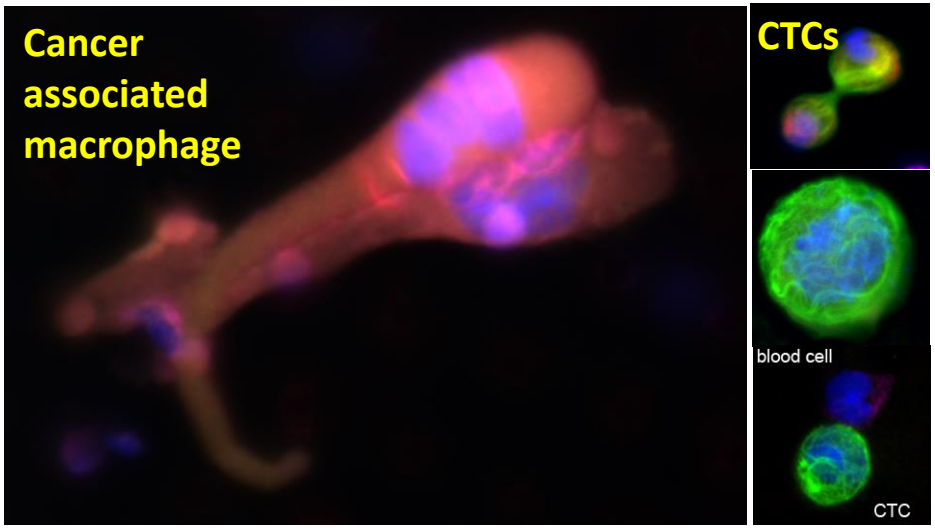
Blood based biopsies in the age of Immunotherapy:

How do we utilize circulating cells?



Daniel Adams
Senior Research Scientist/Head of Clinical Core Laboratory
Creatv MicroTech, Inc.

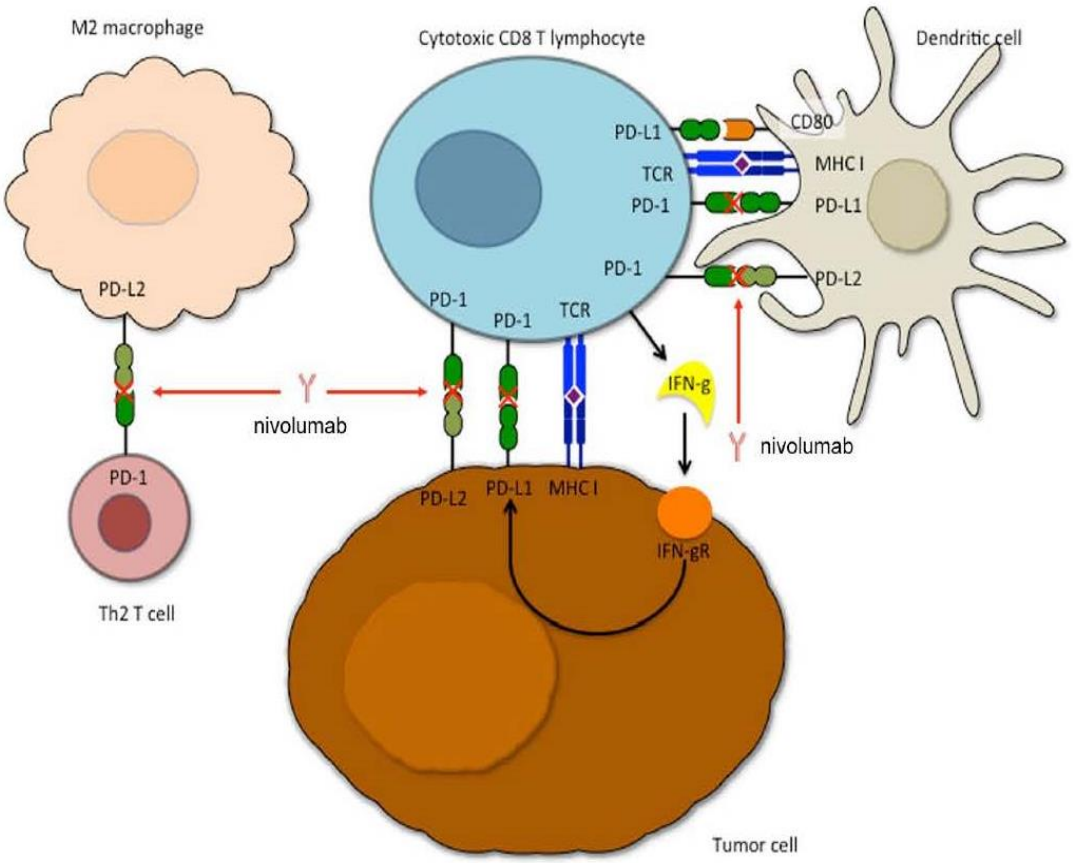
Disclosures

- **Employee of Creatv MicroTech, Inc.**
- **Multiple patents on the technologies discussed**

Immunotherapies involve multiple cell types (Ex: PD-1/PD-L1 pathway)

PD-L1 can be found on:

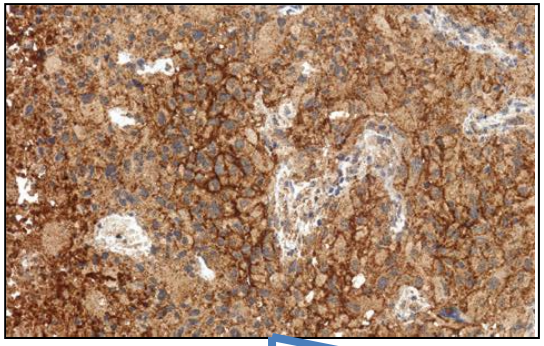
- **Tumor cells**
- **Stromal macrophages**
- **Stromal Tc cells**
- **Stromal Th cells**
- **Stromal Dendritic cells**
- **Tumor fibroblasts**
- **Others**



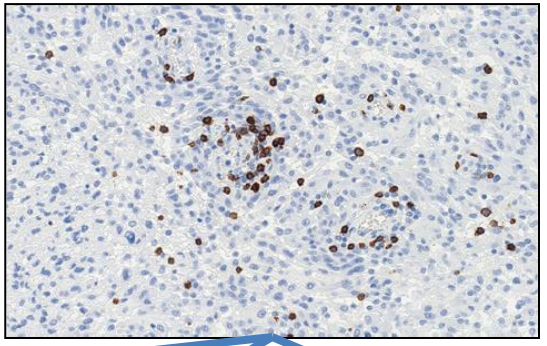
Sundar et al. Ther Adv Med Oncol 2015

We must rethink how companion diagnostics work

Biopsy tumor cells

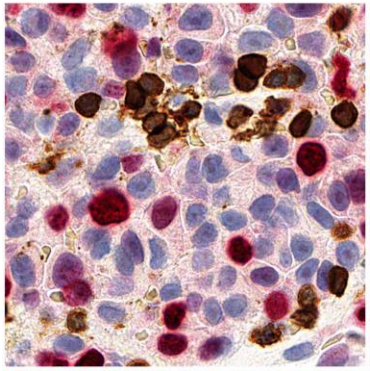


Analyze Immune cells

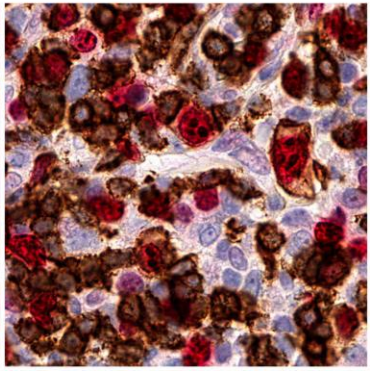


Response

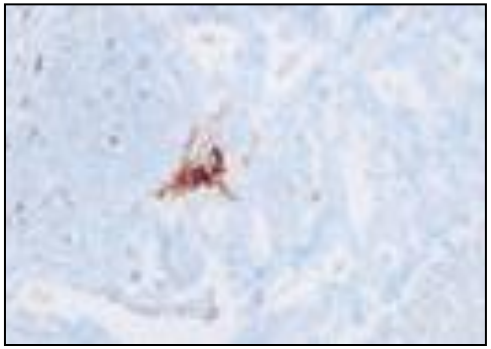
Before Treatment



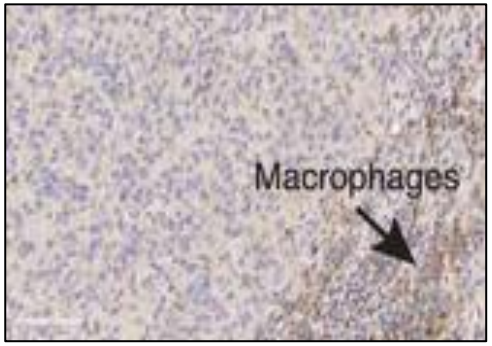
After Treatment



Analyze heterogeneity

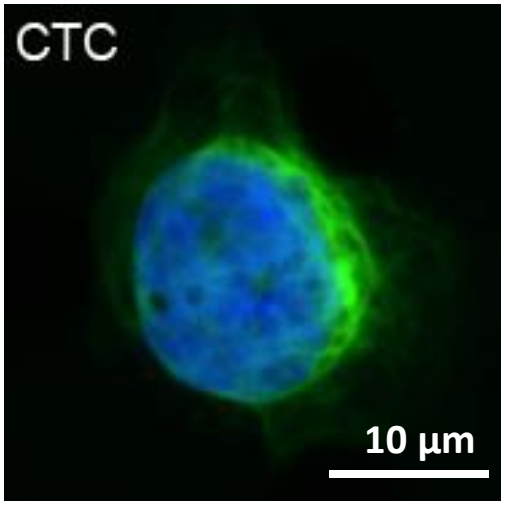


Does biopsy contain stroma?



Tumeh, et al. Nature 2014; Preusser et al. Nat Rev Neurol 2015;
Ilie et al. Ann Onco. 2015; Victor et al. Nature 2015

Advantages of Circulating Tumor Cells (CTCs)

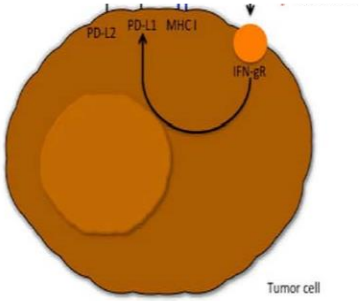


- **Advantages**
  - **Provides prognostic information**
  - **Tracks response to therapy**
  - May provide:
    - Genomic profiling of tumor/metastases
    - Proteomic profiling of the tumor/metastases

Pathologically defined CTCs (PDCTC)

- CK 8, 18, 19 (+) and filamentous
- DAPI (+) cancerous morphology or in division
- CD45 (-)

Disadvantages of CTCs



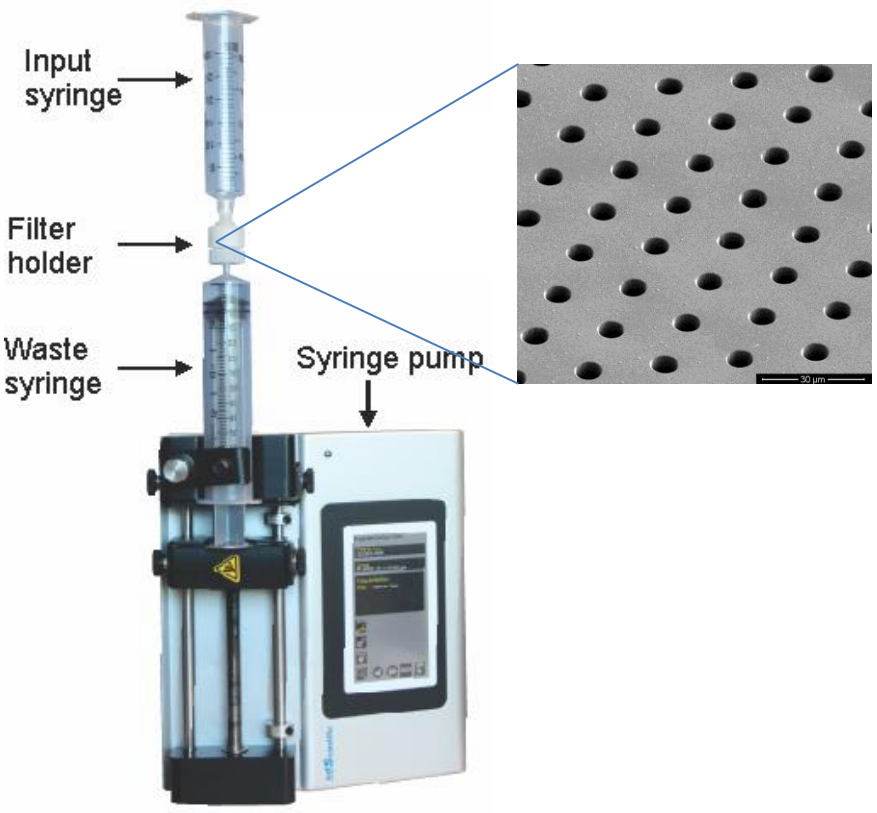
- **Disadvantages**
  - Uncommon (~0-10 per mL blood)*
  - Low frequency (19%-57% of malignant carcinomas)*
  - Only found in late stage/metastatic
  - Tumor cells alone do not represent the stromal environment

*Allard, et al. *Clin Can Res* 2004

Blood based biopsies must have multi-analyte cell biomarker capabilities

- **Circulating Tumor cells (CTCs)**
- **Circulating Stromal cells (CStCs)**
  - **Tumor derived endothelial cells**
  - **Epithelial-mesenchymal transition cells (EMTs)**
  - **Tumor associated macrophage-like cells (CAMLs)**
  - **Tumor derived T cells**
  - **Tumor associated fibroblasts**

Cell isolation based on size



CellSieve™ Microfilters

- **Uniform 7 µm pore size and distribution with high porosity**
  - Rapid, consistent and gentle flow
  - 3 min to filter 7.5 ml of blood
  - Small (100uL) and large (>30mL) sample size
- **Non-fluorescence**
- **CellSave tubes are run ≤ 96 hrs**

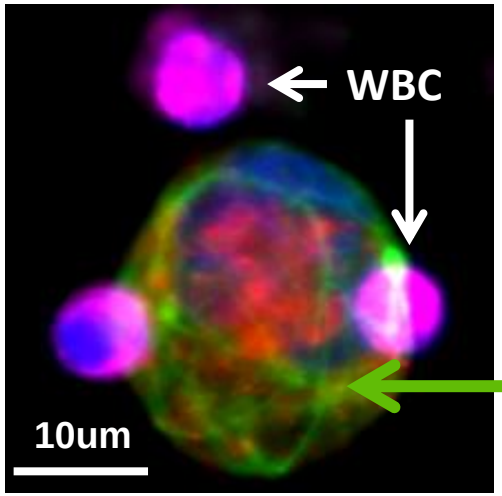
Analysis of Immunotherapy

Nucleus(dark blue)/CK(green)/ EpCAM(red)/CD45(violet)

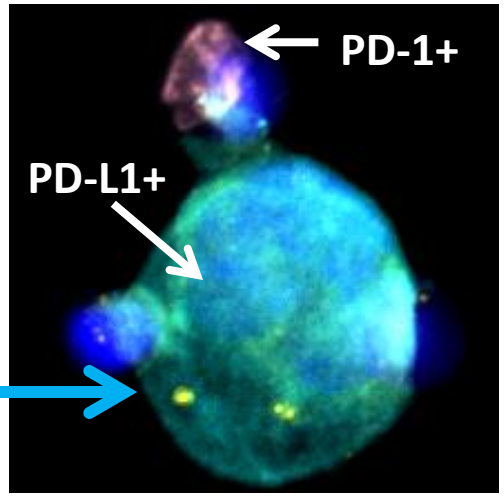
Nucleus(dark blue)/PD-L1(turquoise)/ PD-1(pink)

Breast

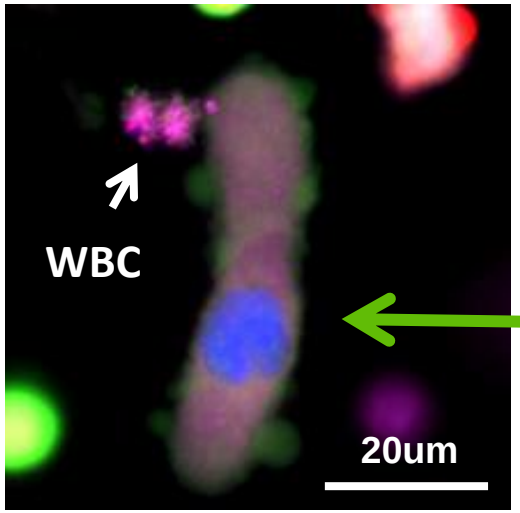
CTC with bound white blood cells



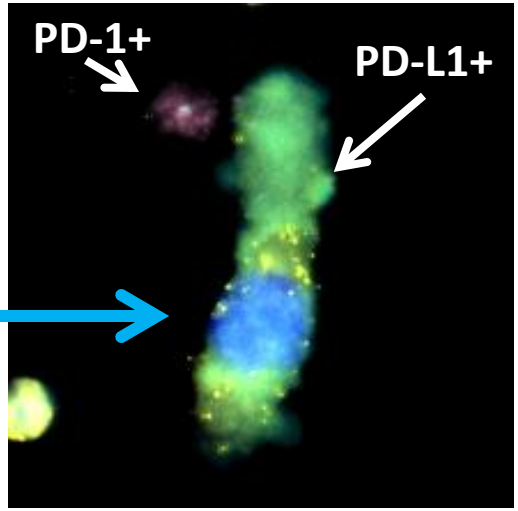
CTC



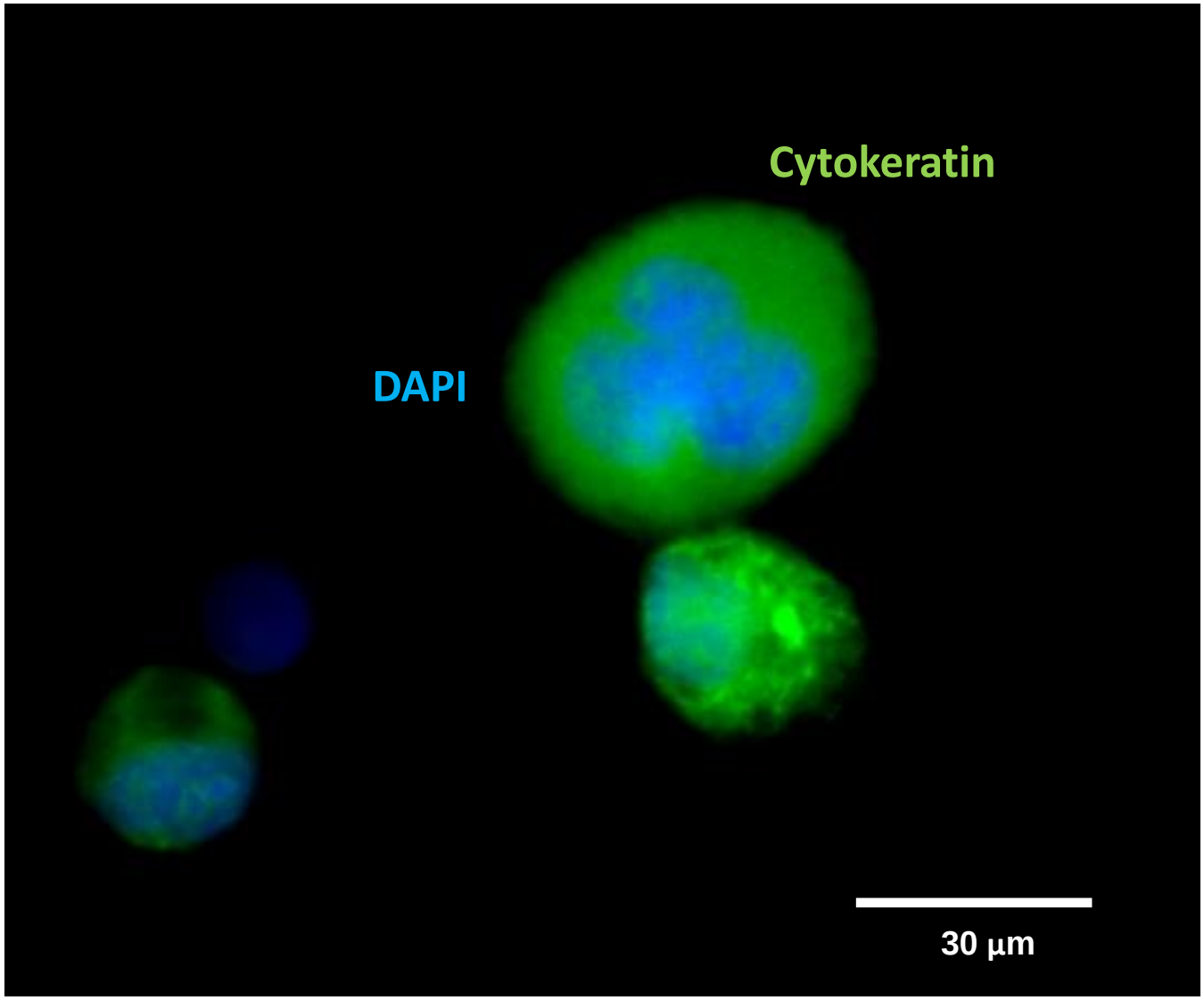
Cytokeratin positive cell with bound white blood cell



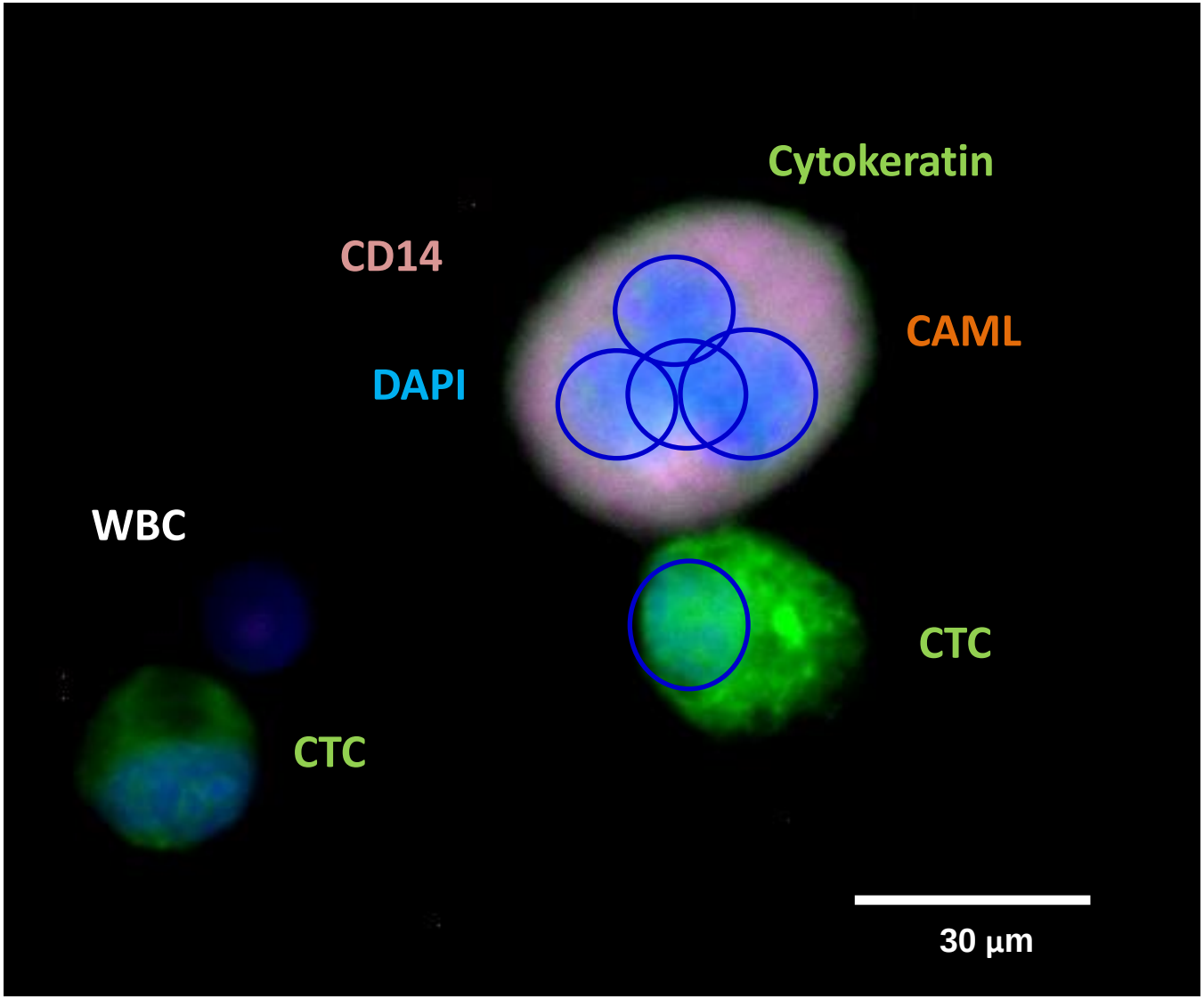
CAML



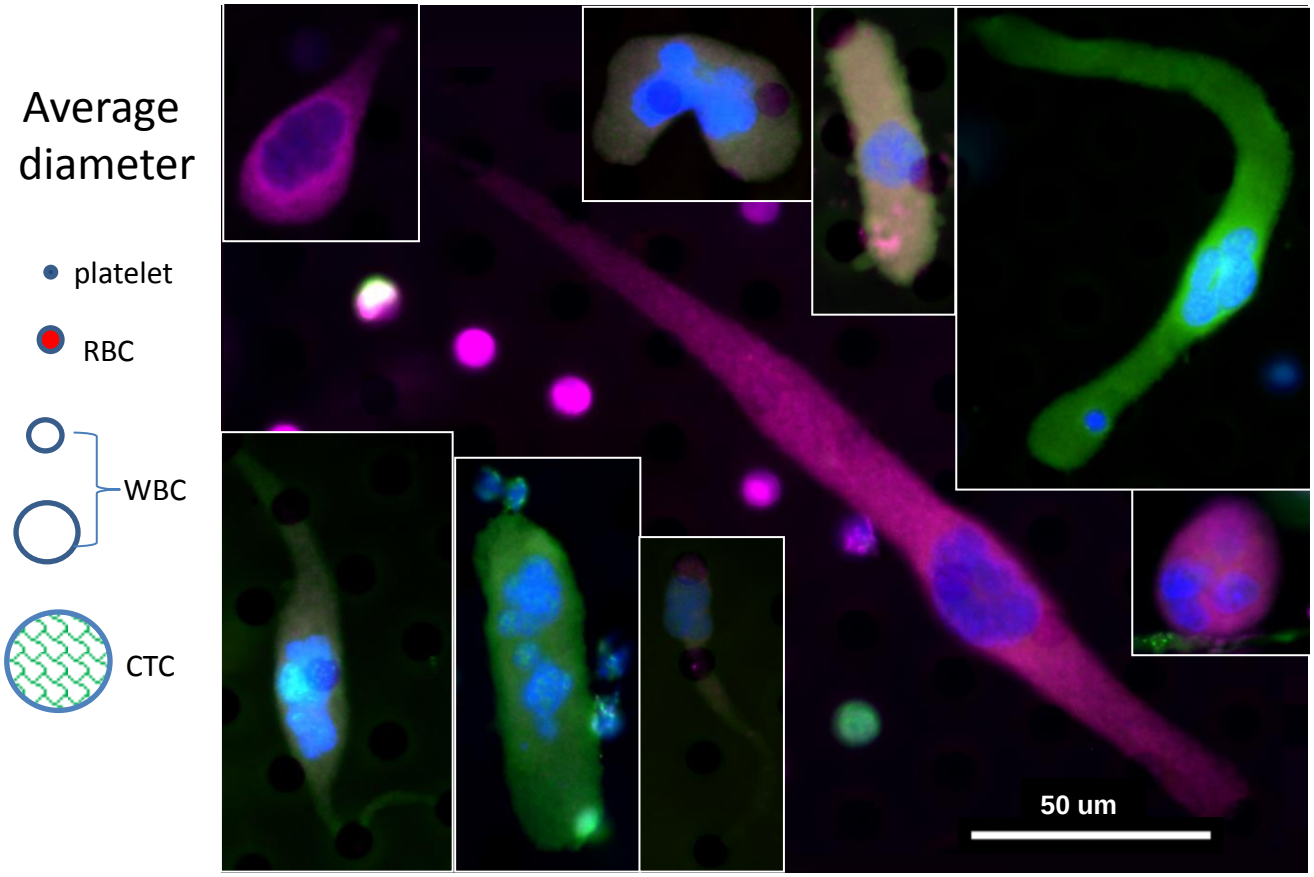
Discovery



Discovery



Circulating Cancer Associated Macrophage-like Cells (CAMLs)

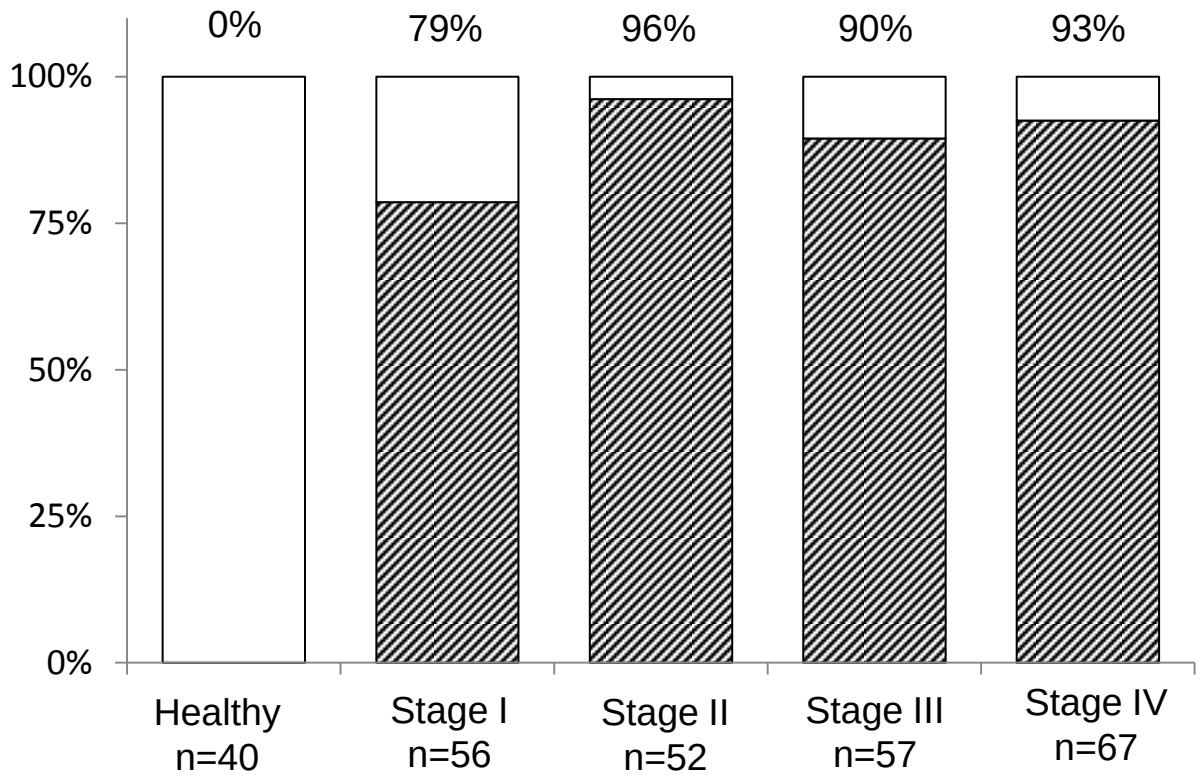


- Large, atypical nucleus
- May express CK and EpCAM
- Contain tumor markers
- Most are CD45 positive
- Large: 30 - 300 µm
- Express CD11c/CD14
- Express endothelial markers CD146, TIE-2

Adams, et al. PNAS 2014

CAMLs in Cancer Patients

None in healthy controls



Total
n=272

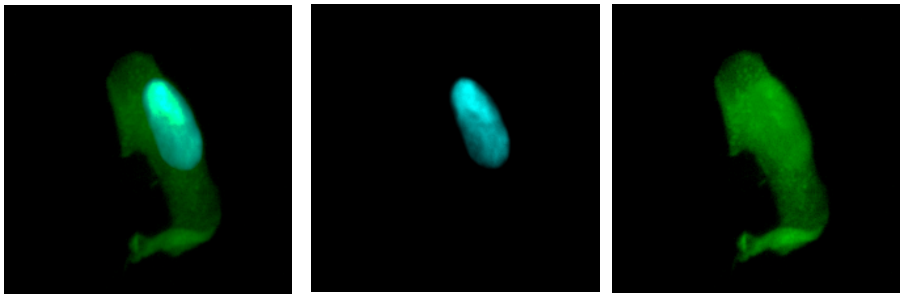
Cancer types

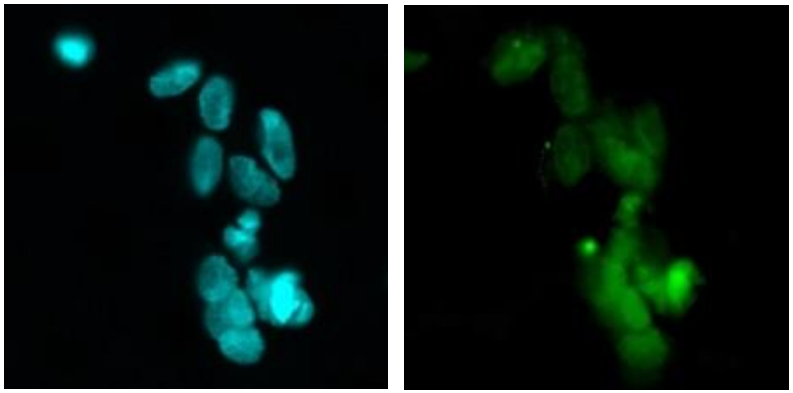
- Breast
- Prostate
- Pancreatic
- Lung (NSCLC)
- Colon
- Esophageal

Sensitivity 89% (95% CI 85-93%)
Specificity 100% (95% CI 91-100%)
PPV 100% (95% CI 98-100%)

EMT like Cells

EMT like Cells

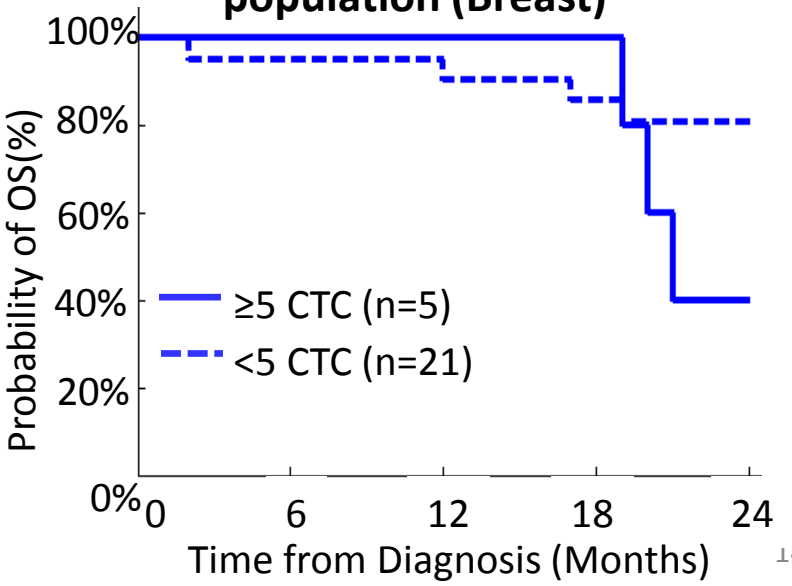




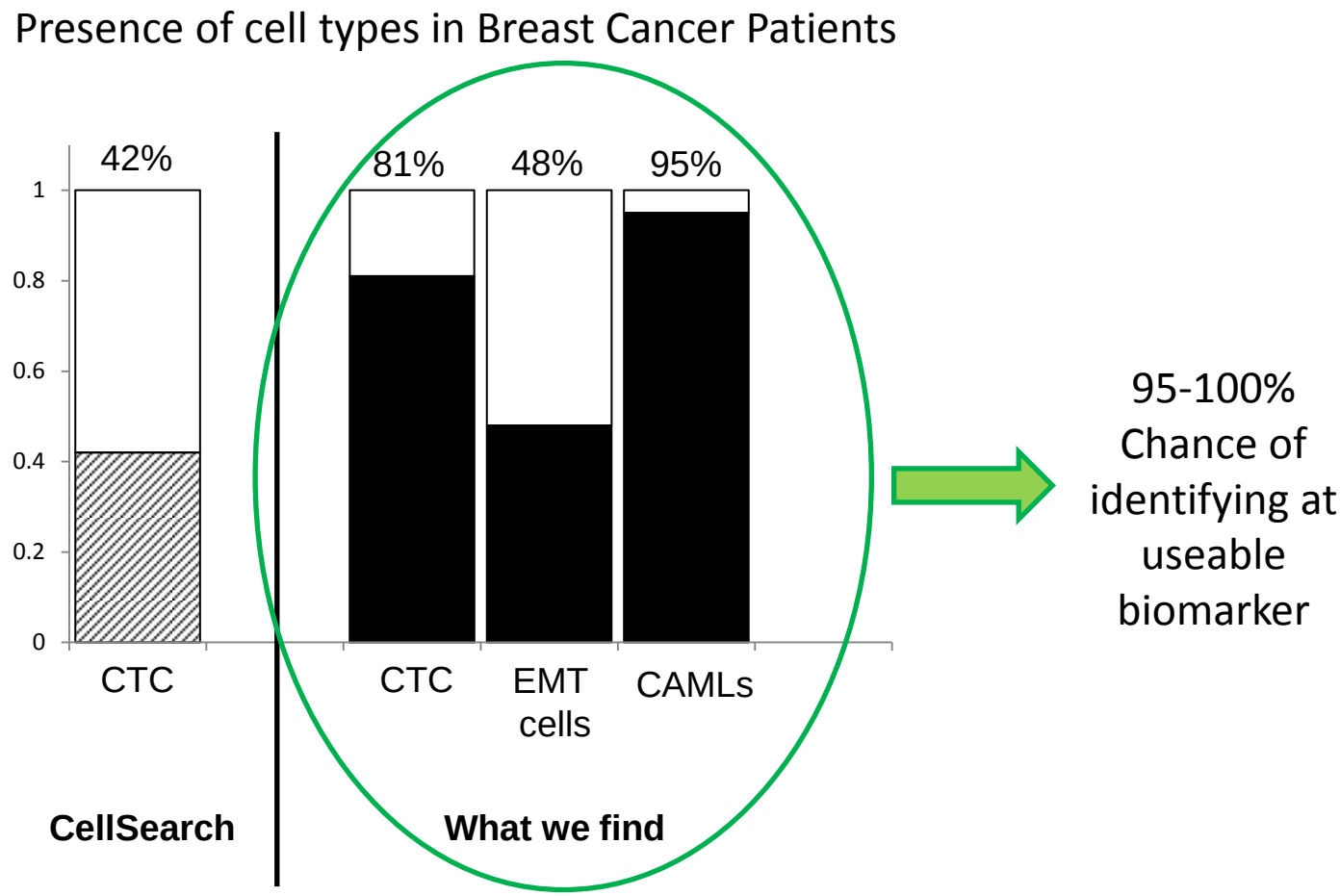
Criteria unique to high resolution imagery

- CK 8, 18, 19 (+) diffuse/non-filamentous
- DAPI (+) cancerous morphology
- CD45 (-)

Overall Survival for EMT Cells population (Breast)



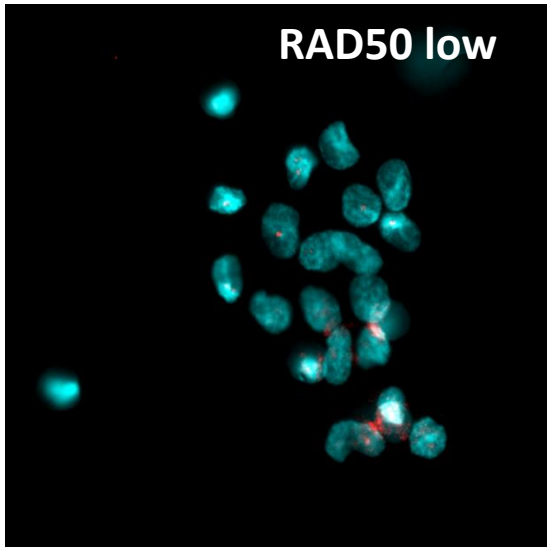
We analyze CTCs and CStCs to maximize useable biomarkers



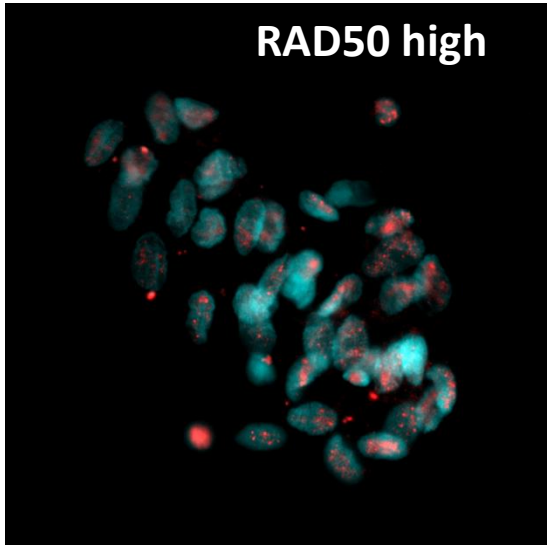
Adams et al. PNAS 2014 and Cytometry A 2015

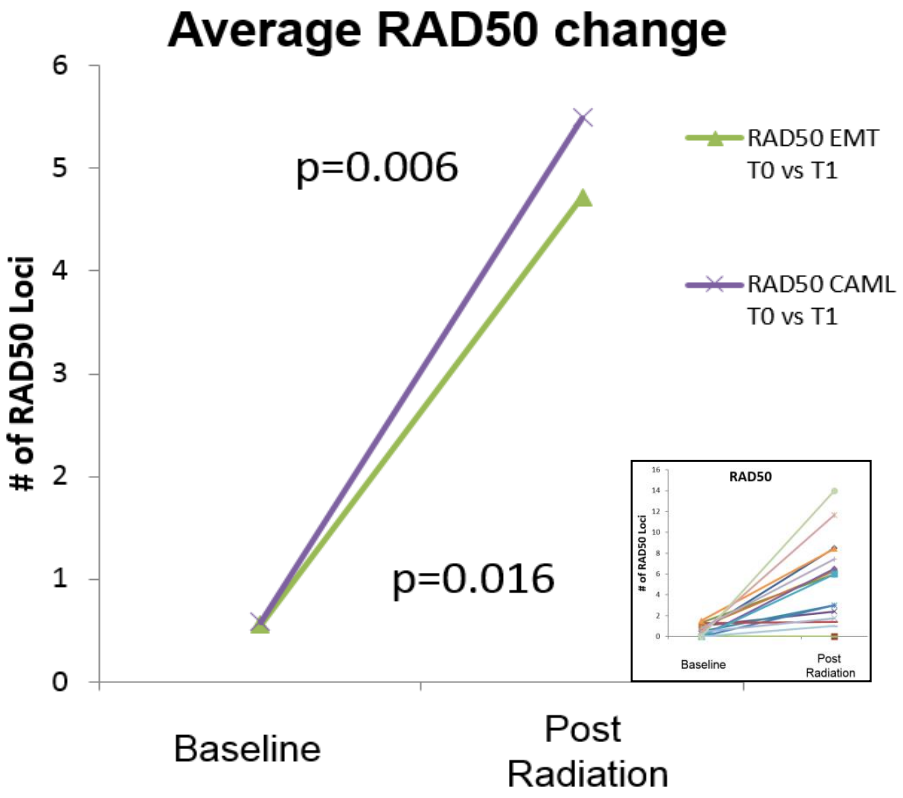
Tracking origin of CStCs

Low Before Radiation



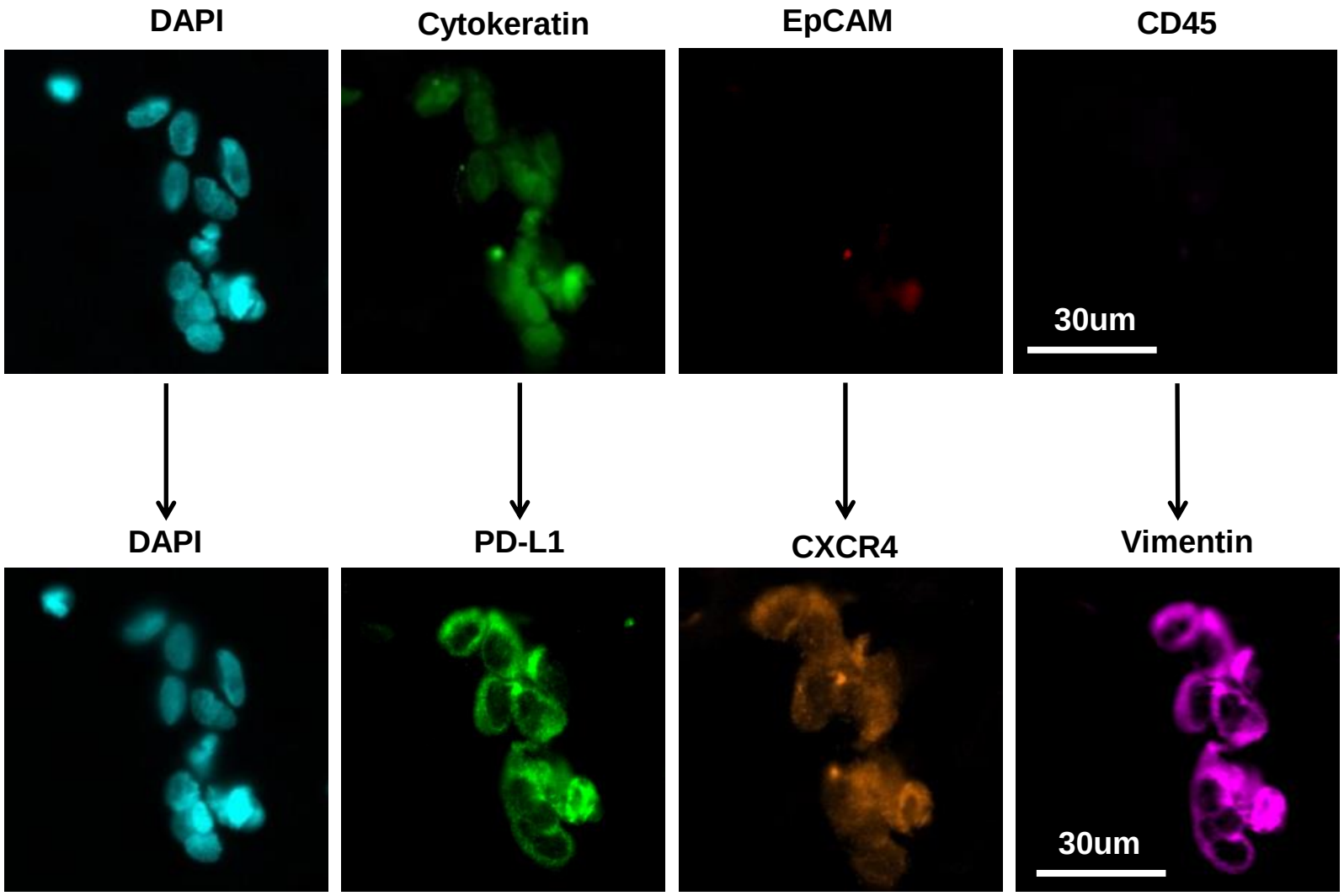
High Post Radiation





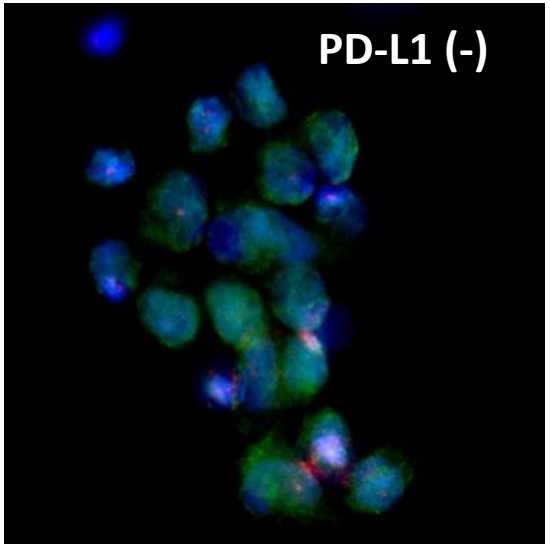
RAD50 foci ranged from 0-20 per cell, with an average of 0.57 at T0 that increased to 5.11 at T1 ($p<0.001$) during radiotherapy

Subtyping CTCs and CStCs by Immunotherapeutic markers

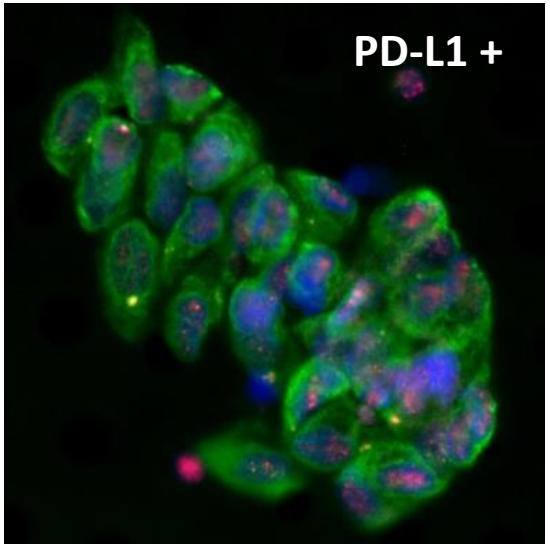


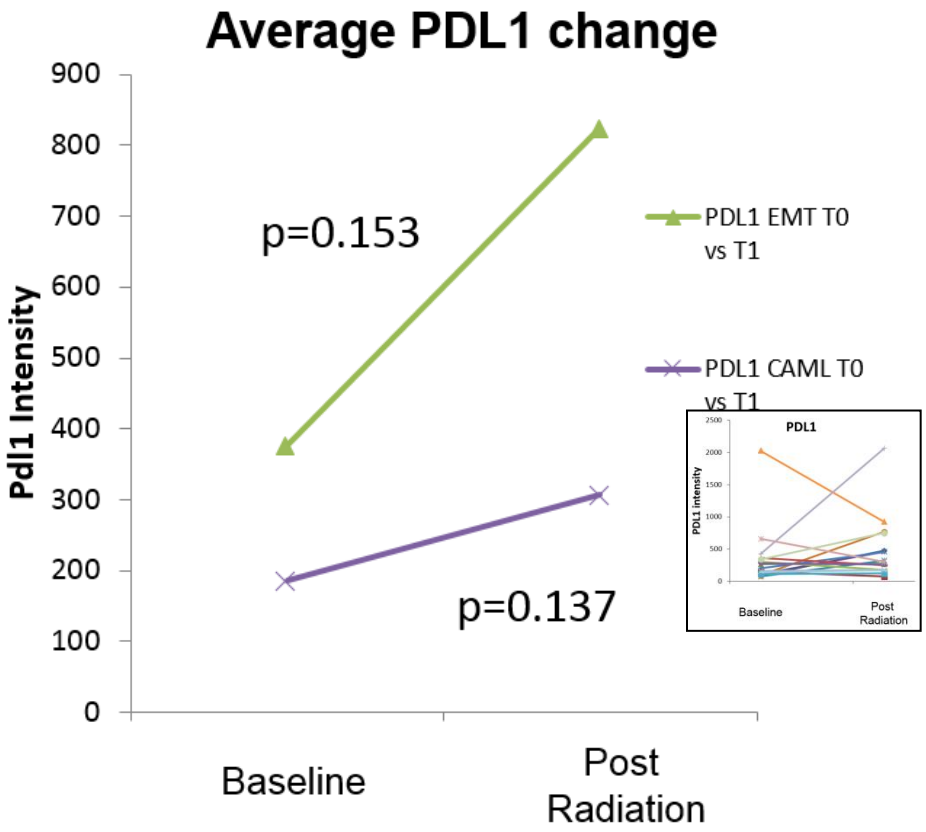
Tracking upregulation and down regulation of biomarkers in real time

Low Before Radiation



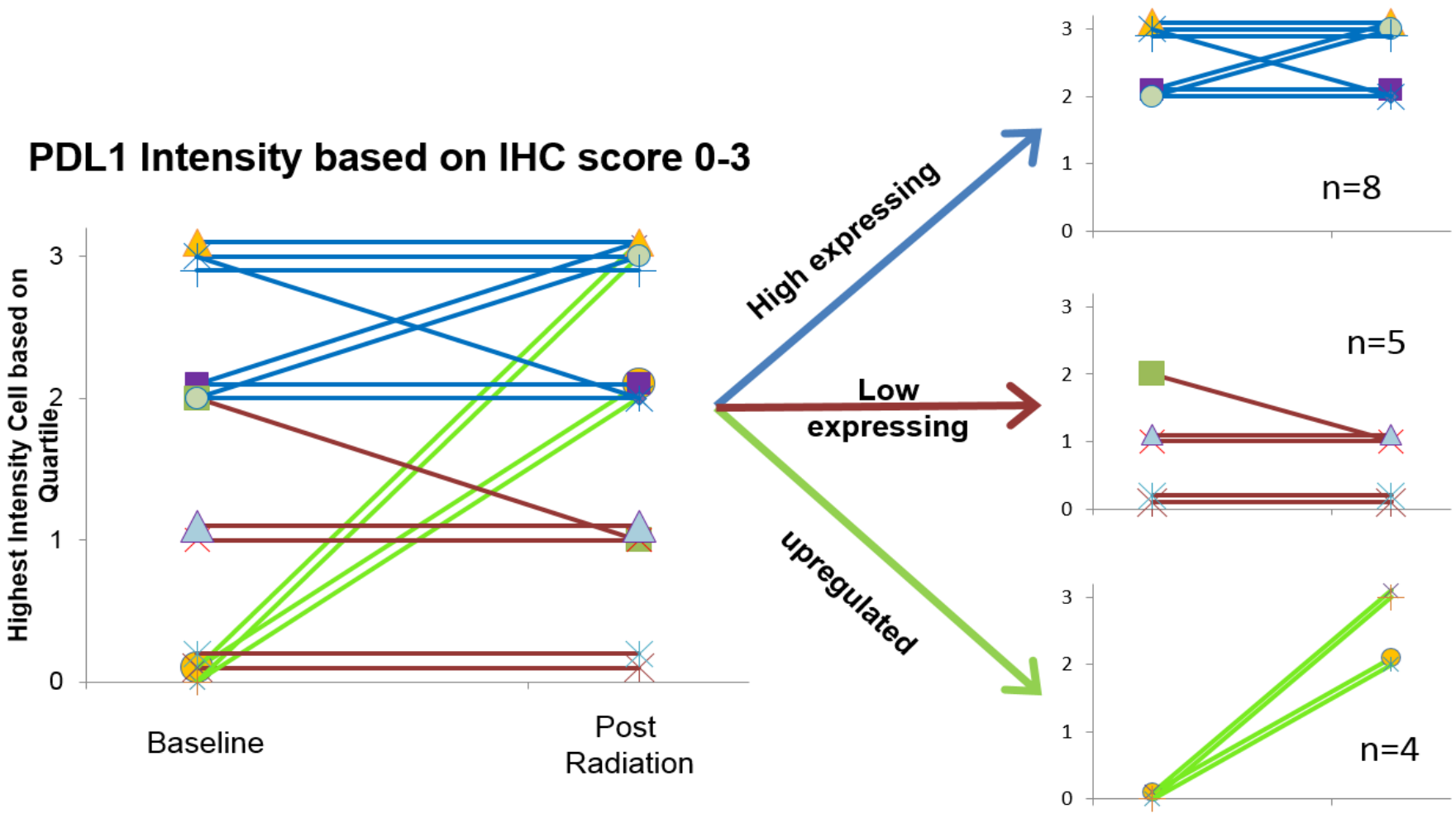
High Post Radiation





PD-L1 expression ranged from 34-2711 pixel intensity, with an average of 281 at T0 and 565 at T1 (p=0.07).

PD-L1 changes in NSCLC patients before and after radiation treatment



Lin/Adams, et al 2015 WCLC

Research Collaborators

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Johns Hopkins University	David Loeb, M.D.
Mayo Clinic Cancer Center	Thai Ho, M.D., Saranya Chumsri, M.D.
MD Anderson	Steven Lin, M.D.
Medical College of Wisconsin	Susan Tsai, M.D.
OHSU Knight Cancer Institute	Raymond C. Bergan, M.D.
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Acknowledgements

- **Maryland TEDCO MTTCF award**
- **The U.S. Army Research Office (ARO) and the Defense Advanced Research Projects Agency (DARPA) (W911NF-14-C-0098)**

The content of the information does not necessarily reflect the position or the policy of the US Government.

Thank you

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