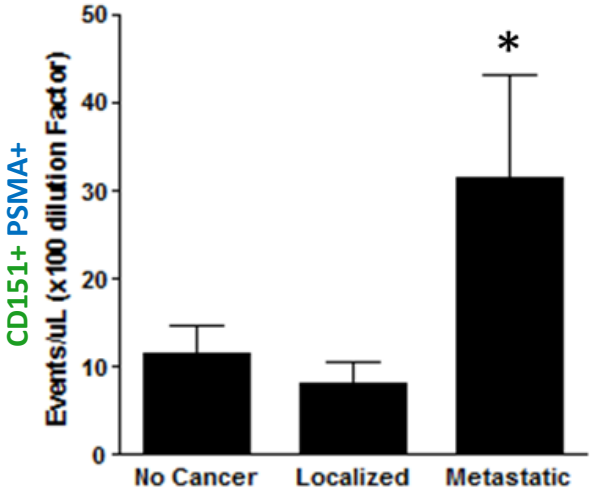
Detection of **prostate-derived EVs** in complex biofluids





66 patient cohort
University Health Network - Toronto



Age, PSA matched

Biofluid: plasma

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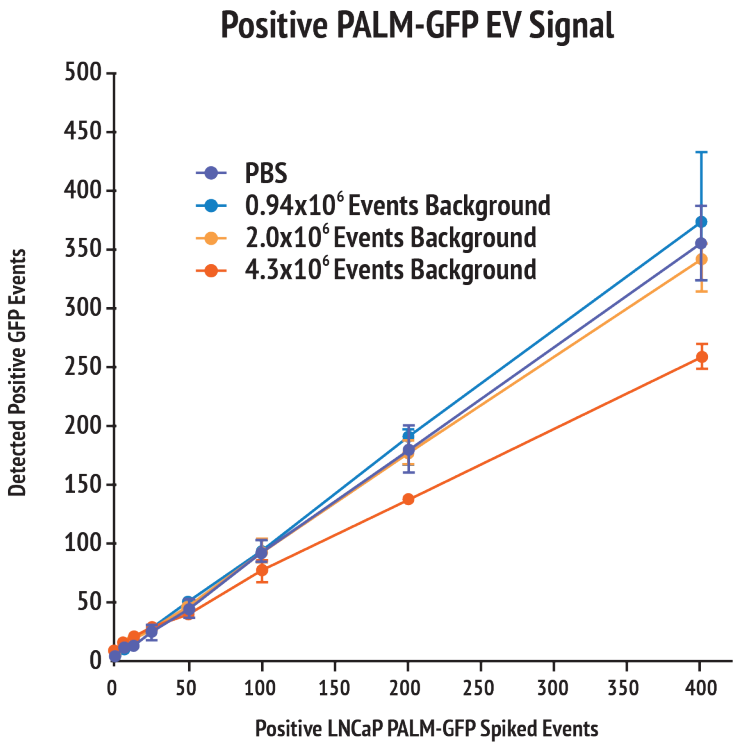
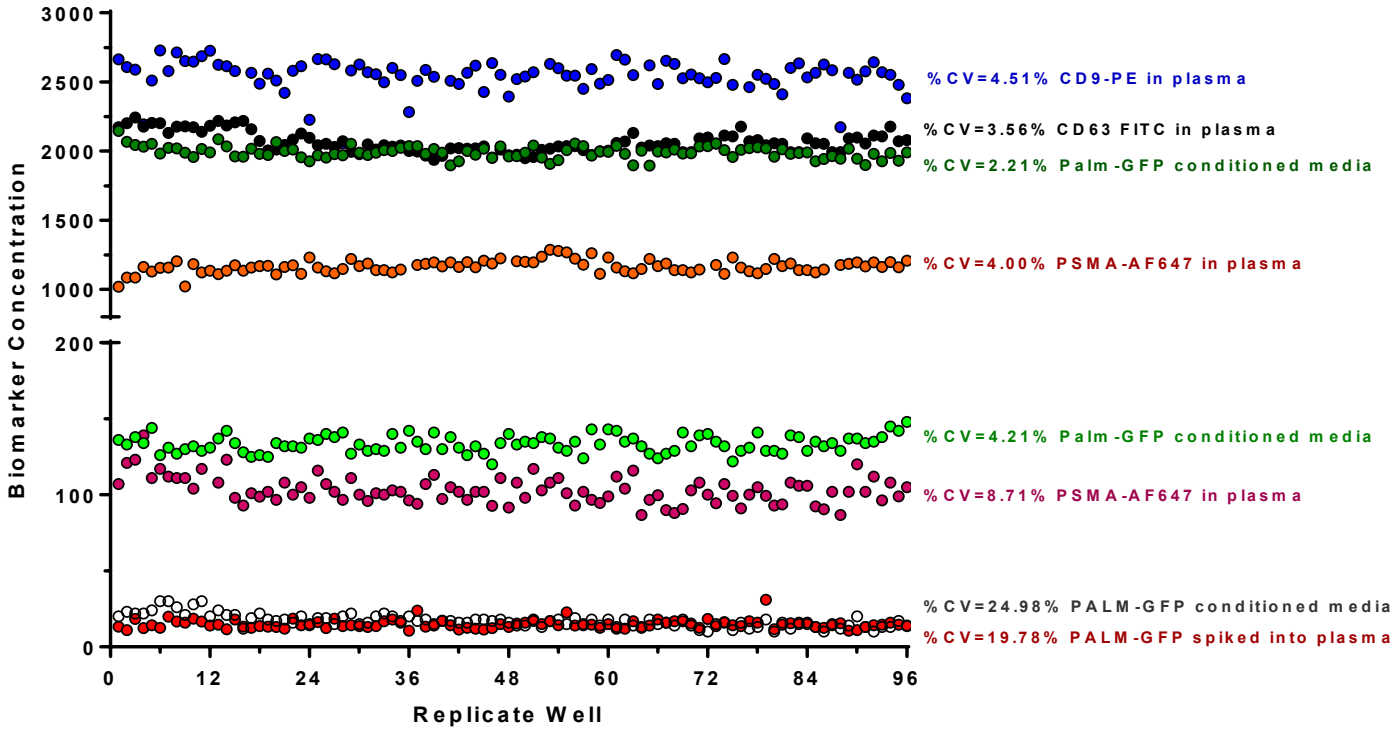
Microflow cytometry of plasma EVs **is highly sensitive and reproducible**



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

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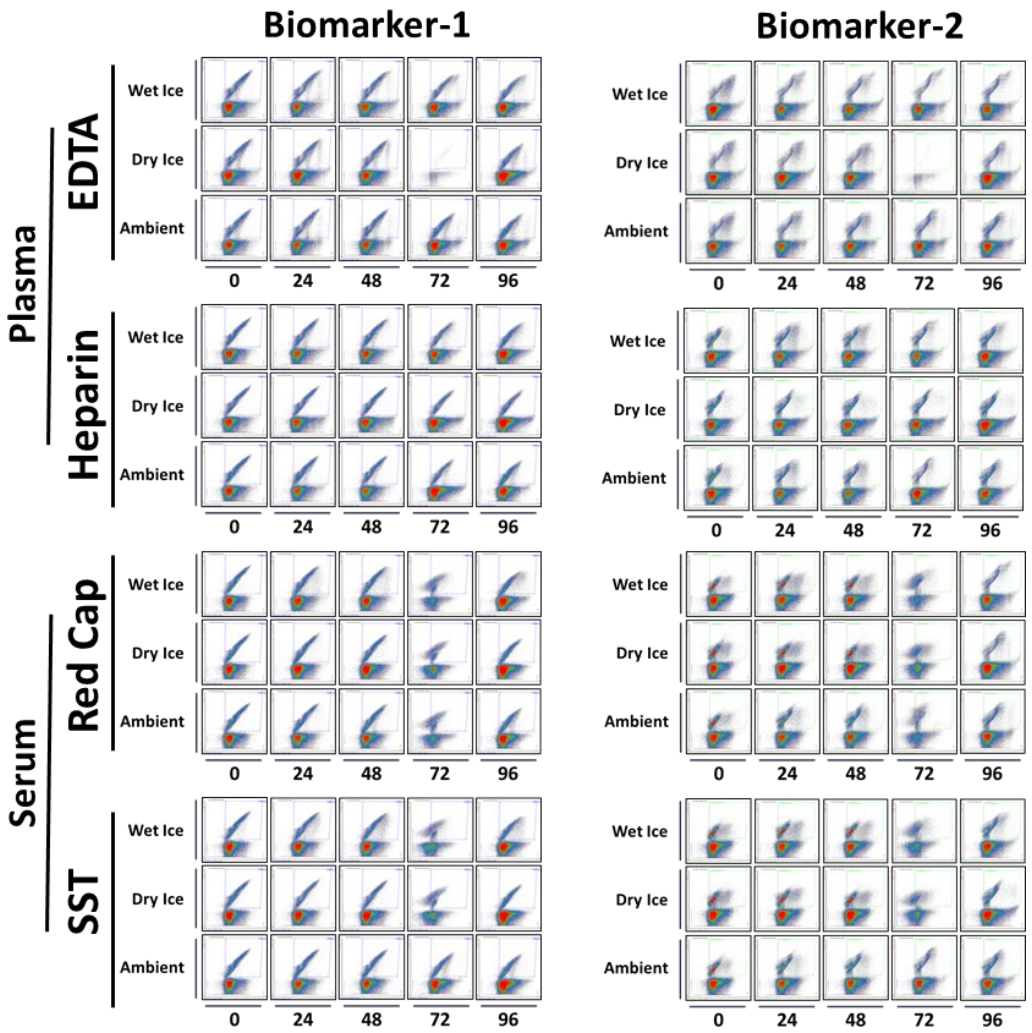
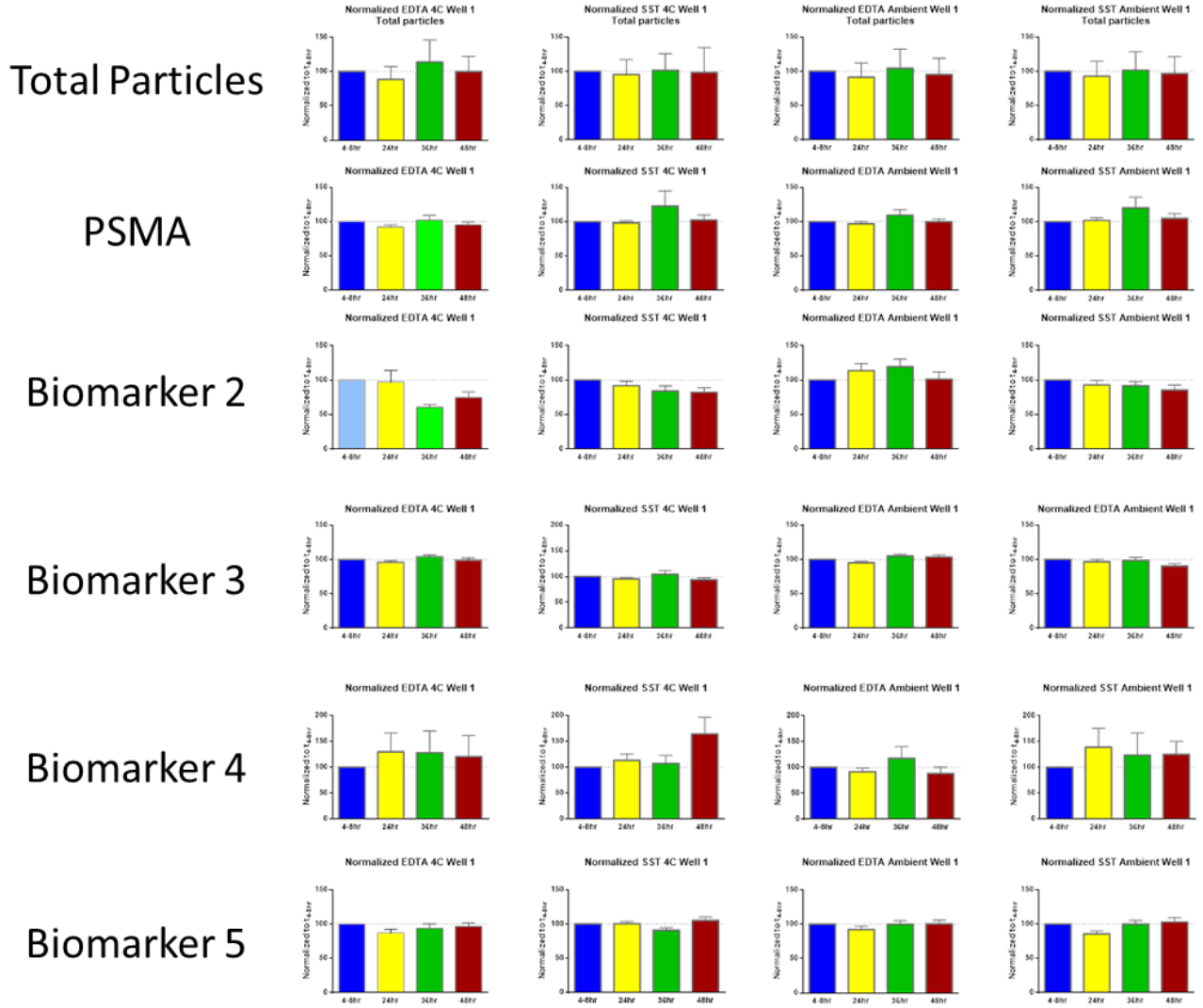
Sample stability of EVs in human plasma and serum

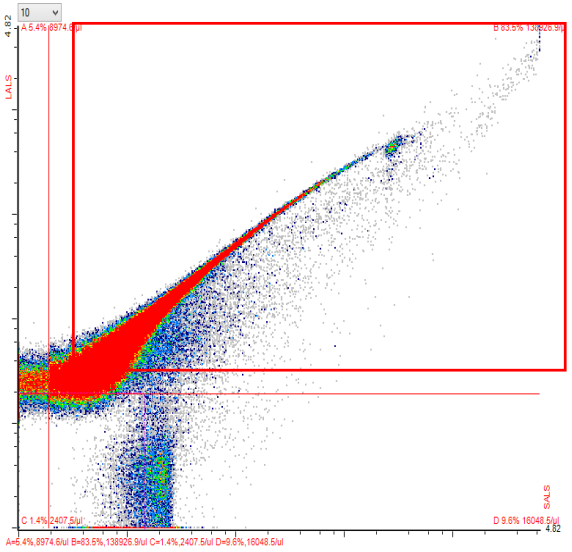
Sample stability matrix



Sample stability quantification



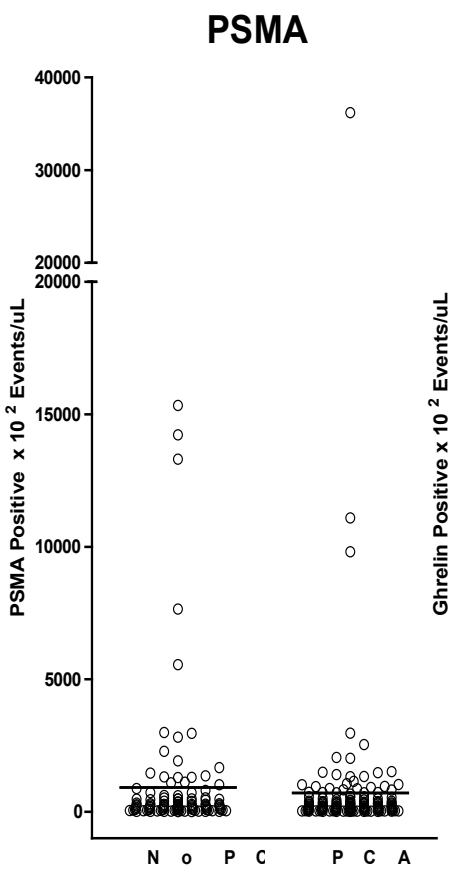
Combining size and surface biomarkers: **disease prediction?**



PSMA+

Ghrelin
(Lu et al, Prostate 2012)

Metastasis-associated biomarkers



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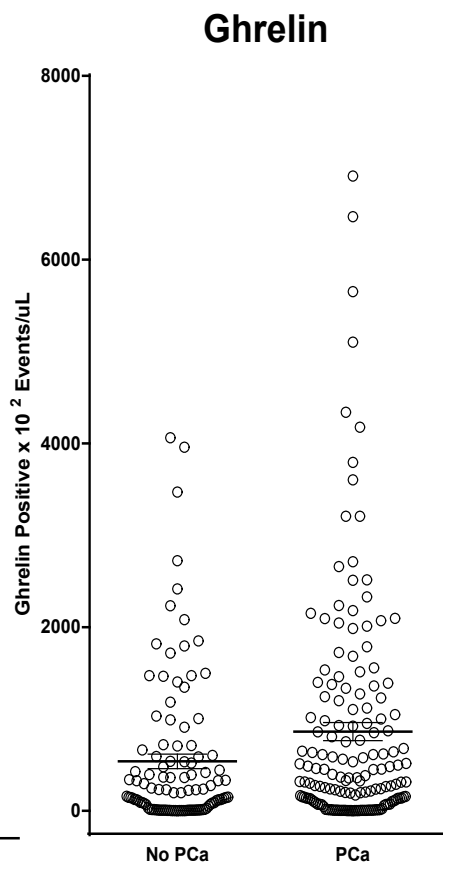

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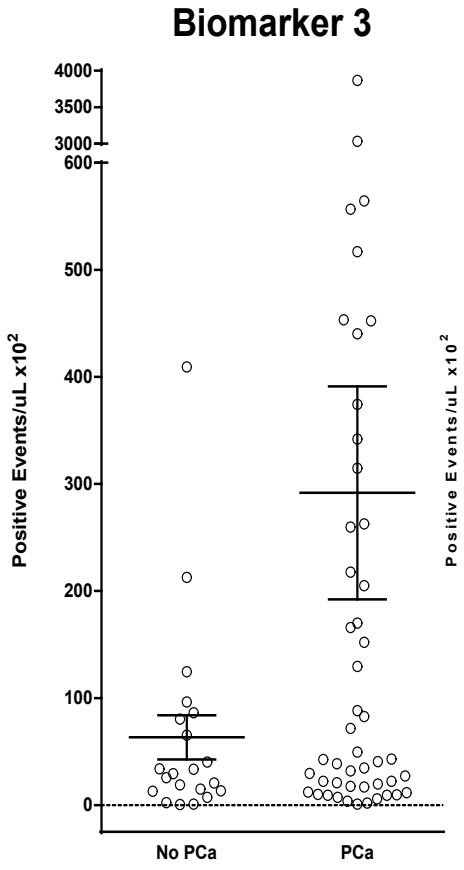
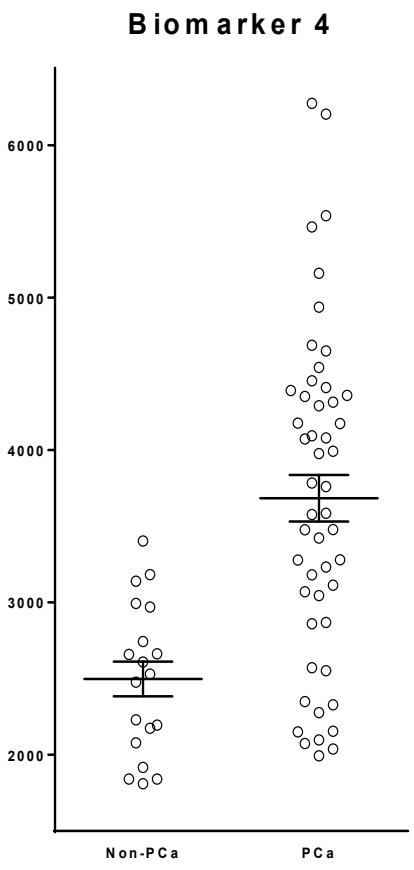


P=0.0144



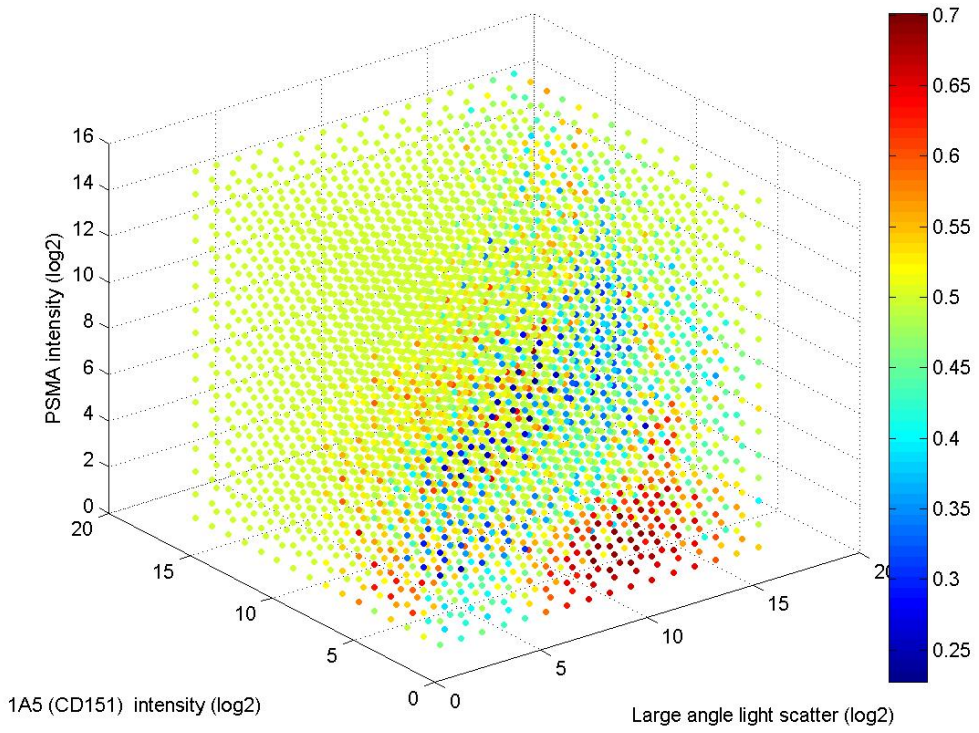
P<0.0001

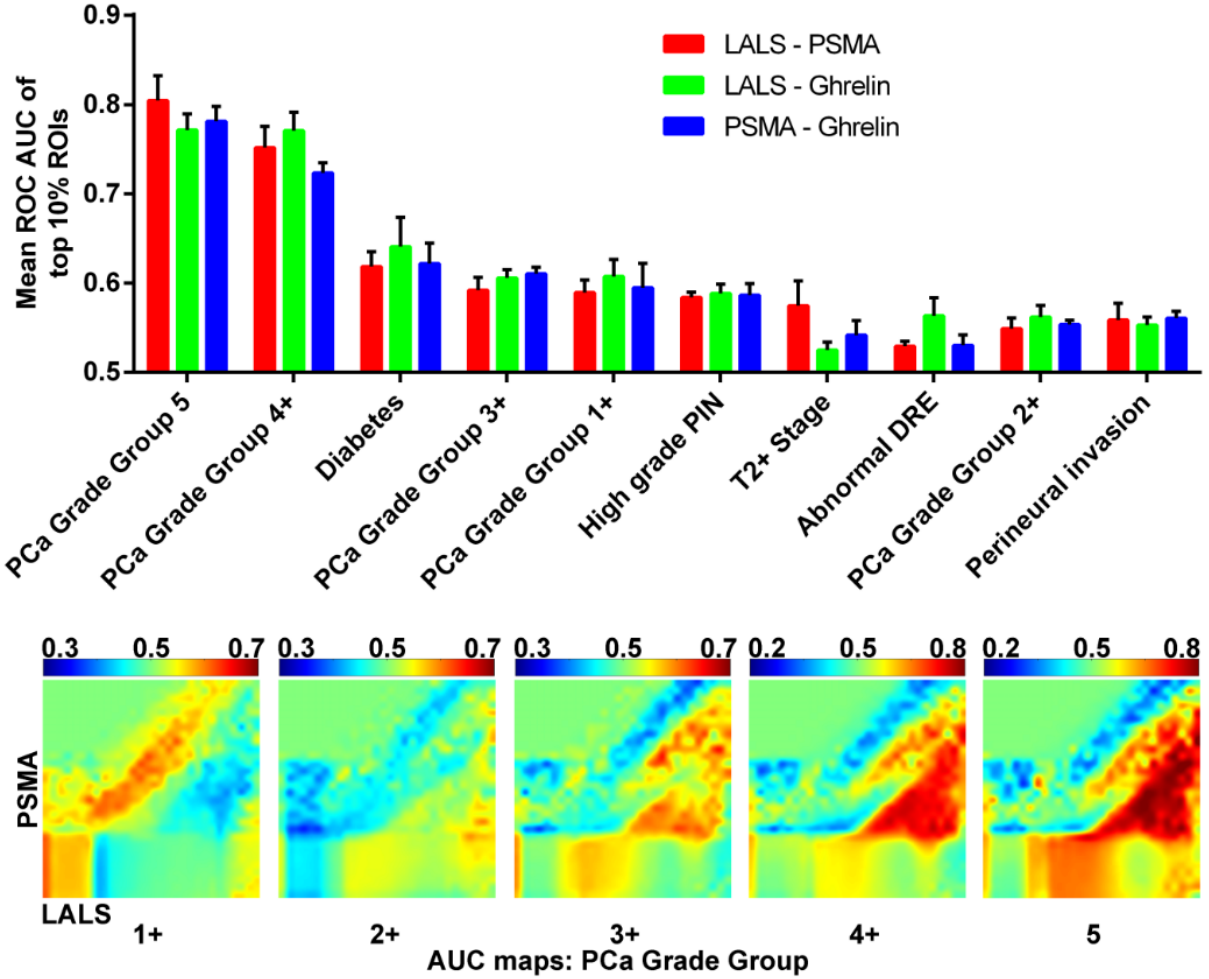
Analyzed using one-tailed t-test with Welch's corrections

NANOSTICS PRECISION HEALTH

Machine learning approach to generate classifiers from multi-dimensional microflow cytometry data

3D plot of ROC area under the curve





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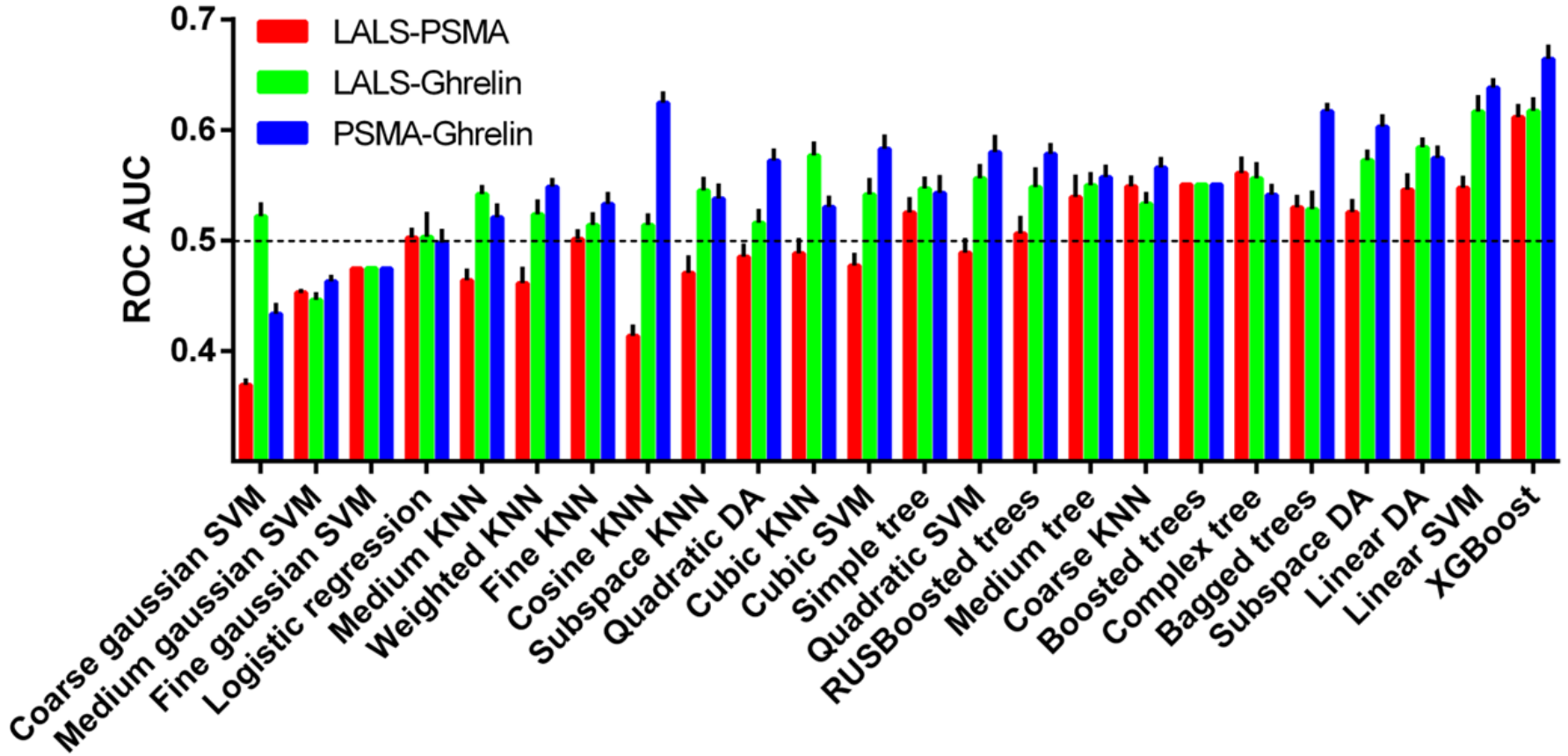
XGBoost provides highest AUCs for predicting clinically significant prostate cancer



Ensembled: Results of multiple decisions trees averaged into 1 result

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

XGBoost is an ensembled, boosted, decision tree-based model.

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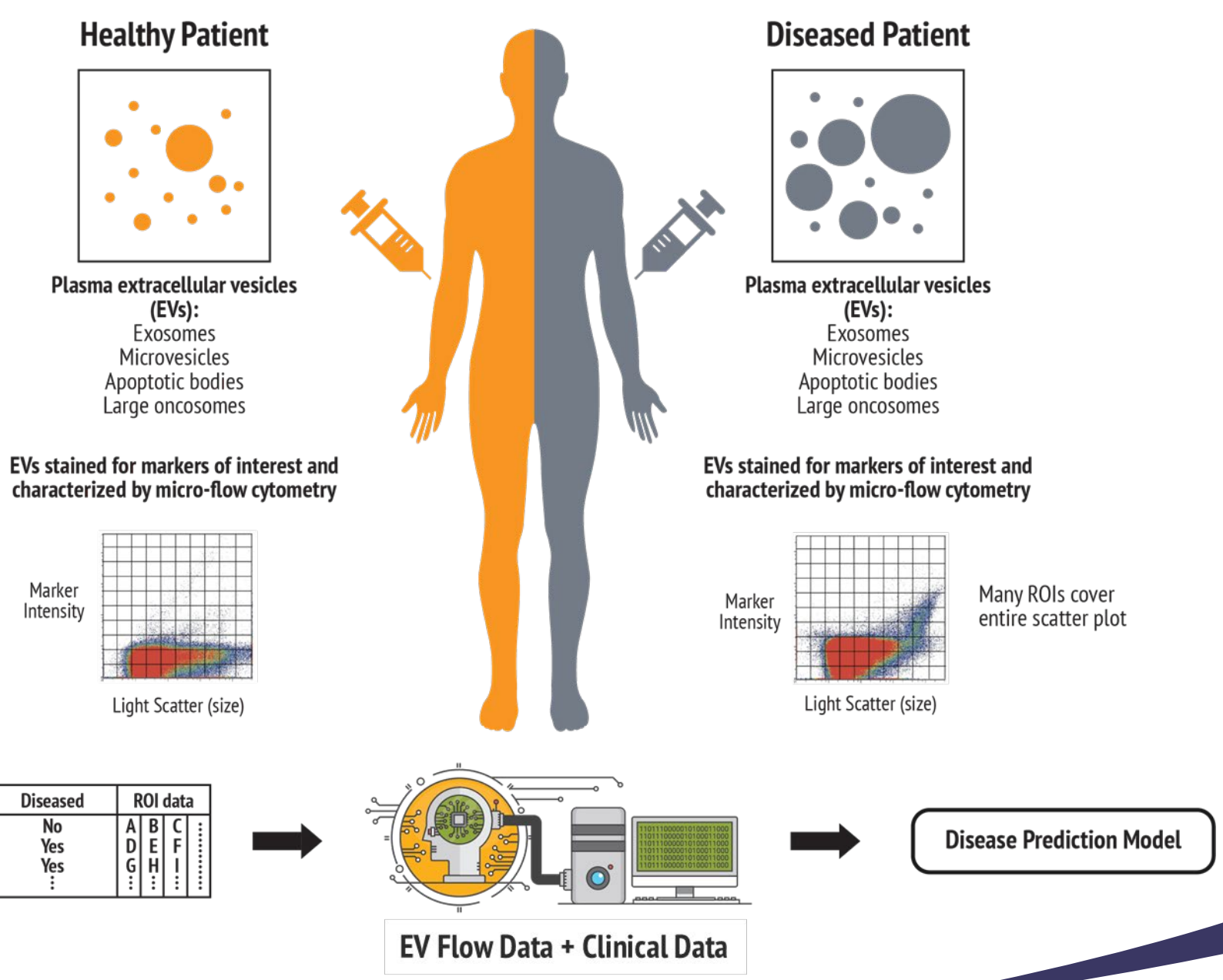
Nanostics' platform technology generates **EV fingerprints to predict disease with a liquid biopsy**



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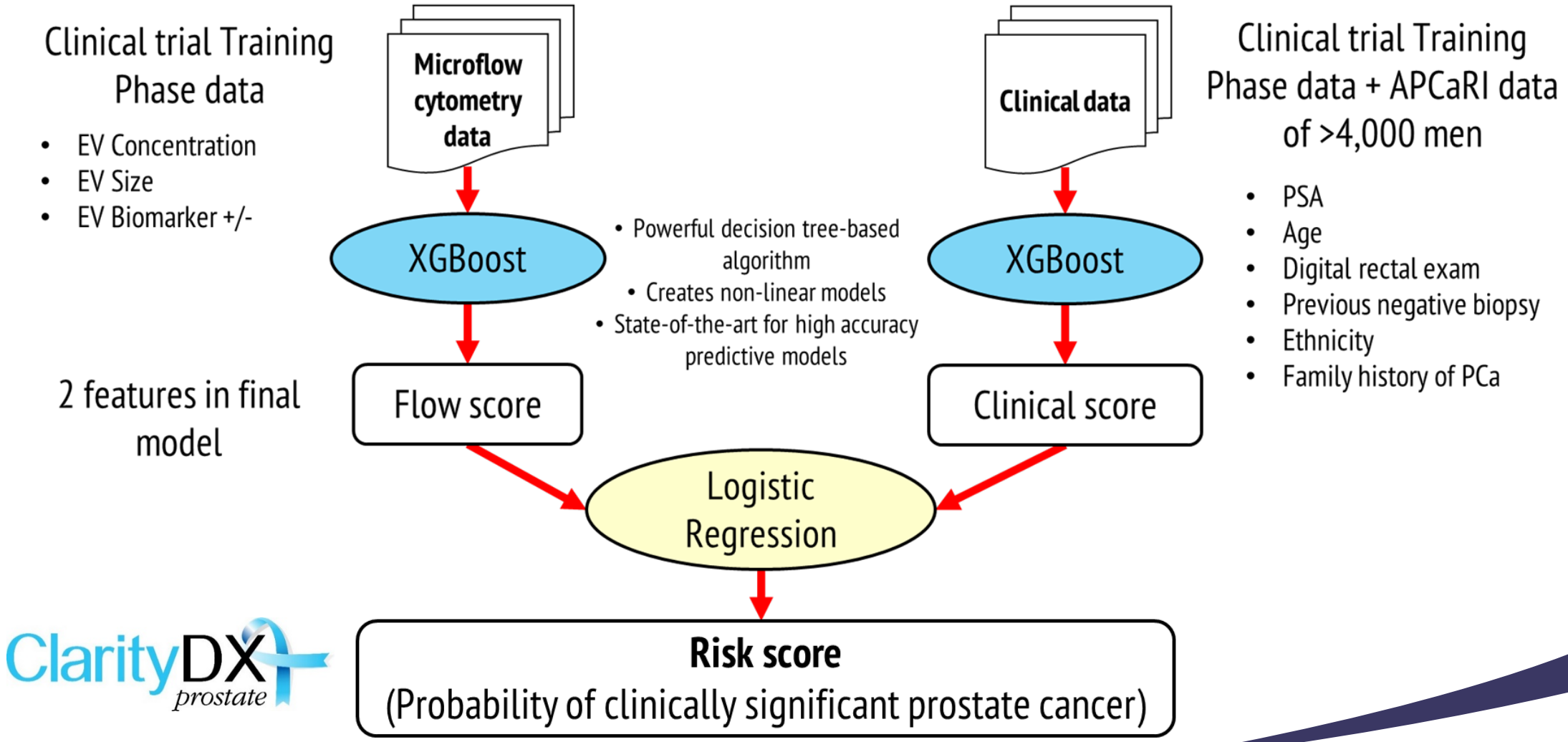
Generating the **ClarityDX Prostate** Risk Score



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Prospective pre-diagnosis cohort
in Alberta, Canada

ALBERTA
PROSTATE CANCER
RESEARCH INITIATIVE
knowledge | action | impact

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

Identified by urologist - refer to CRC at clinical site

⊛Informed Consent Biospecimens are collected
⊛Intake Survey/QOL 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

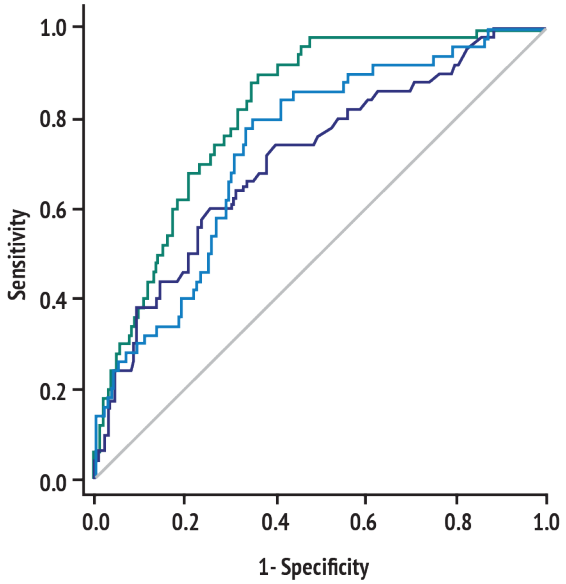
PCa **is** detected

PCa **is not** detected

Validation of **ClarityDX Prostate** in a **377 patient** prospective cohort

Training cohort

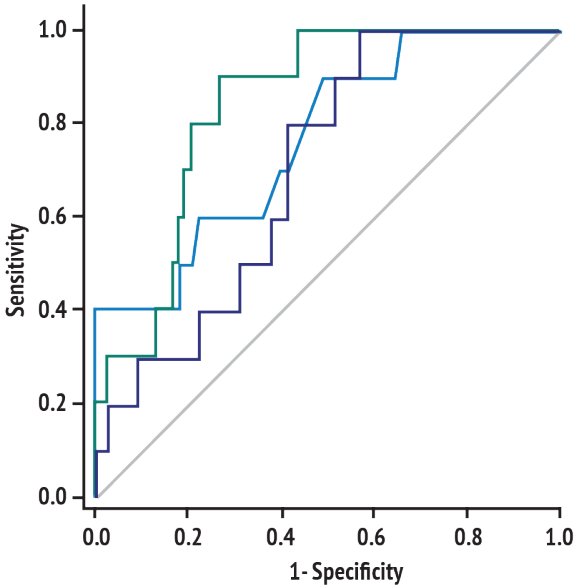
	AUC	95% CI
Flow + PSA Model	0.82	0.77-0.88
Flow Model	0.74	0.67-0.81
PSA	0.71	0.63-0.79



Average AUC = 0.83

Test cohort

	AUC	95% CI
Flow + PSA Model	0.84	0.74-0.94
PSA	0.77	0.62-0.92
Flow Model	0.71	0.57-0.85



All patient characteristics by Gleason score	GS ≤ 3+4	GS ≥ 4+3	p-value	ROC AUC (CI)	Sensitivity, % (CI)	Specificity, % (CI)	NPV, % (CI)
Patients, n	317	60					
Family history of PCa, n (%)	89 (38)	17 (36)	0.87	0.51 (0.42-0.60)	36 (23-51)	62 (55-68)	83 (76-88)
DRE, n (% abnormal)	54 (26)	11 (29)	0.84	0.51 (0.41-0.61)	29 (15-46)	74 (67-79)	85 (79-90)
Previous negative biopsy, n (%)	34 (11)	5 (8.3)	0.82	0.51 (0.43-0.59)	8 (3-18)	89 (85-92)	84 (79-88)
Age, yr, mean (CI)	62 (61 - 63)	64 (62 - 66)	0.10	0.57 (0.49-0.65)	95 (86-99)	13 (10-17)	93 (82-99)
PSA, ng/ml, mean (CI)	7.5 (6.7 - 8.3)	20 (9.4 - 31)	< 0.0001	0.72 (0.65-0.8)	**95 (86-99)**	**17 (13-22)**	**95 (86-99)**
Flow assay score, mean (CI)	5.8 (4.8 - 6.8)	17 (11 - 22)	< 0.0001	0.74 (0.68-0.8)	95 (86-99)	28 (23-33)	97 (91-99)
Flow assay + PSA score, mean (CI)	5.7 (4.6 - 6.8)	20 (15 - 26)	< 0.0001	0.83 (0.78-0.88)	**95 (86-99)**	**56 (51-62)**	**98 (95-100)**

39% higher specificity for clinically significant prostate cancer than PSA alone

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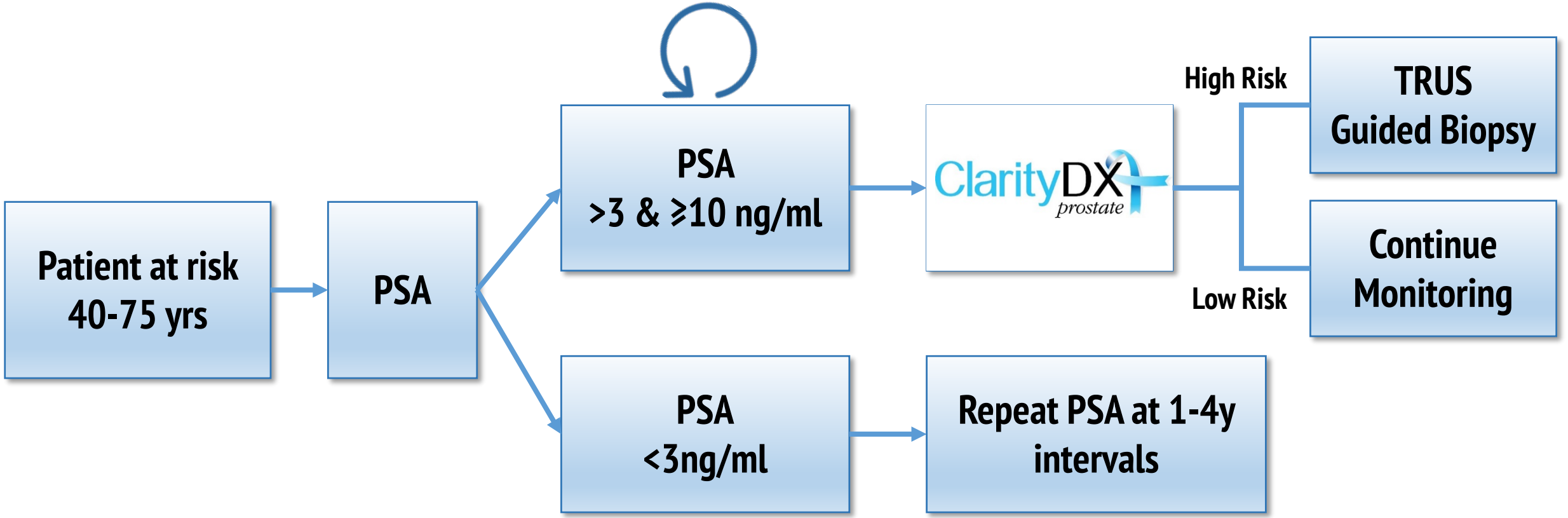
Proposed prostate cancer diagnosis model



IHE INSTITUTE OF HEALTH ECONOMICS ALBERTA CANADA

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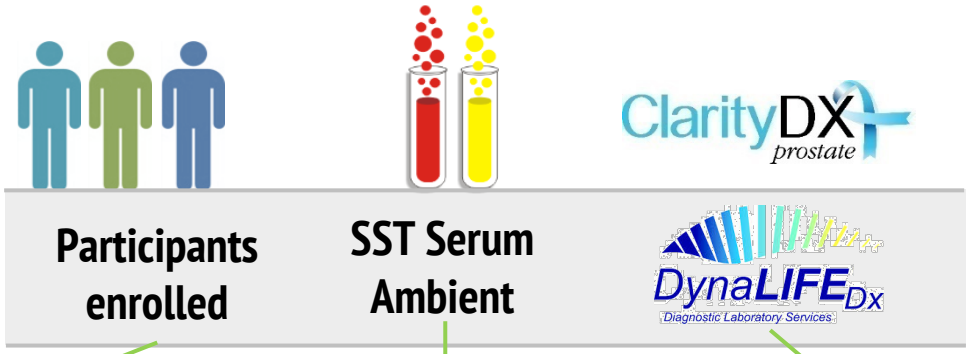
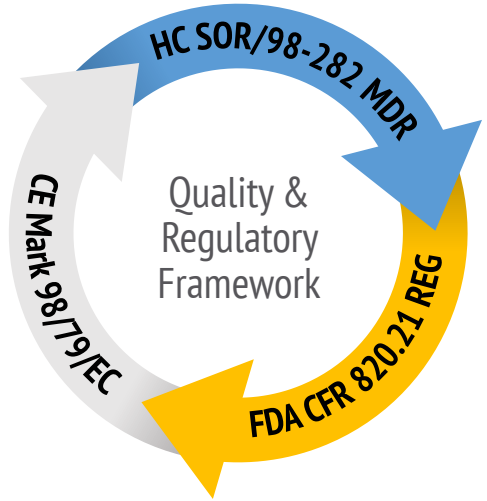
Pivotal Clinical Validation Study



Annual ~ #s	Shipping	Processing
NAUC: 500 PCC: 500 YUK: 100 US: 250	NAUC: DynaLIFE	Within 48h
Interim analysis in 6m ~700 pts	PCC: Overnight Express door to door service	Flow Score
	YUK & US: TBD	



ISO 13485:2016
Standard / framework to address regulatory requirements

FDA CFR 820.21
HC SOR/98-282 CE Mark 98/79/EC Regulations

NANOSTICS PRECISION HEALTH

Acknowledgements

ALBERTA
PROSTATE CANCER
RESEARCH INITIATIVE
knowledge | action | impact

Alberta Cancer
FOUNDATION

Alberta
Innovates
Health
Solutions

CANADA
RIDE FOR DAD

Bird Dogs
For Prostate Cancer Research
Pointing to the cure

Prostate Cancer
Canada

MOVEMBER
FOUNDATION

DynaLIFE Dx
Diagnostic Laboratory Services

APOGEE
FLOW SYSTEMS

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