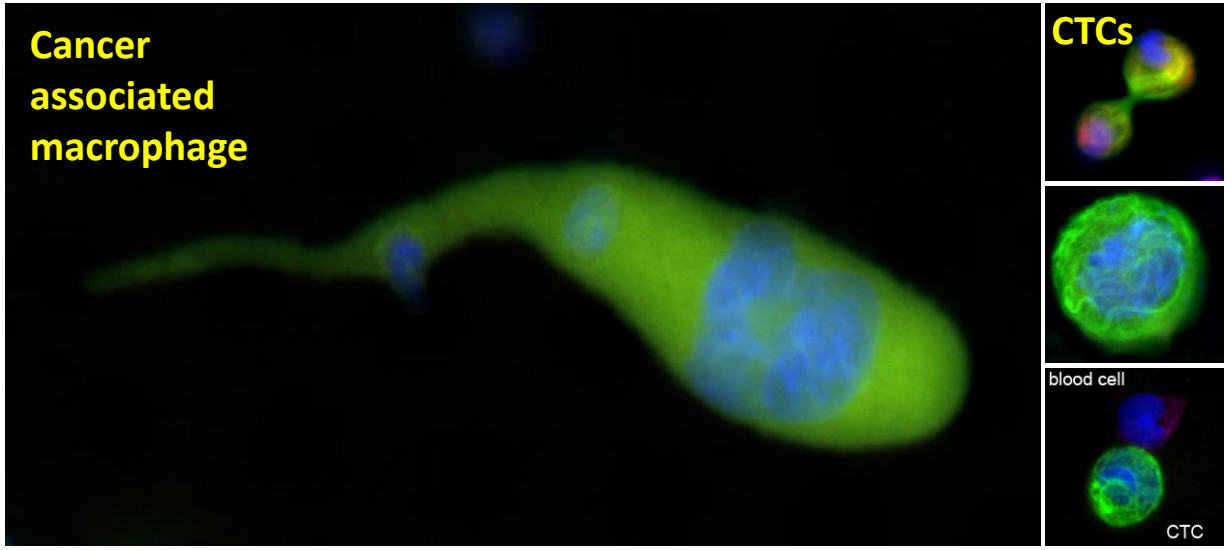
Eighth Annual Next Generation Dx SUMMIT
August 23- 26, 2016 | Grand Hyatt Washington | Washington, DC
Moving Assays to the Clinic

# Circulating Stromal Cells in Immunotherapy

## Utilizing a Total Blood Based Biopsy




**Daniel Adams**
Senior Research Scientist/Head of Clinical Core Laboratory
**Creatv MicroTech, Inc.**
August 24, 2016

# Disclosures

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

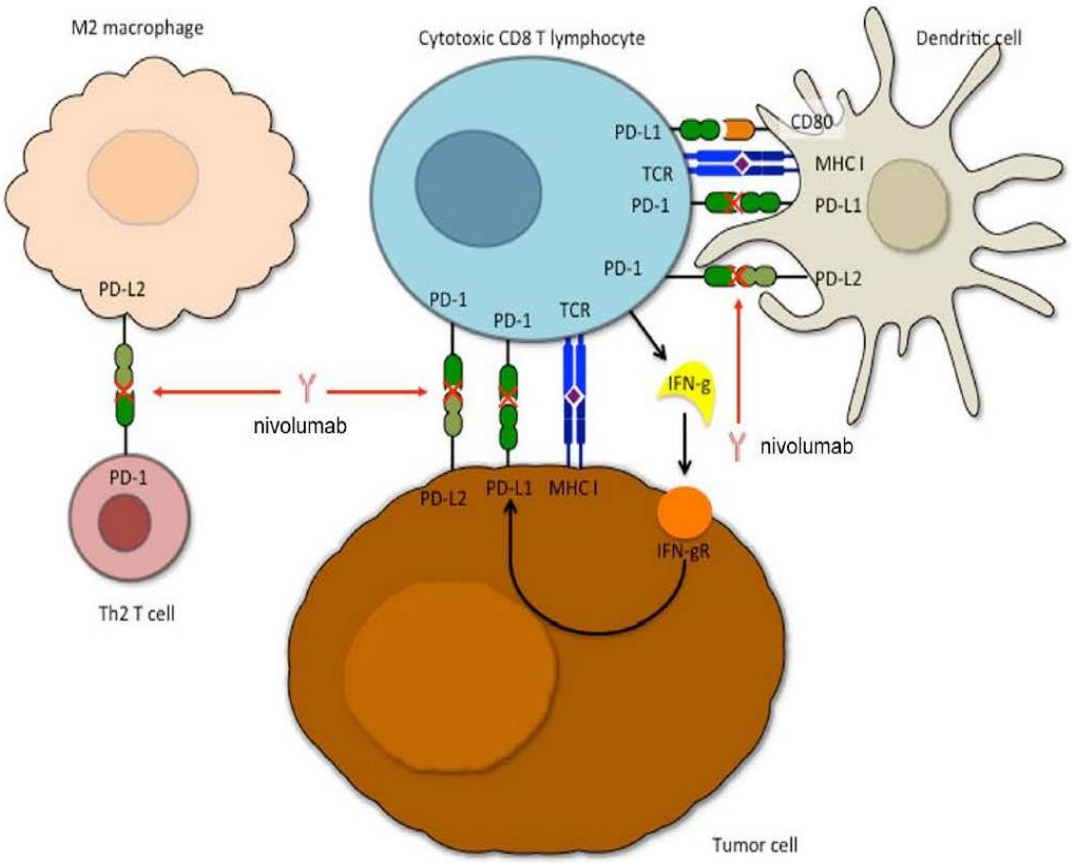
# Utilities of Blood Based Biopsy
## for cancer diagnostics

- **Circulating Tumor Cells (CTCs) if present**
  - Sequential tracking of therapy
  - Genomic and proteomic profiling of tumor
- **CTCs alone not enough and additional needs**
  - EARLY and late stage diseases (CTCs only late)
  - Majority of patient population (CTC only certain pop.)
  - Immunotherapy (targets non-malignant cells)
  - Assist drug development

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

**PD-L1 can be found on:**

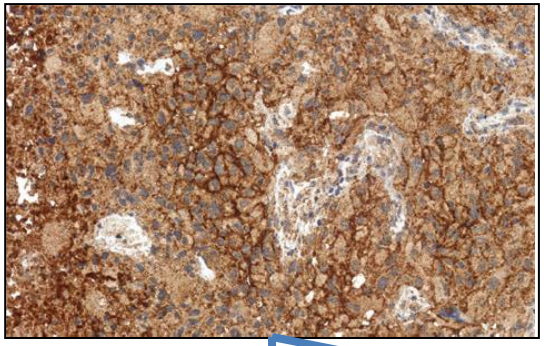
- **Tumor cells**
- **Stromal macrophages**
- **Stromal Tc cells**
- **Stromal Th cells**
- **Stromal Dendritic cells**
- **Tumor fibroblasts**
- **Others**



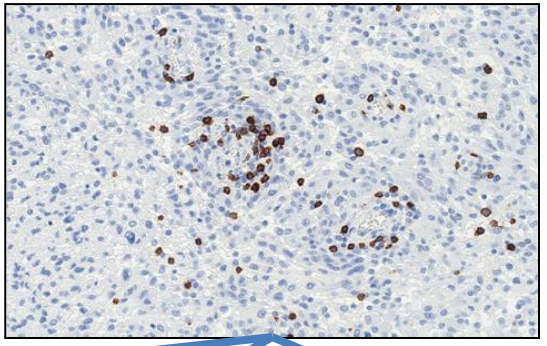

Sundar et al. Ther Adv Med Oncol 2015

# We must rethink how companion diagnostics work

**Biopsy tumor cells**

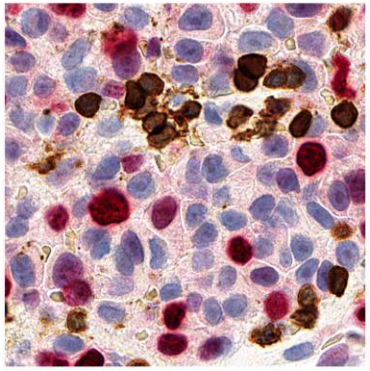


**Analyze Immune cells**

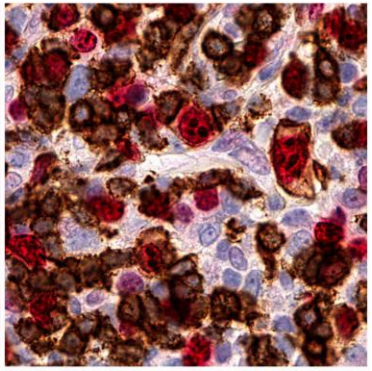


Response

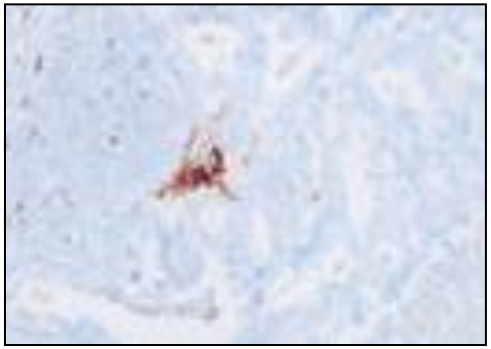
Before Treatment



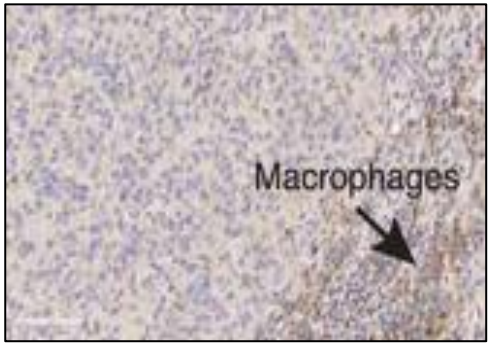
After Treatment



**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

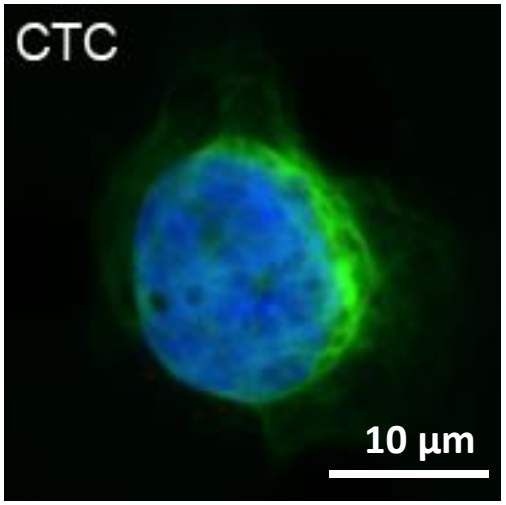
# Immunotherapeutic biomarkers must have multi-cellular capabilities

- **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**

# Requirements of Blood Based Biopsy for Immunotherapy

- **Applicable to sequential tracking of therapy**
- **Allow genomic and proteomic profiling of tumor**
- **Applicable to all stages of disease**
- **Must isolate heterogeneous populations of Multiple cell types**
- **Must isolate heterogeneous populations of stromal cells** (Targeting non-malignant cells)

# Advantages of Circulating Tumor Cells (CTCs)

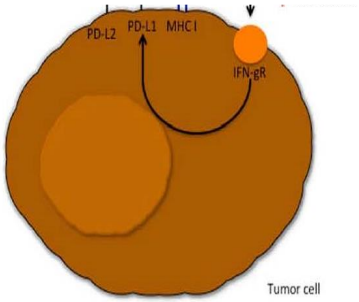



- **Advantages**
  - **Provides prognostic information**
  - **Tracks response to therapy**
  - May provide:
    - Genomic profiling of tumor/metastases
    - Proteomic profiling of the tumor/metastases

**Pathologically defined CTCs (PDCTC)**

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

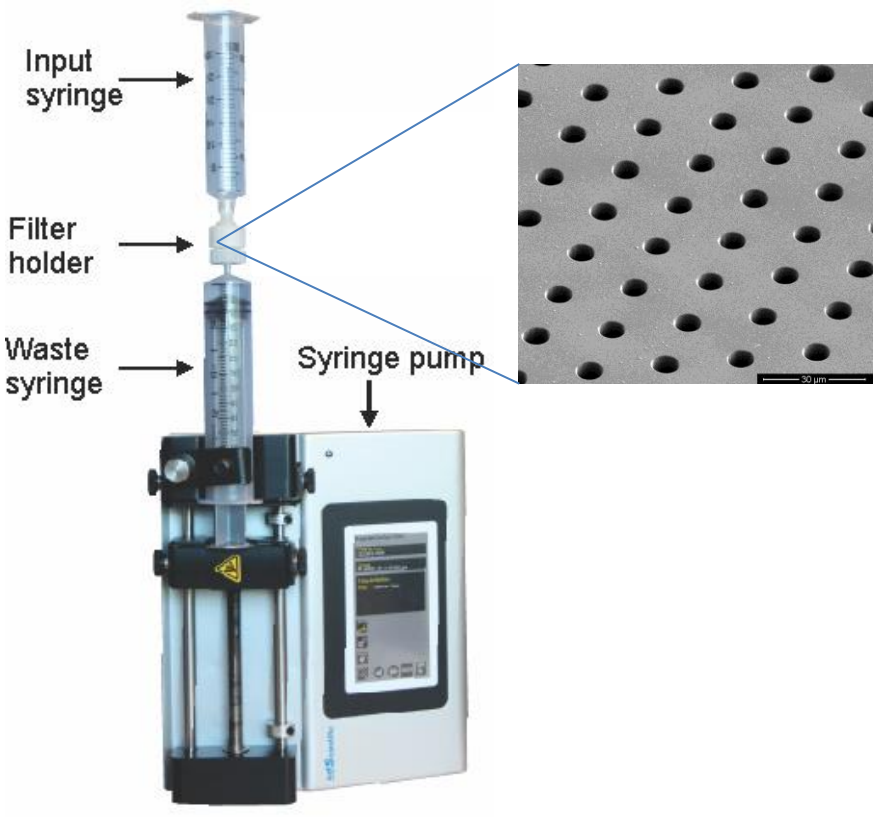
# Disadvantages of CTCs




- **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
Sundar et al. Ther Adv Med Oncol 2015

# Cell Isolation Based on Size

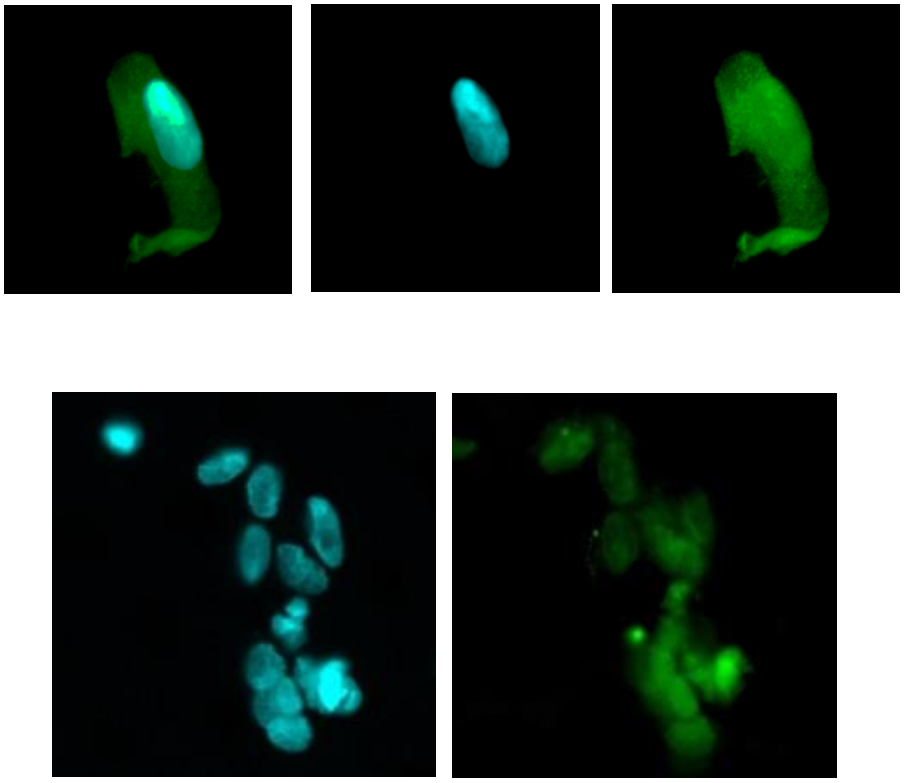



## CellSieve™ Microfilters

- **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**

# EMT like Cells

## EMT like Cells



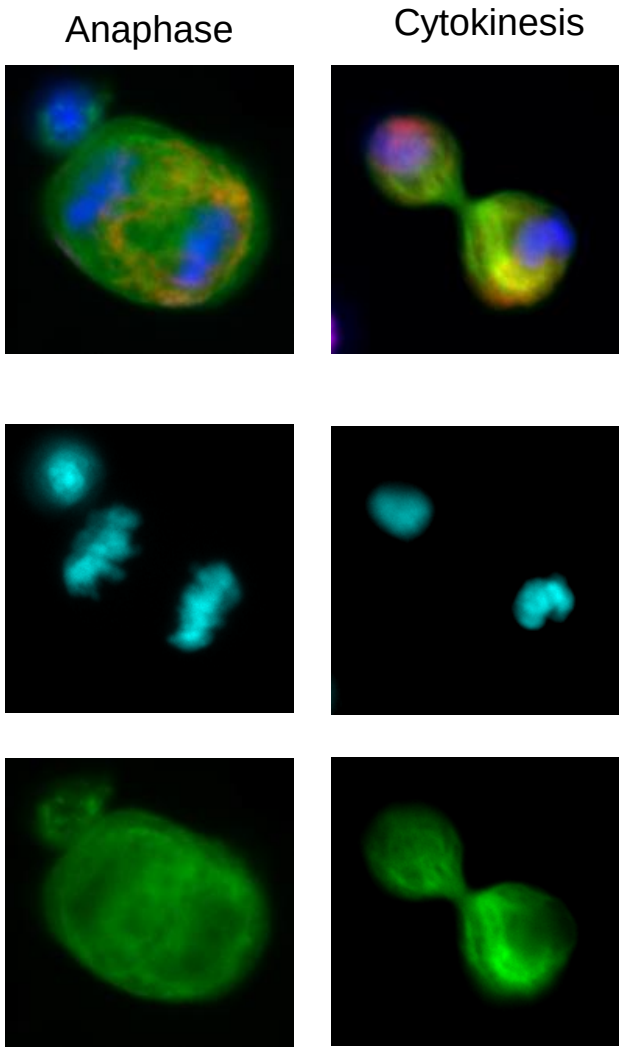
**Criteria unique to high resolution imagery**

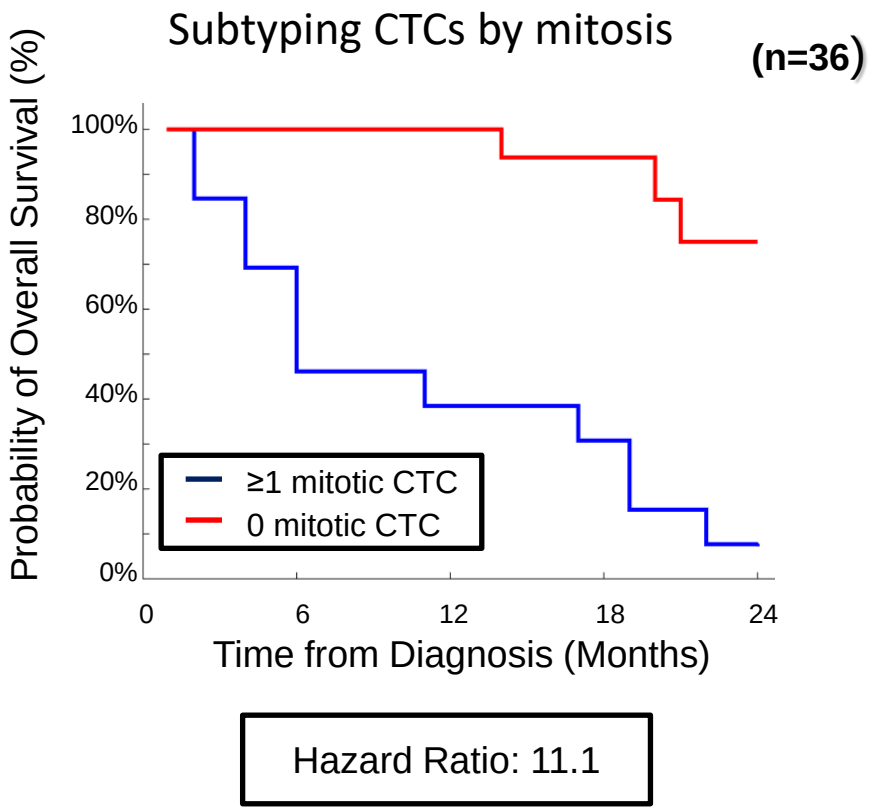
- CK 8, 18, 19 (+) diffuse/non-filamentous
- DAPI (+) cancerous morphology
- CD45 (-)

Adams, et al. Cytometry A 2015

# CTCs in Division

## Cytokeratin (green), DAPI(blue)



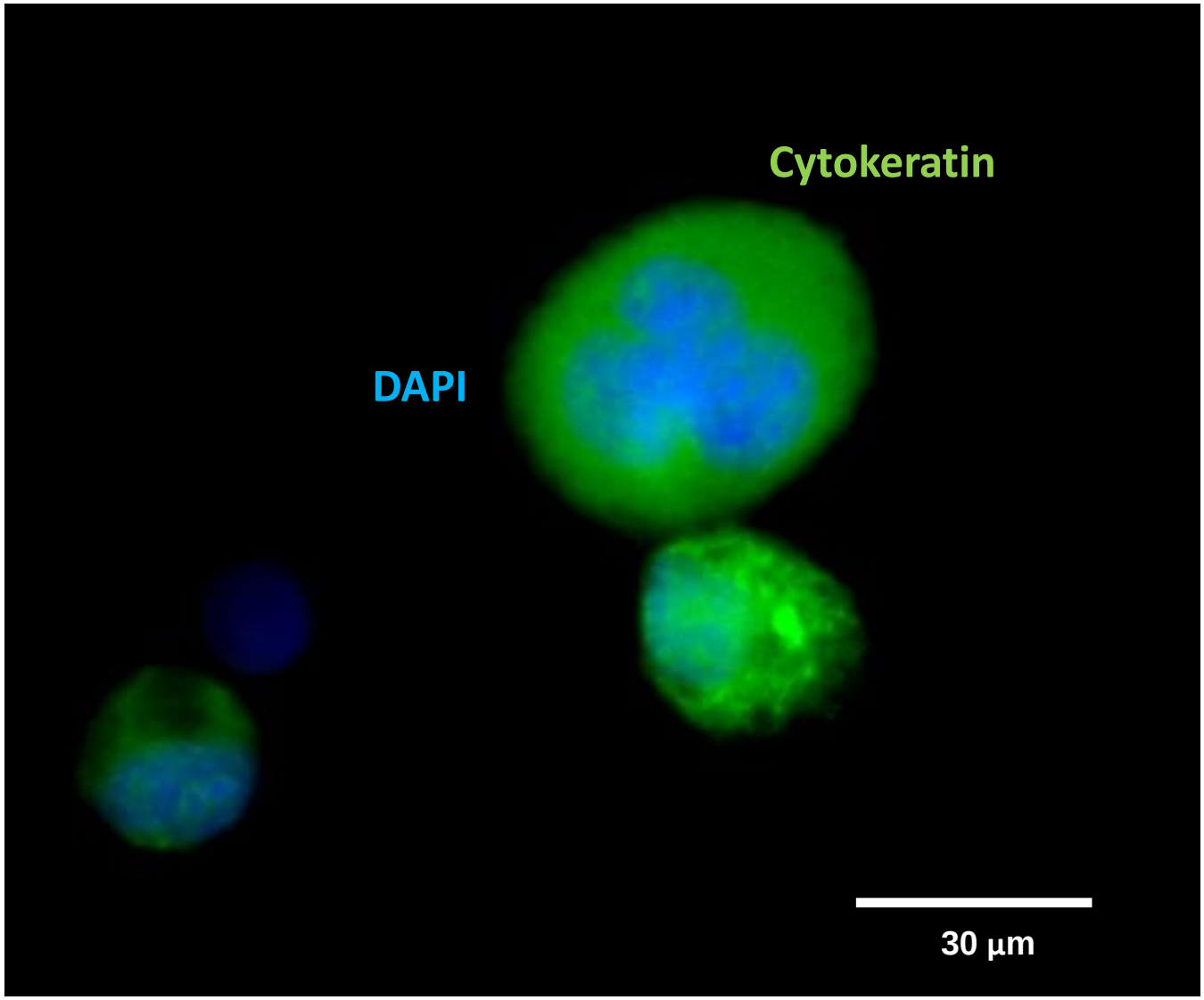




Hazard Ratio: 11.1

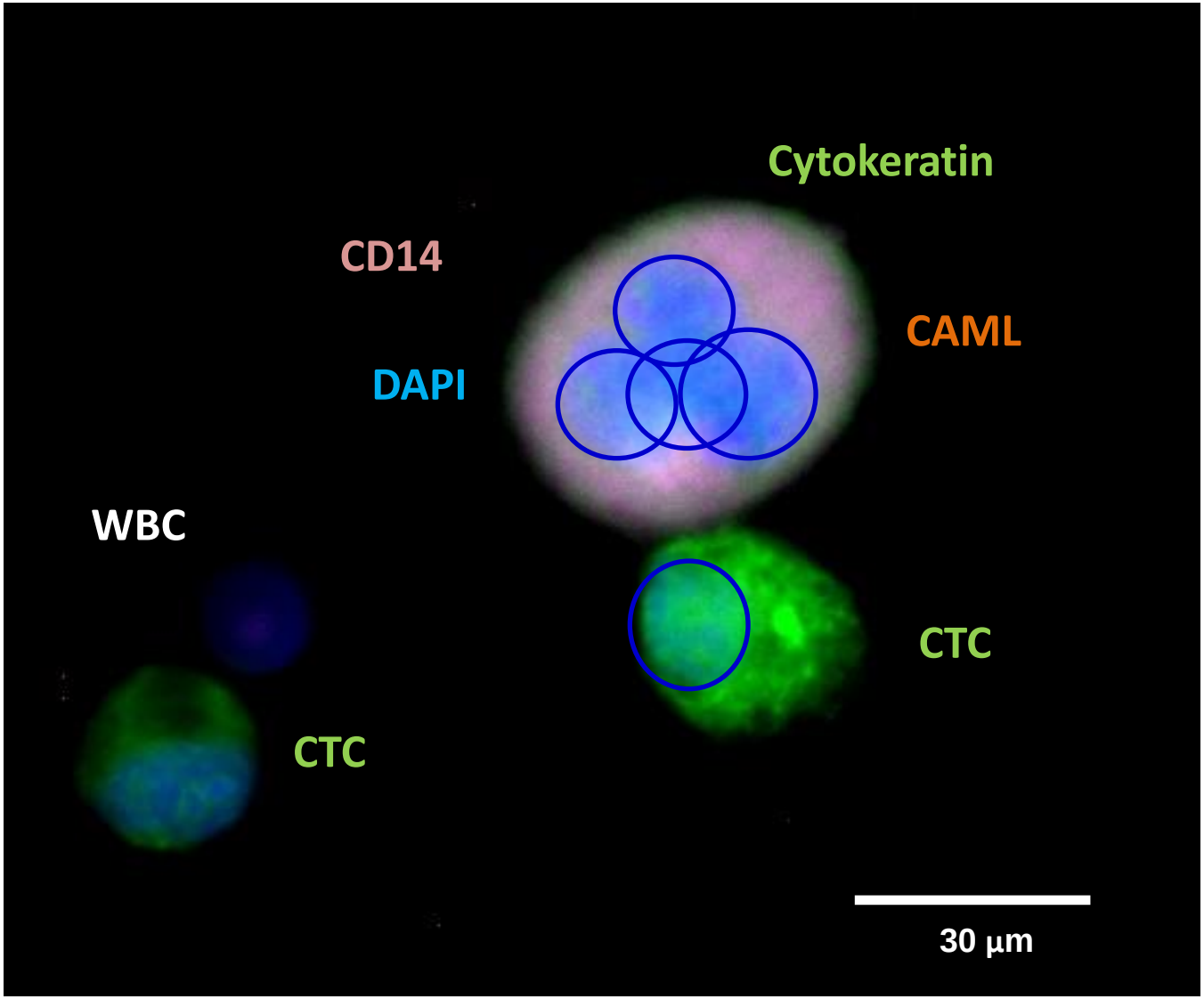


Adams et al. 2016 Breast Cancer Research

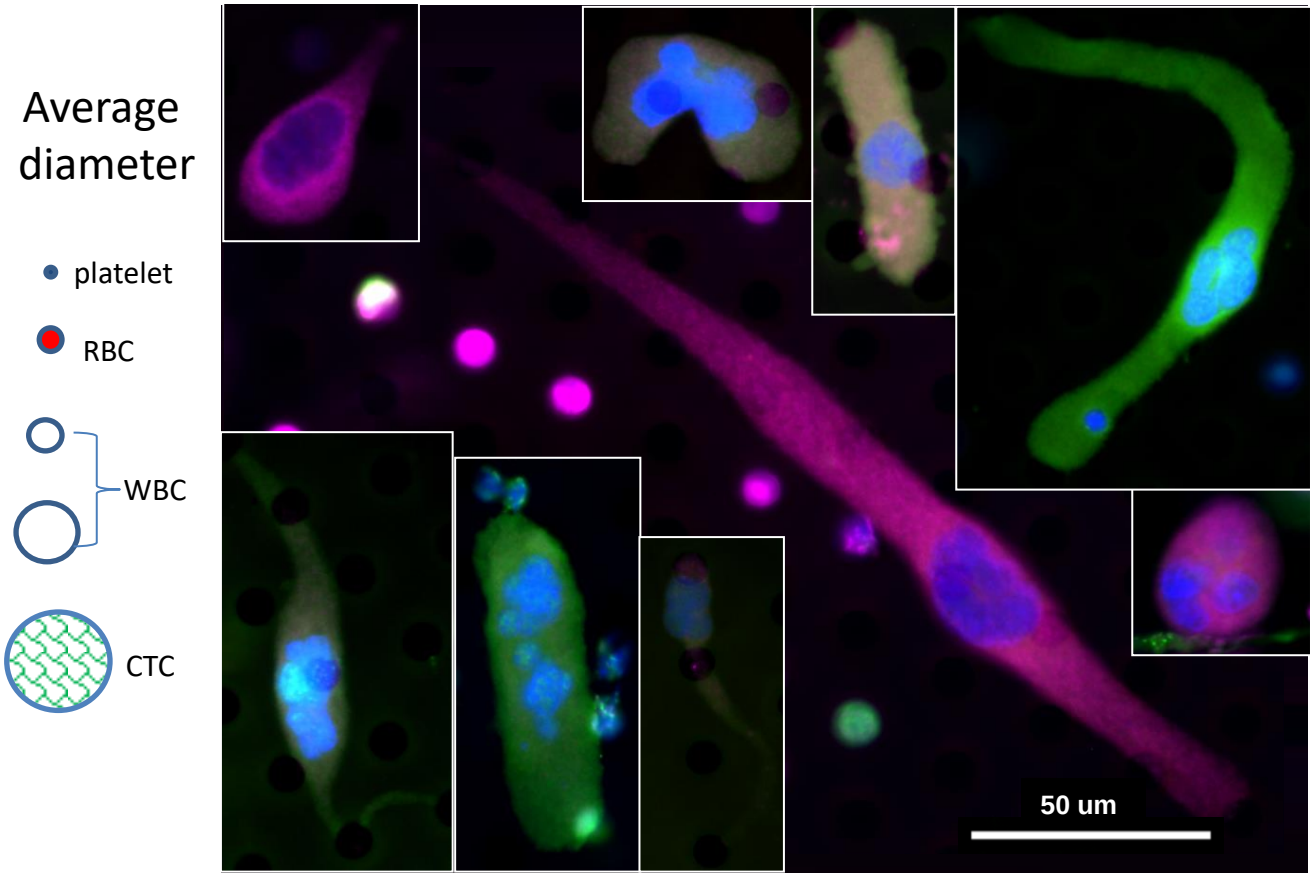
# Discovery



# Discovery



# Circulating Cancer Associated Macrophage-like Cells (CAMLs)

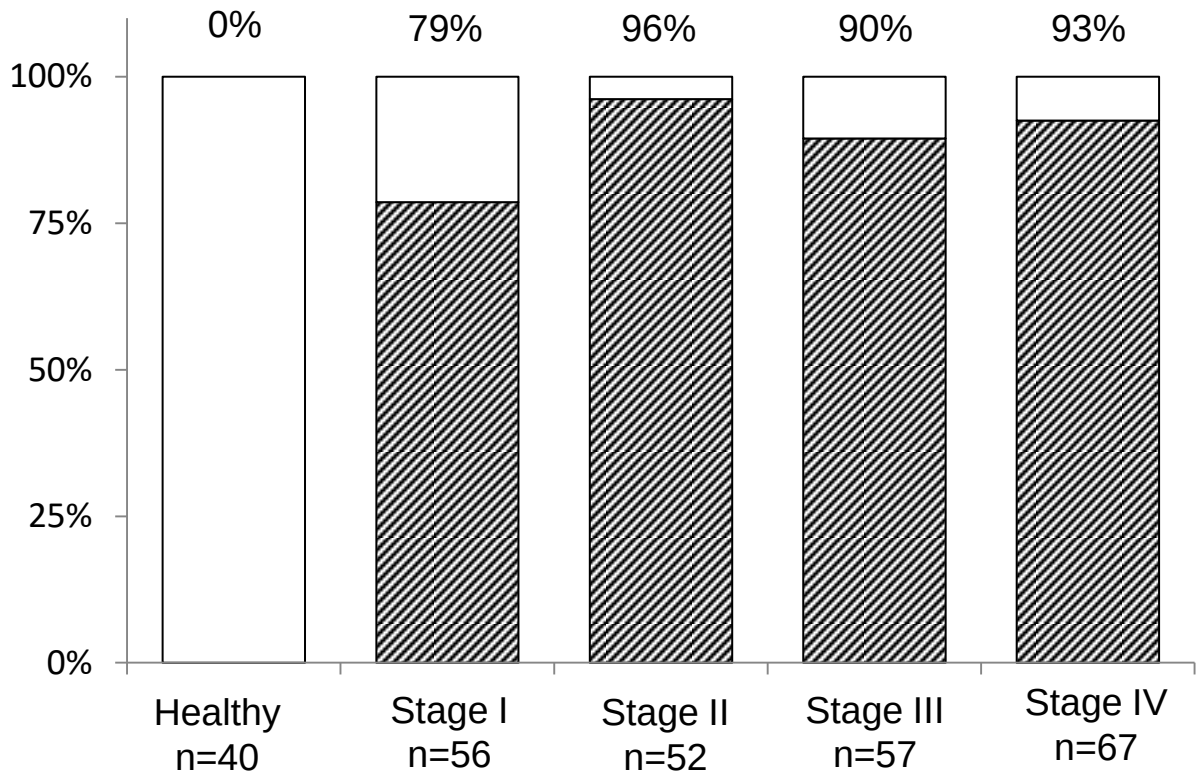



- Large, atypical nucleus
- May express CK and EpCAM
- Contain tumor markers
- Most are CD45 positive
- Large: 30 - 300 µm
- Express CD11c/CD14
- Express endothelial markers CD146, TIE-2

Adams, et al. PNAS 2014

# CAMLs in Cancer Patients

## None in healthy controls




Total
n=272

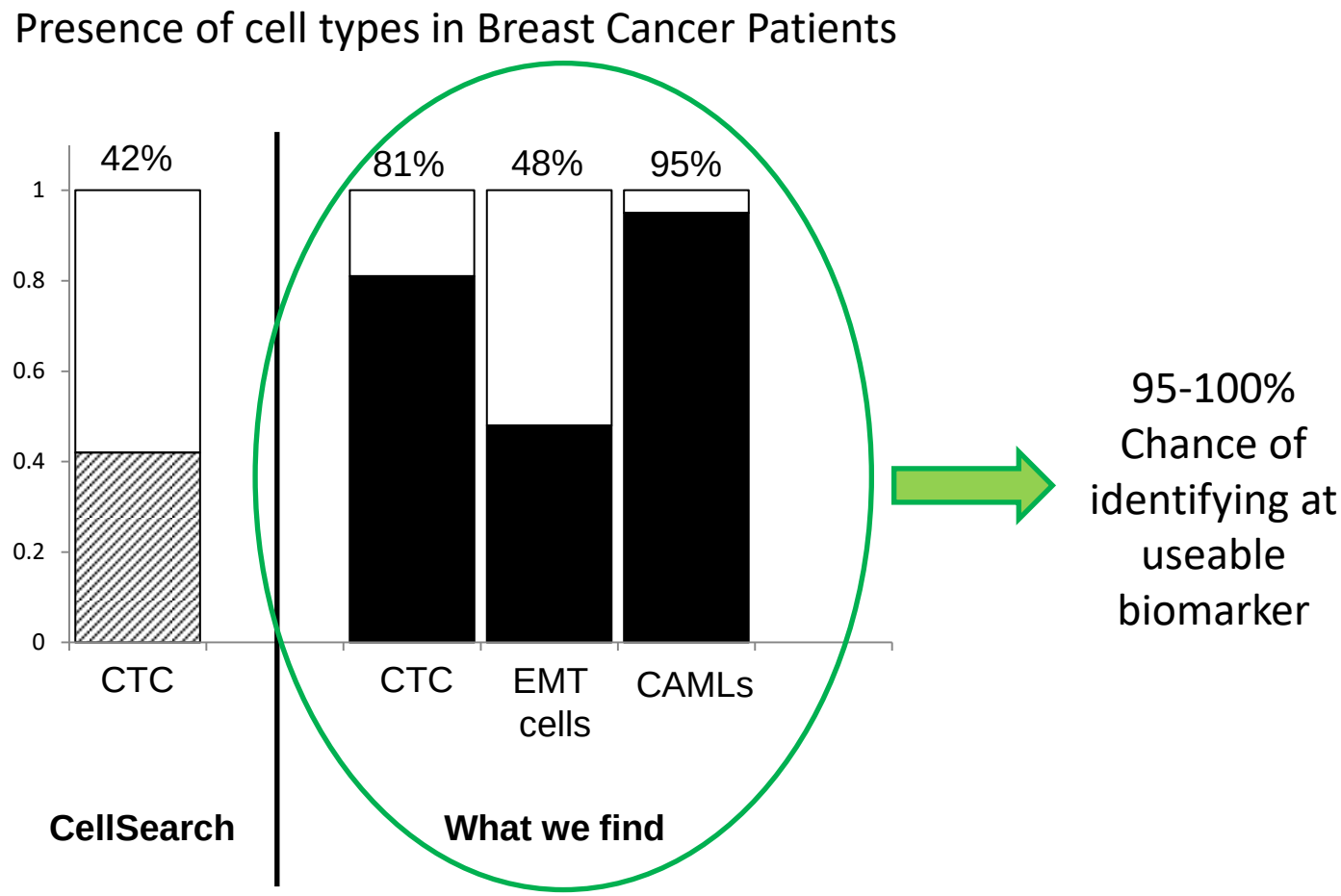
Cancer types

- 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%)**

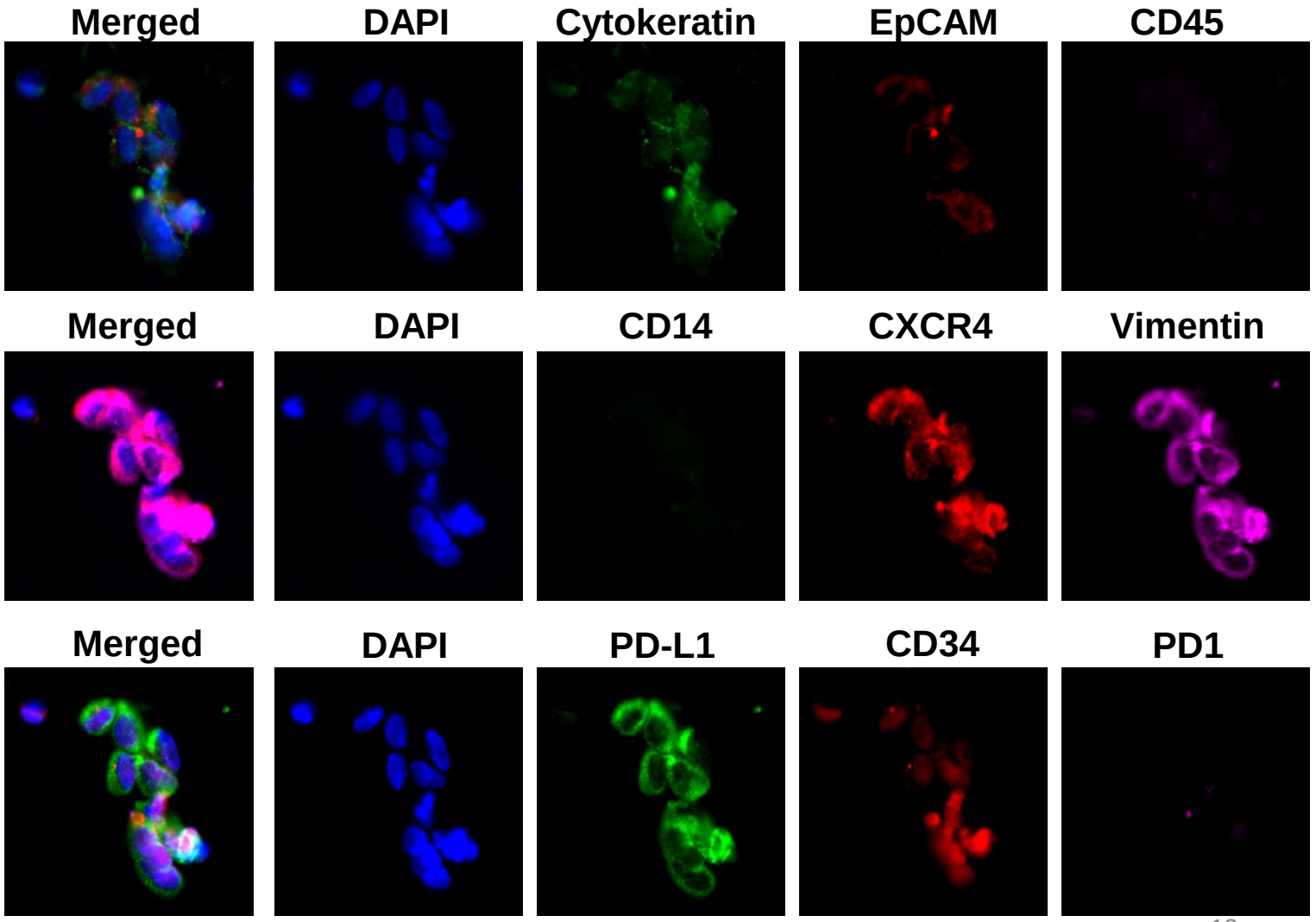
Adams, et al. ASCO 2015
Adams et al CEBP 2016

# We analyze CTCs and CStCs to maximize useable biomarkers




Adams et al. PNAS 2014 and Cytometry A 2015

# Multi-analyte Subtyping CTCs and CStCs using a single sample




Adams et al. Scientific Reports 2016 (in press)

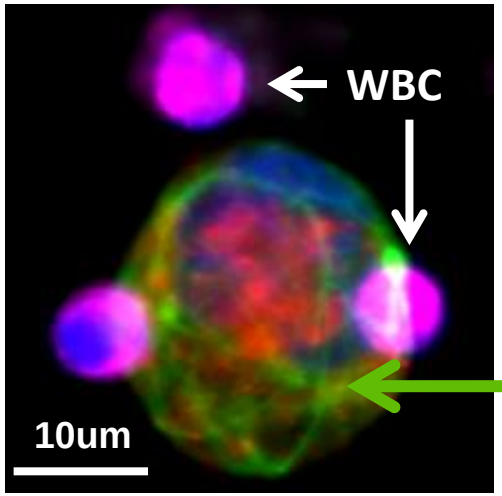
# Analysis of Immunotherapy

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

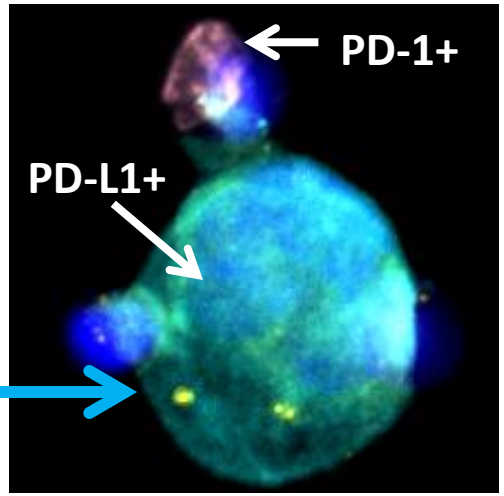
Nucleus(dark blue)/PD-L1(turquoise)/ PD-1(pink)

**Breast**

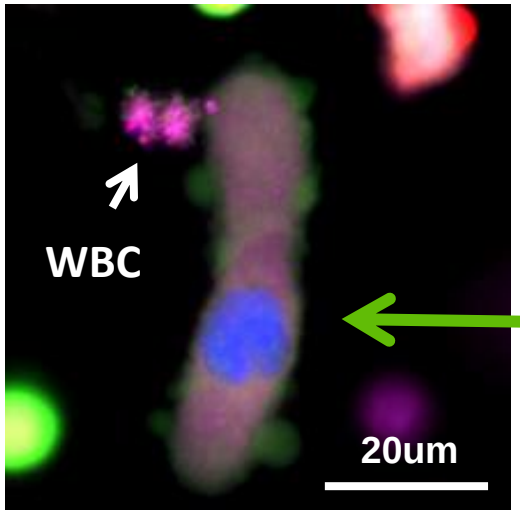
**CTC with bound white blood cells**



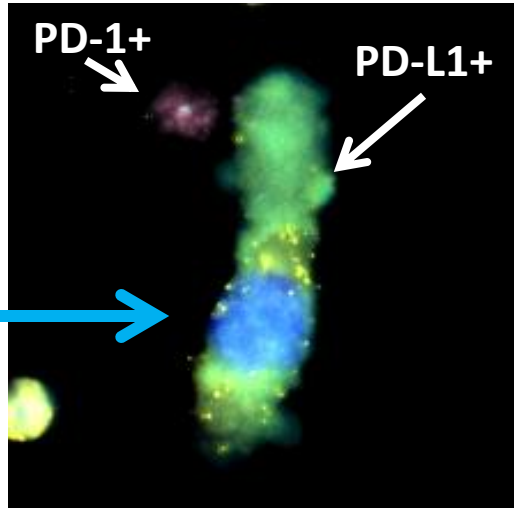

CTC



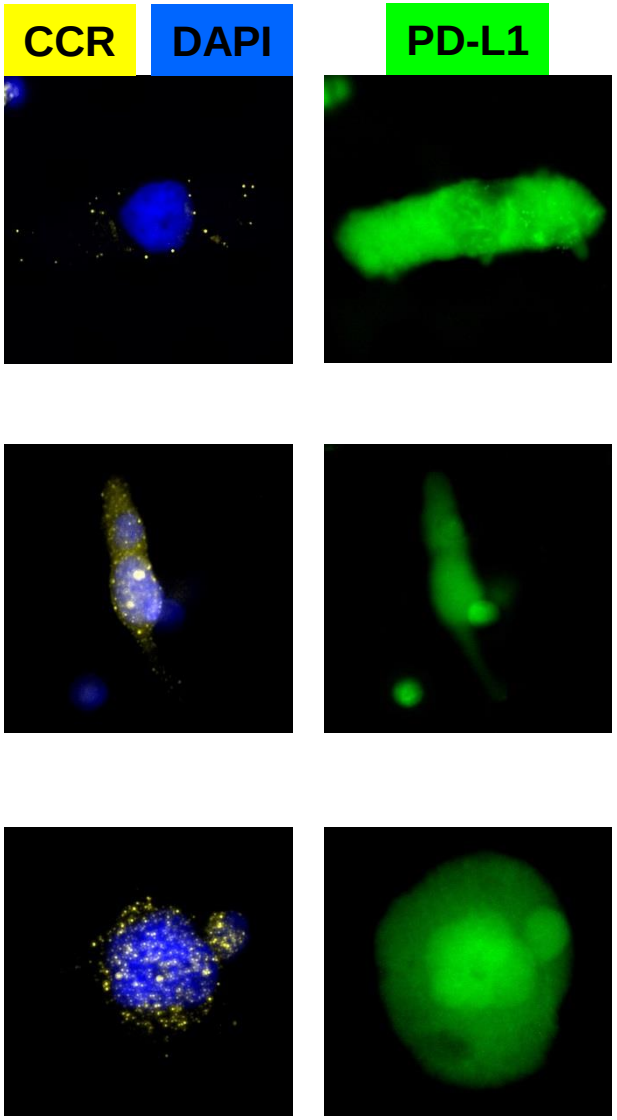

**Cytokeratin positive CStC with bound white blood cell**




CAML



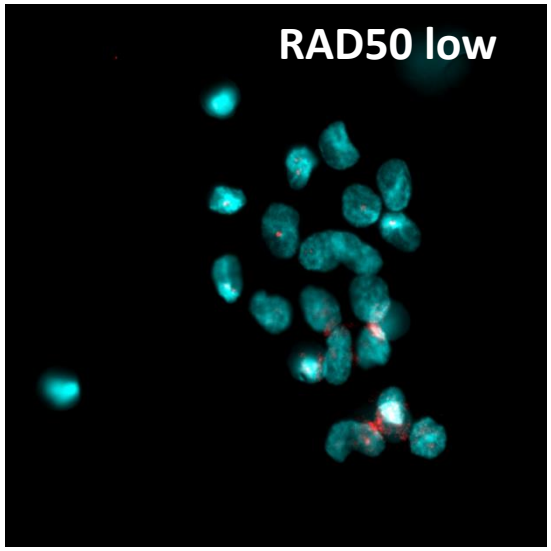
# Proteomic profiling of each individual patient in Real-Time



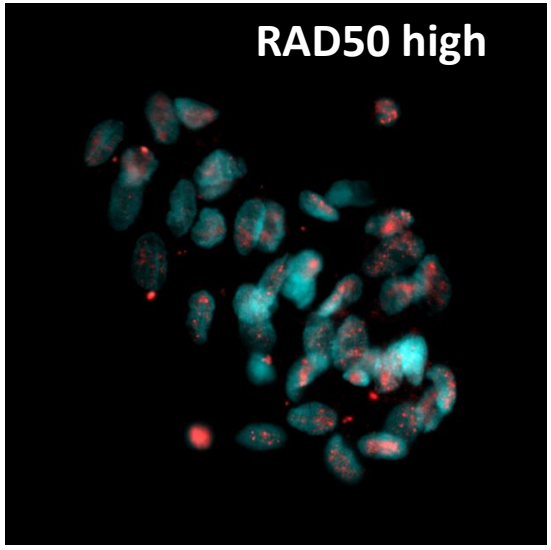

| | CK | VM | PD-L1 | CXCR4 | CD34 | EpCAM | CD45 | PD-1 | CD14 |
|---|---|---|---|---|---|---|---|---|---|
| P4 | | | | | | | | | |
| P6 | | | | | | | | | |
| P2 | | | | | | | | | |
| P12 | | | | | | | | | |
| P11 | | | | | | | | | |
| P10 | | | | | | | | | |
| P1 | | | | | | | | | |
| P3 | | | | | | | | | |
| P5 | | | | | | | | | |
| P7 | | | | | | | | | |
| P9 | | | | | | | | | |
| P8 | | | | | | | | | |

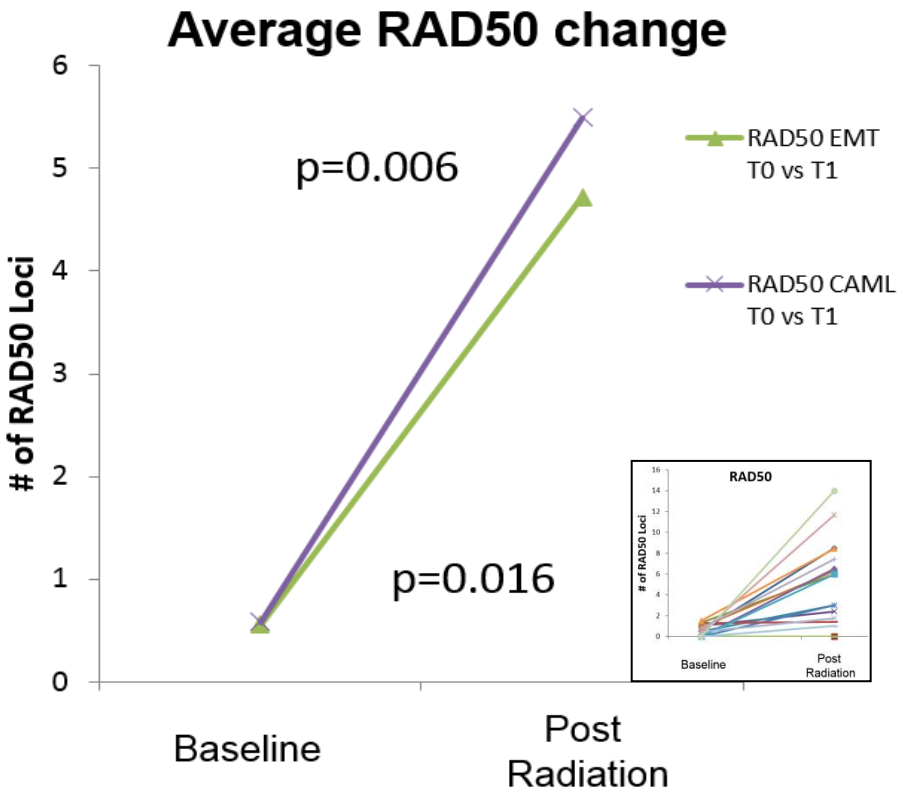
# Tracking Origin of CStCs

**Low Before Radiation**




**High Post Radiation**

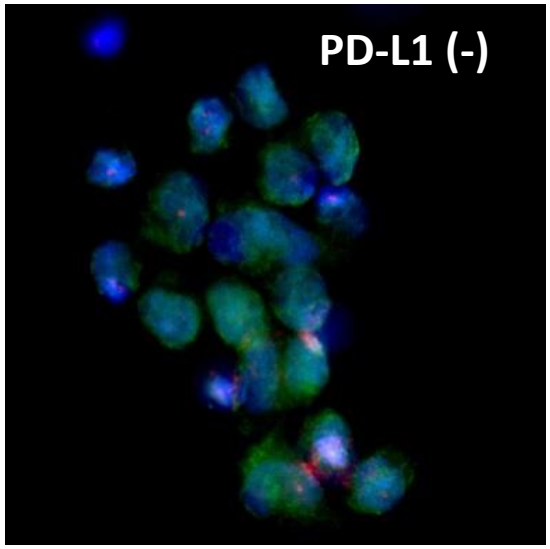


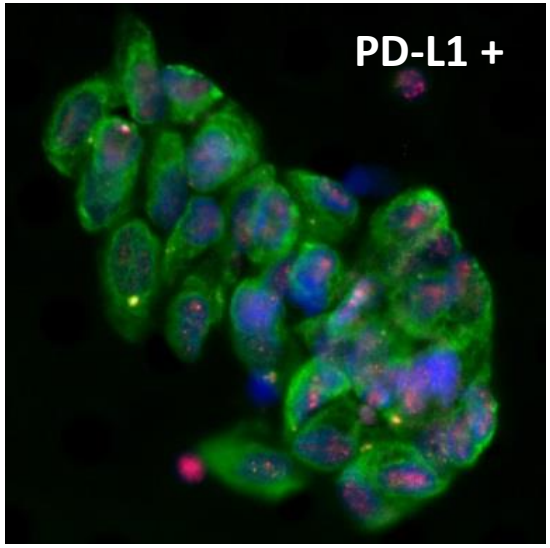

RAD50 foci ranged from 0-20 per cell, with an average of 0.57 at T0 that increased to 5.11 at T1 ($p<0.001$) during radiotherapy

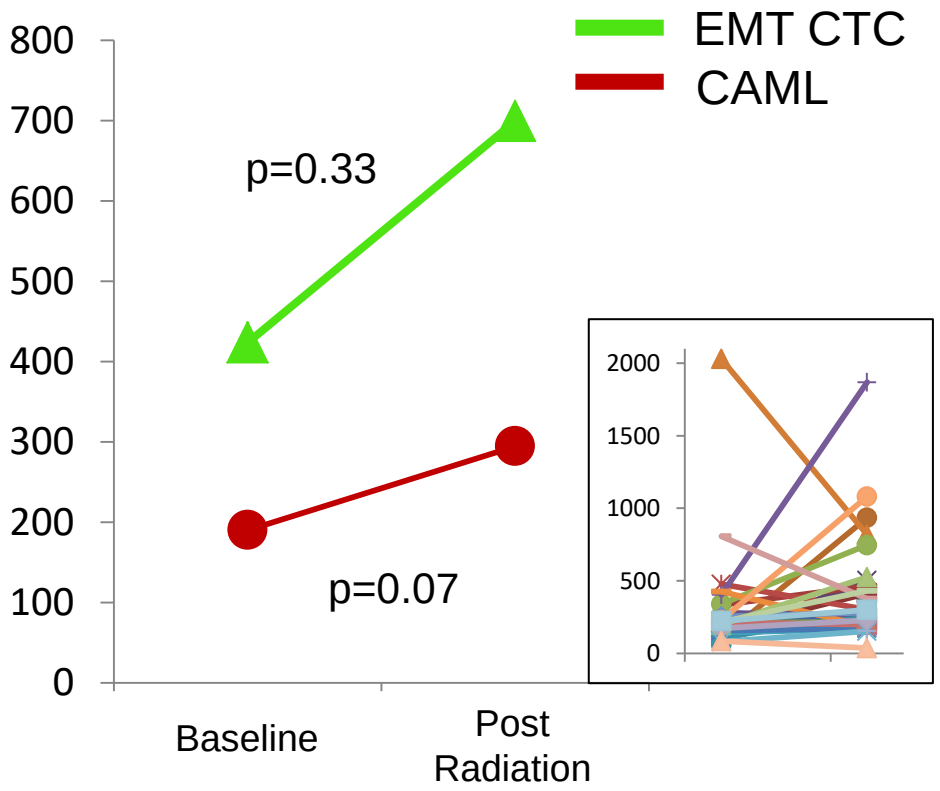
# Tracking upregulation and down regulation of biomarkers in real time

**Low Before Radiation**



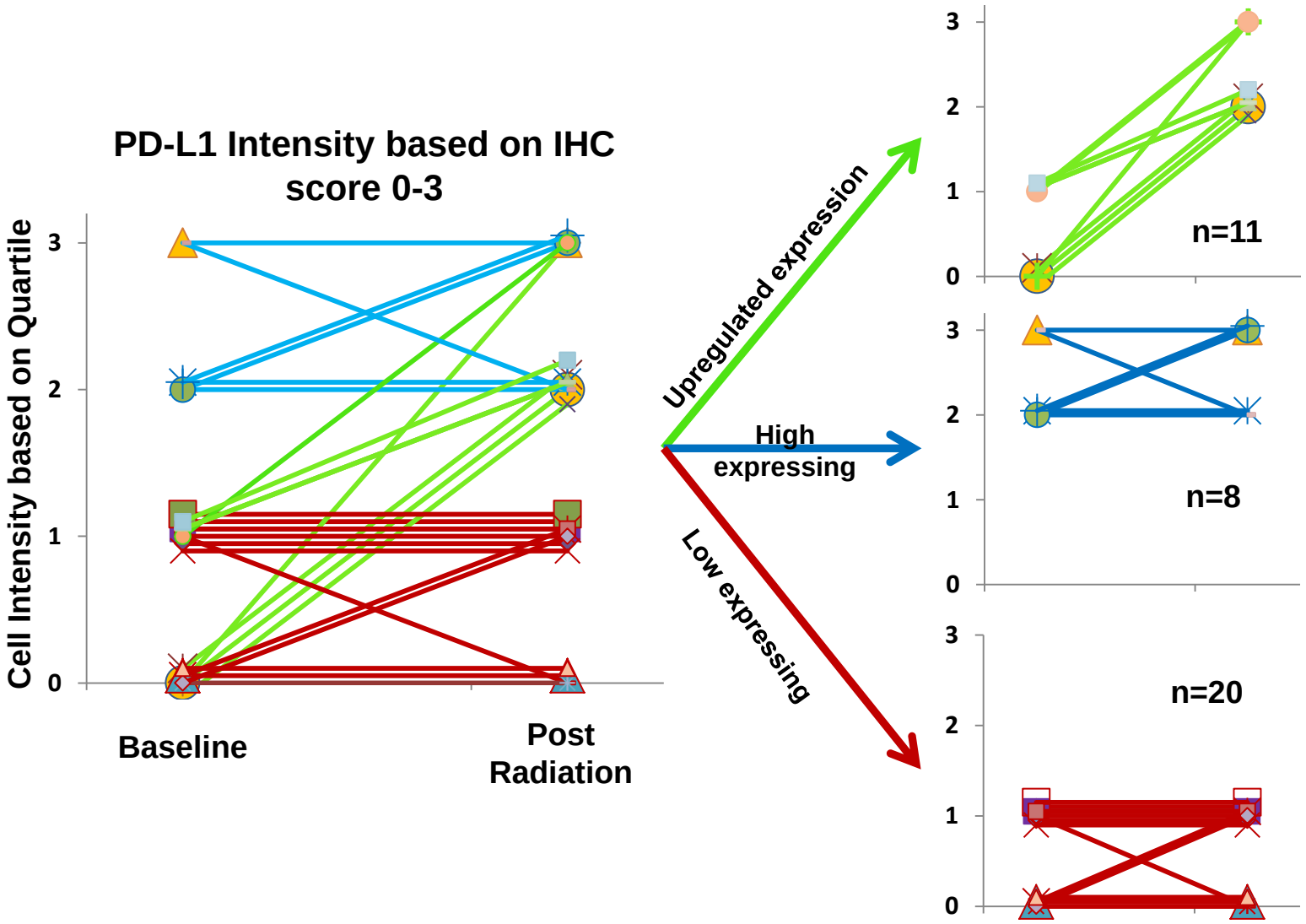

**High Post Radiation**




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 + AACR

# Ongoing Research and Clinical Trials with Creatv Partners

| | 4th Q 2015 | 1st Q 2016 | 2nd Q 2016 | 3rd Q 2016 |
|---|---|---|---|---|
| MEK inhibitors + PD-1 therapy (IV breast) | | | | |
| PD-1 therapy (stage IV lung) | | | | |
| PD-1 therapy (Renal Cell) | | | | |
| Platinum based chemo+PD-1 (IV breast) | | | | |
| PD-1 therapy + Radiation (stage III lung) | | | | |
| PD-1 therapy + Radiation (stage I lung) | | | | |

Research Trial

Clinical Trial

**All trials are tracking CTCs, EMTs, and CAMLs**

# Summary of Creatv's Capabilities

- **CellSieve™ Blood Based Biopsy – isolates CTCs and CStCs**
  - Provides sequential tracking of cancer-baseline through treatment
  - Applicable to ALL Stages of cancer (screening through prognosis)
  - Allows genomic and proteomic profiling of multiple cell types
  - Useful in most solid tumors
- **Analyze all subpopulations of tumor cells and stromal cells**
  - Not marker specific, i.e. multicellular analysis
  - Parallel subtyping of cells and drug targets
- **Wide variety of cancer specific cell analyzes (CTCs and CStCs)**
  - Companion diagnostics
  - Monitor treatment
  - Cancer screening

# Research Collaborators

| Research Institute | Collaborators |
|---|---|
| University of Maryland Baltimore | Stuart Martin, Ph.D.,Monica Charpentier, M.D. Martin Edelman, M.D., Rena Lepidus. Ph.D. |
| Northwestern University | Massimo Cristofanilli, M.D. |
| Fox Chase Cancer Center | R. Katherine Alpaugh, Ph.D. |
| 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. |
| Duke University | Jeffery Marks, Ph.D. |
| Memorial Sloan Kettering Cancer Center | Daniel Danila, M.D. |
| Washington University | Rebecca Aft, M.D. |
| University of Chicago | Susan Cohn, M.D. |
| George Washington University | Christian C. Haudenschild, M.D. |
| Hememics Biotechnologies | Steigrimur Stefansson, Ph.D. |

# Acknowledgements


- **Thank the volunteers who contributed to these studies**
- **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

**Company Contact**

301-983-1650

cmtang@creatvmicrotech.com

www.creatvmicrotech.com

Booth # 47