CREATV MicroTech

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

# Blood Based Cell Biopsy
# For Cancer Diagnostics


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**August 26, 2016**

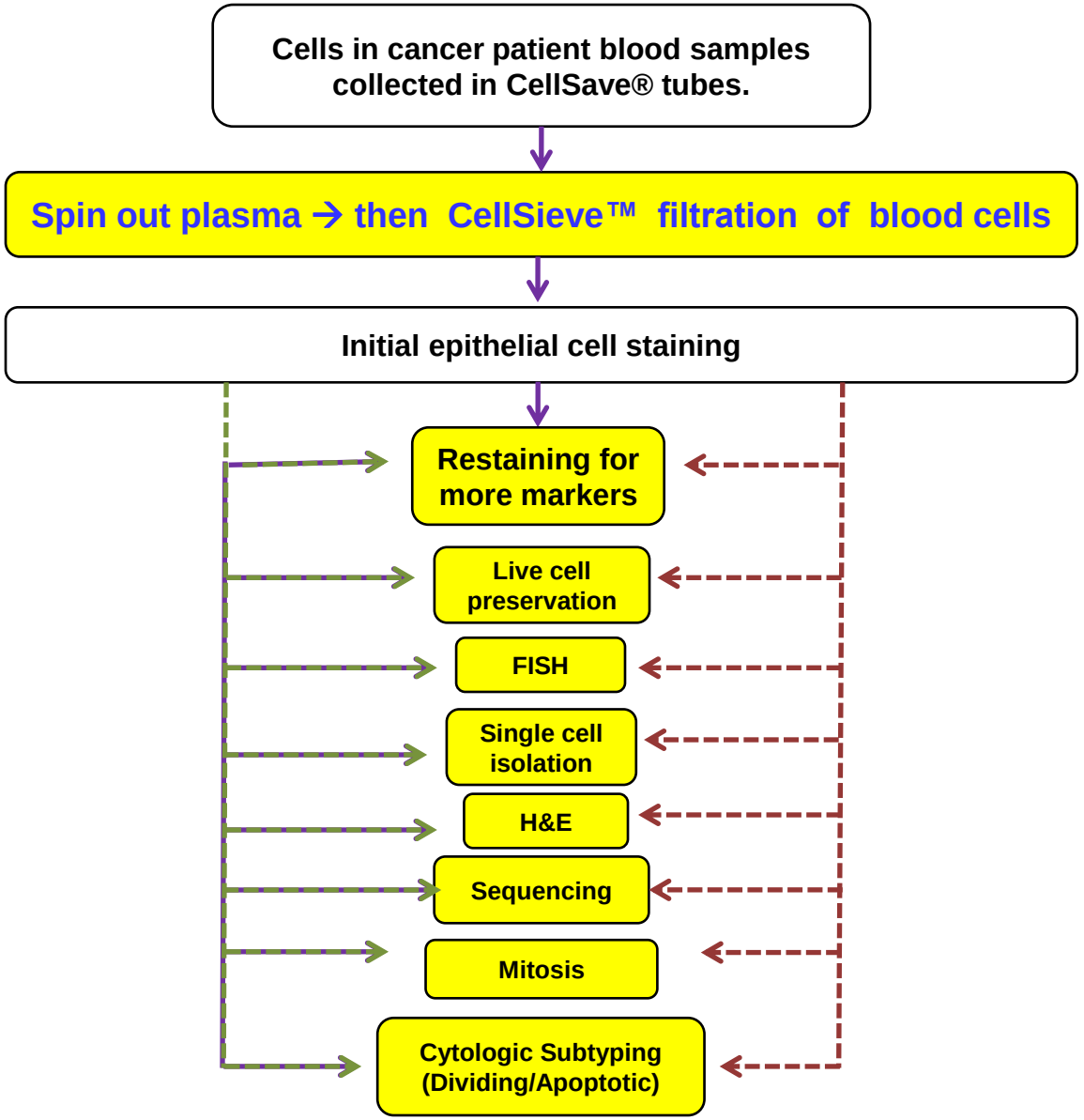
# Outline

- **Overview**
- **Technical background**
- **Clinical application examples**

# Unmet Needs

- **Blood based companion diagnostics**
  - **– example immunotherapy**
- **Monitor treatment response**
- **Early detection of cancer**
- **Early detection of recurrence**
- **R&D and drug development applications**

# Overview of Assays Capabilities

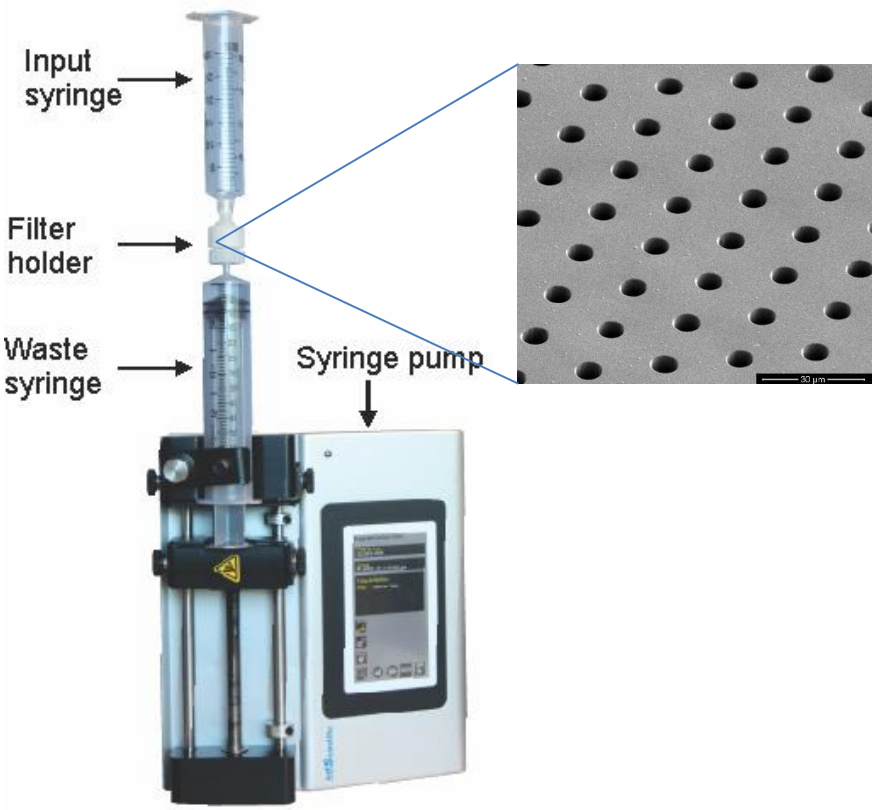


# Clinical Sample Type

- Peripheral blood
- Bone marrow
- Cryo preserved samples
- Urine

# CellSieve™ Microfilters

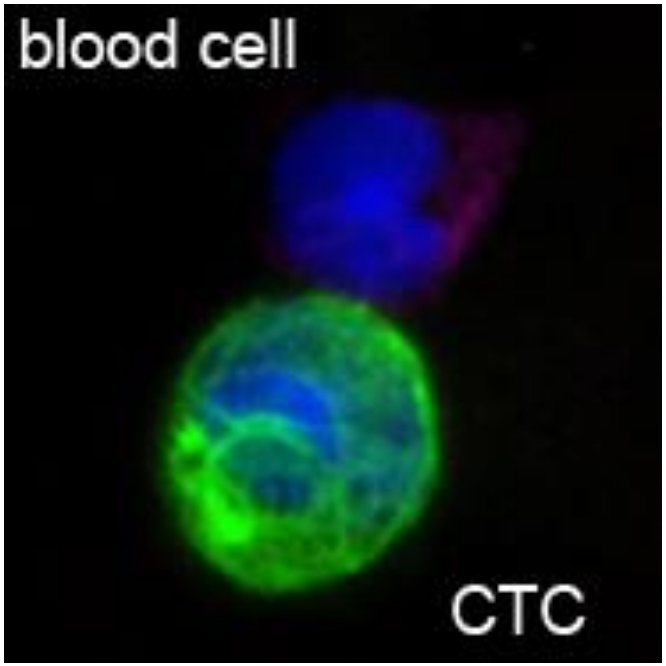
**Not based on surface markers**




- **Uniform 7 µm pore size and distribution with high porosity**
  - **Rapid, consistent and gentle flow**
  - **3 min to filter 7.5 ml of blood**
  - **Eliminates all red blood cells**
  - **Eliminates ~99.99% of white blood cells**
- **Low fluorescence background**
- **CellSave tubes good ≤ 96 hrs**

# "Pathologically Defined" CTCs

## Creatv's criteria to improve accuracy

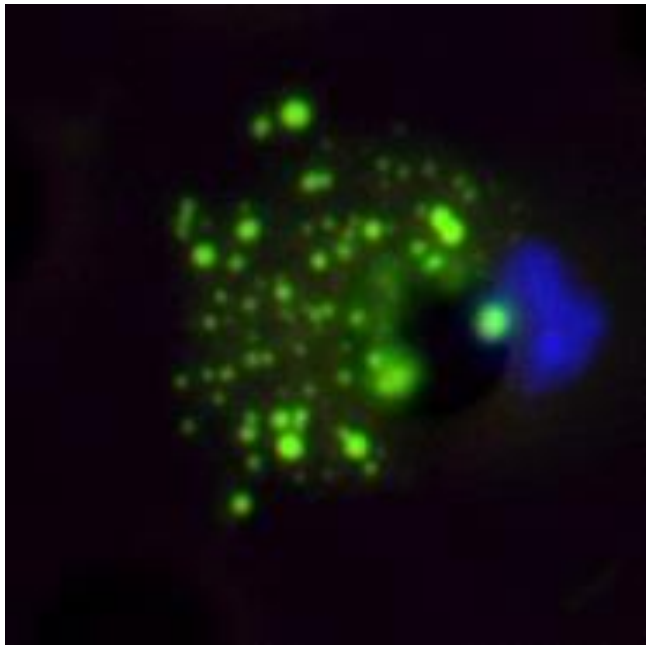



- DAPI positive
  - **and cancerous looking**
- To identify CTC
  - **CK 8, 18, 19 (+)**
  - **Cytoskeletons → filamentous pattern**
- To rule out white blood cells
  - **CD45 (-)**

Feasible with CellSieve™ microfiltration system

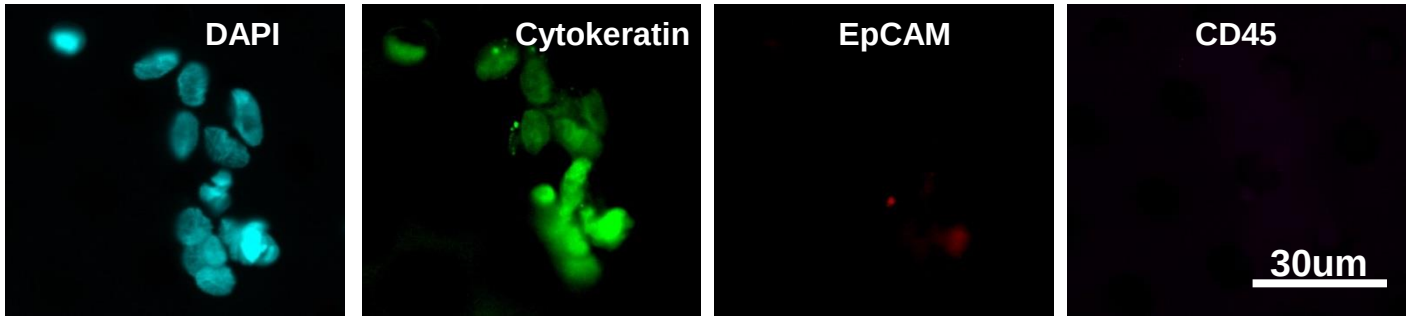
# Apoptotic CTCs

## Creatv's criteria to improve specificity



- DAPI positive
  - **and nucleus degrading**
- CK 8, 18, 19 (+)
  - **and fragmented into spots**
- To rule out white blood cells
  - CD45 (-)

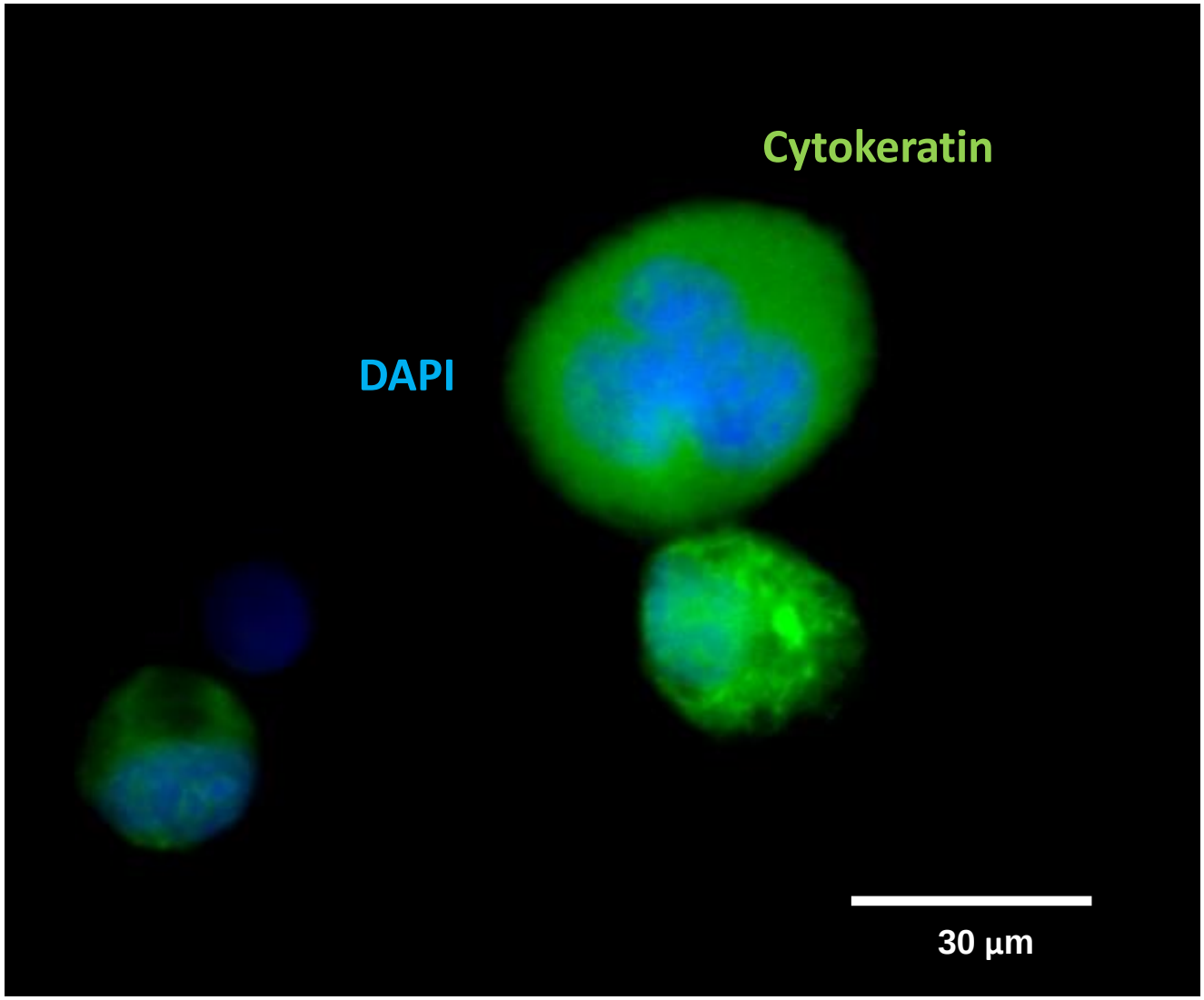
# Epithelial Mesenchymal Transition Cells (EMTs) – cells can later become cancer cells




- DAPI positive
  - **Smaller nuclei than CTCs**
  - **Usually form clusters**
- Identification of EMTs
  - **Weakly CK 8, 18, 19 (+)**
  - **No EpCAM**
- To rule out white blood cells
  - **CD45 (-)**

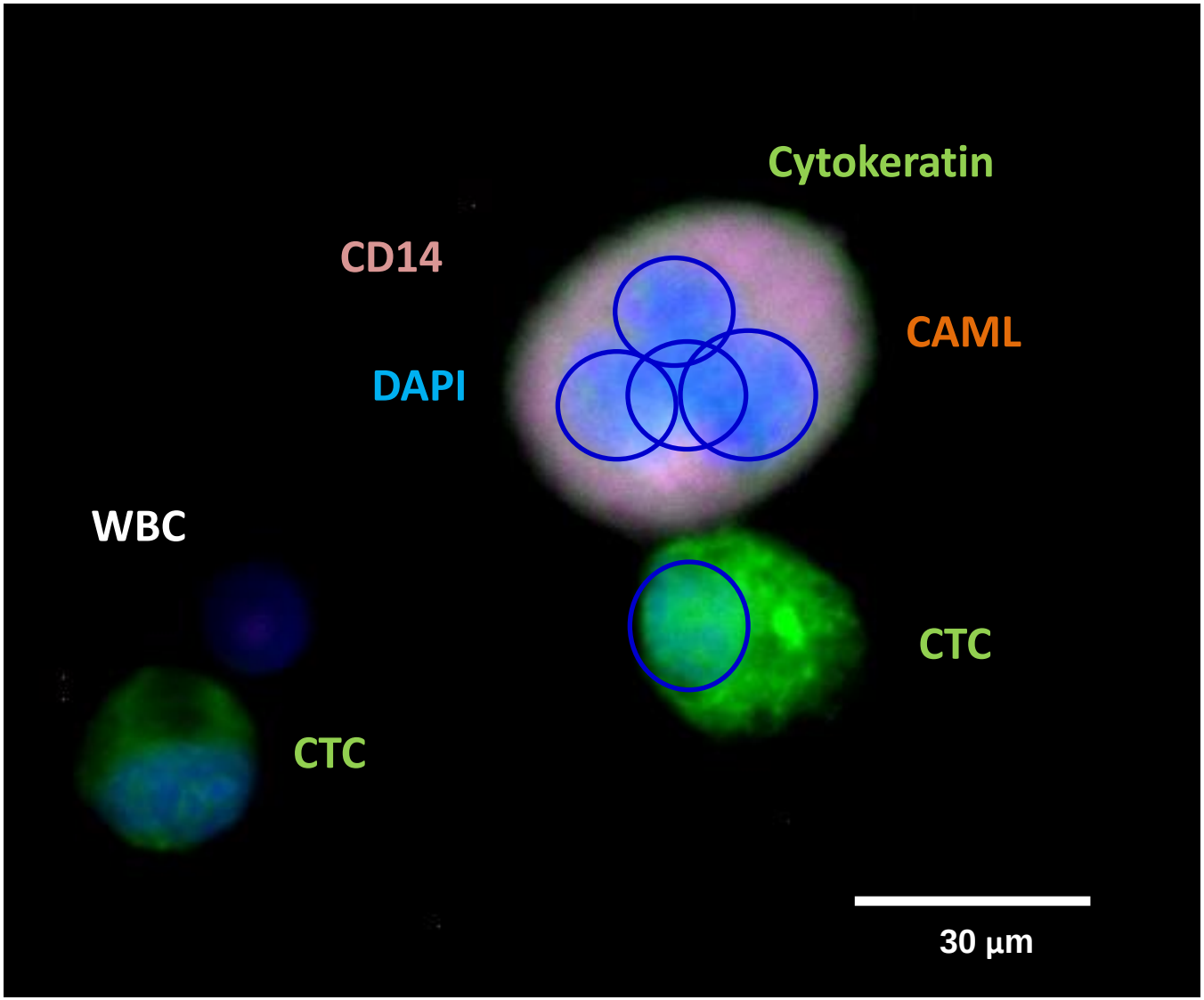
# Creatv Discovers a New Cell Type

**Cytokeratin:** marker for epithelial cell
**DAPI:** marker for the nucleus

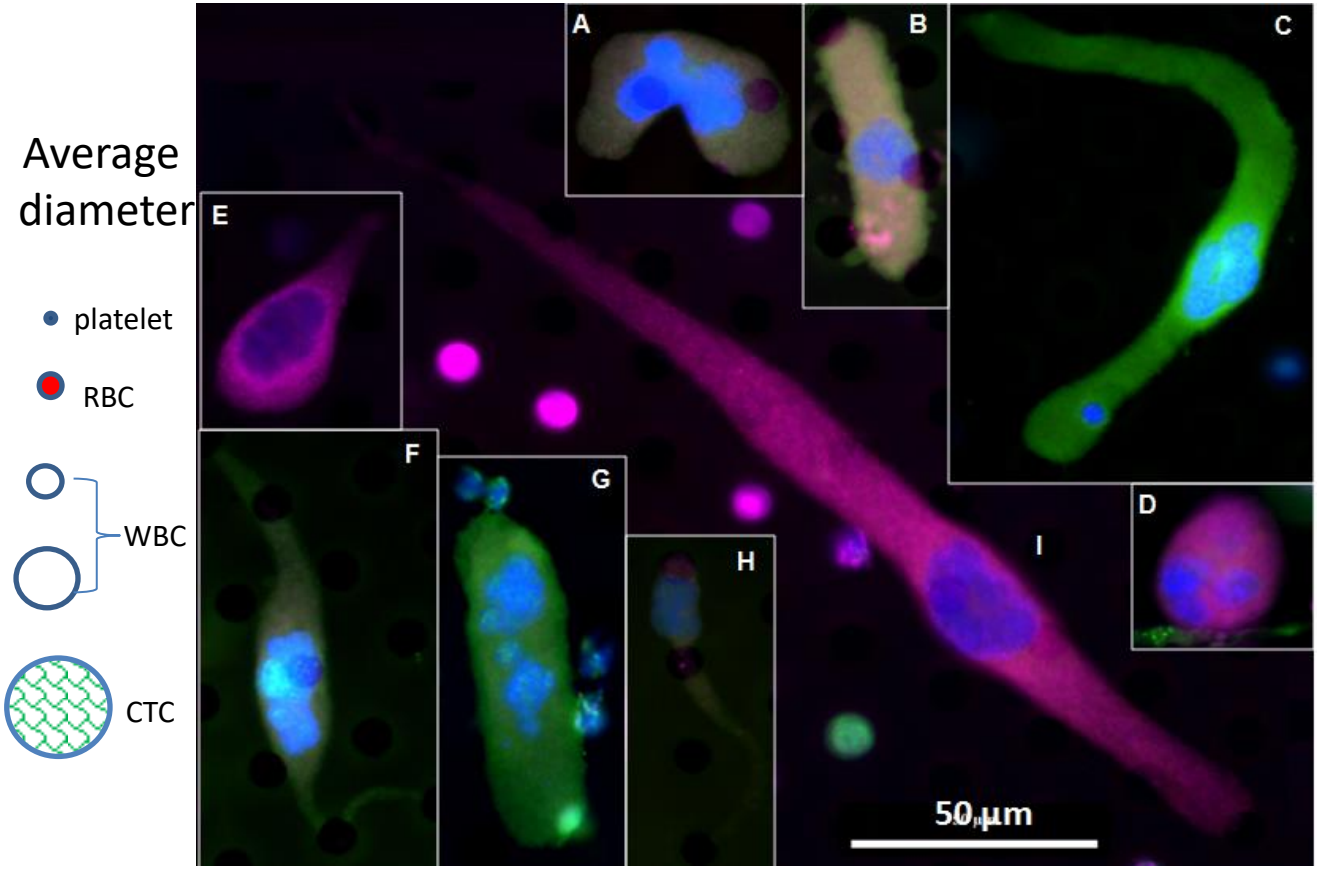


# Discovery

**CTC:** circulating tumor cells
**CAML:** circulating cancer associated macrophage-like cells



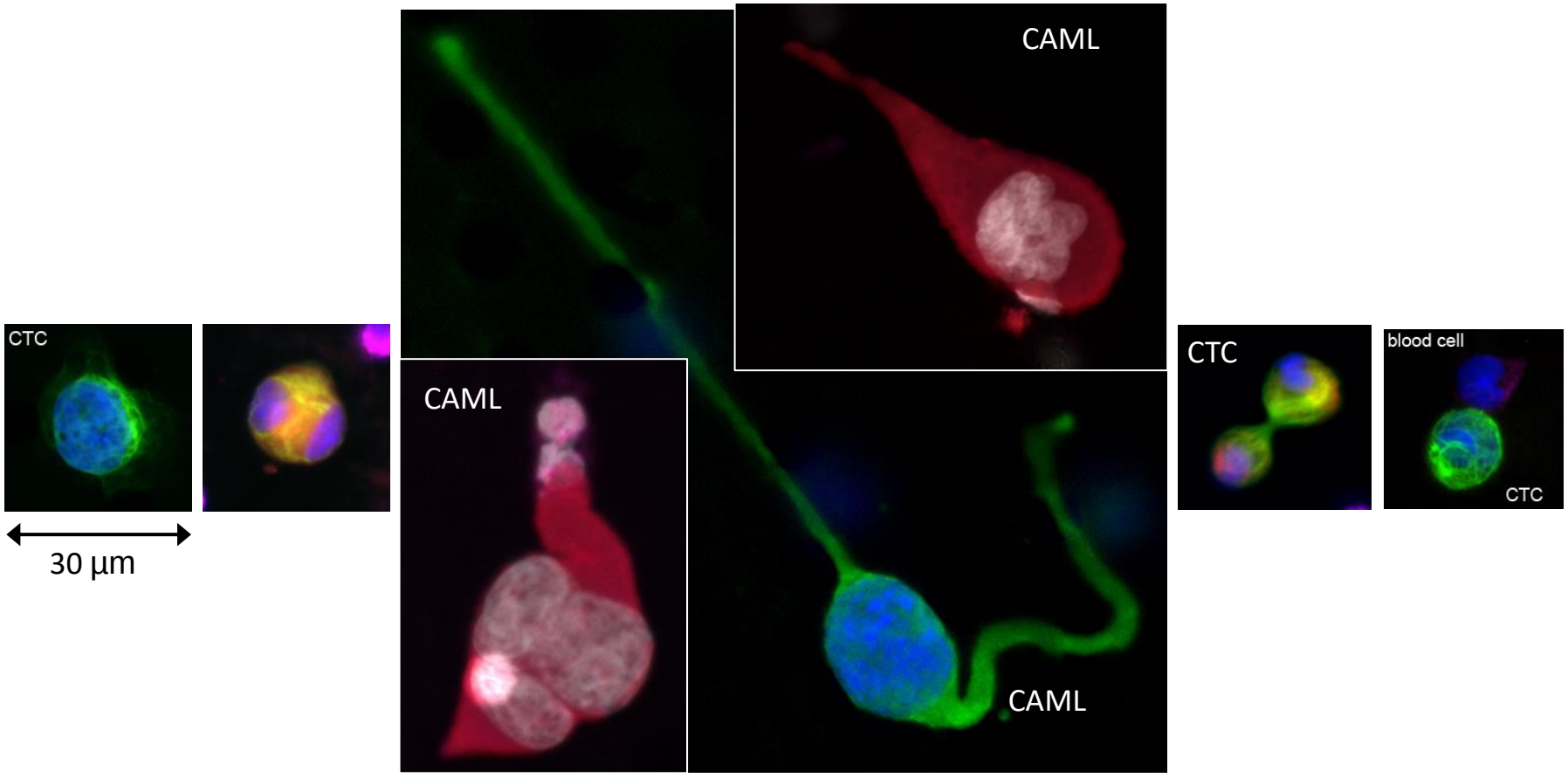
# Circulating Cancer Associated Macrophage-like Cells (CAMLs)




- Large, atypical nucleus
- May express CK and EpCAM
- Contain tumor markers
- Most are CD45 positive
- Large: 25 - 300 µm
- Express CD11c/CD14
- Express endothelial markers CD146, TIE-2

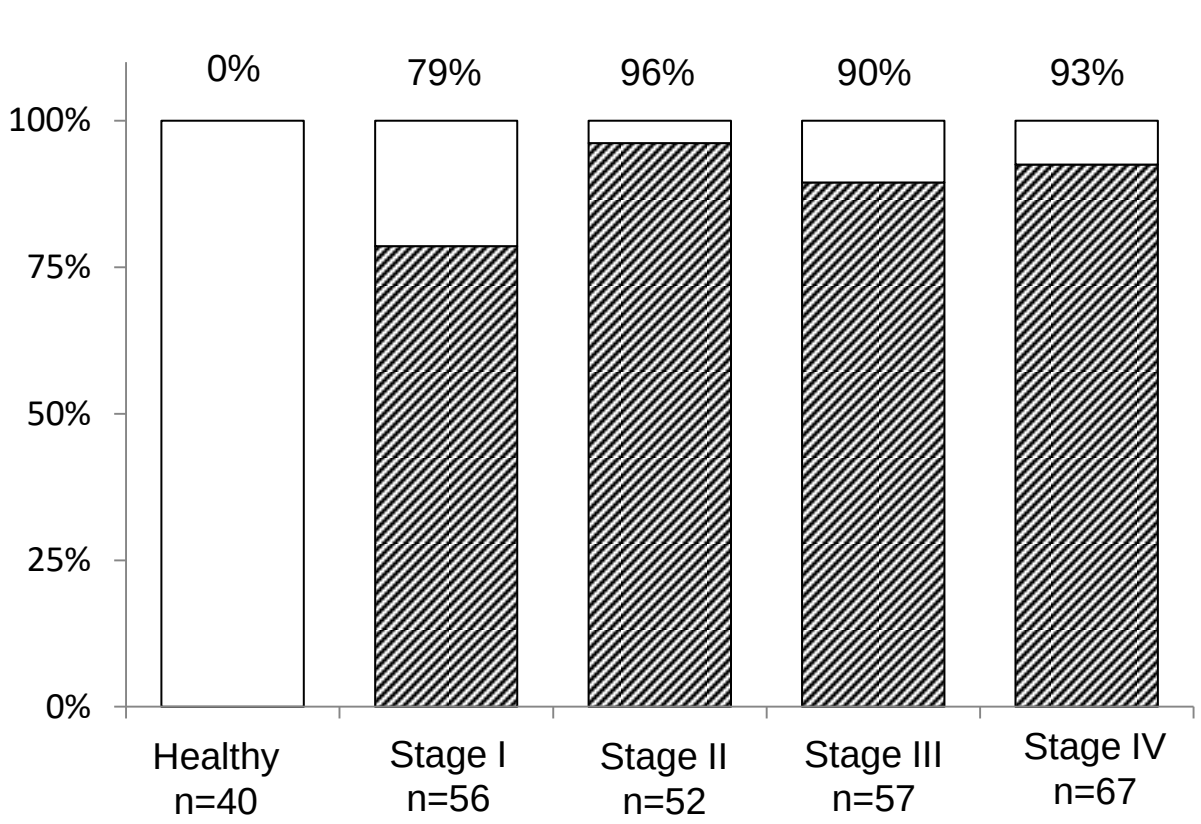
Adams, et al. PNAS 2014

# CAMLs Engulf Tumor Cells and Debris



# CAMLs only occur in Cancer Patients

**None in healthy controls**




Total n=272*

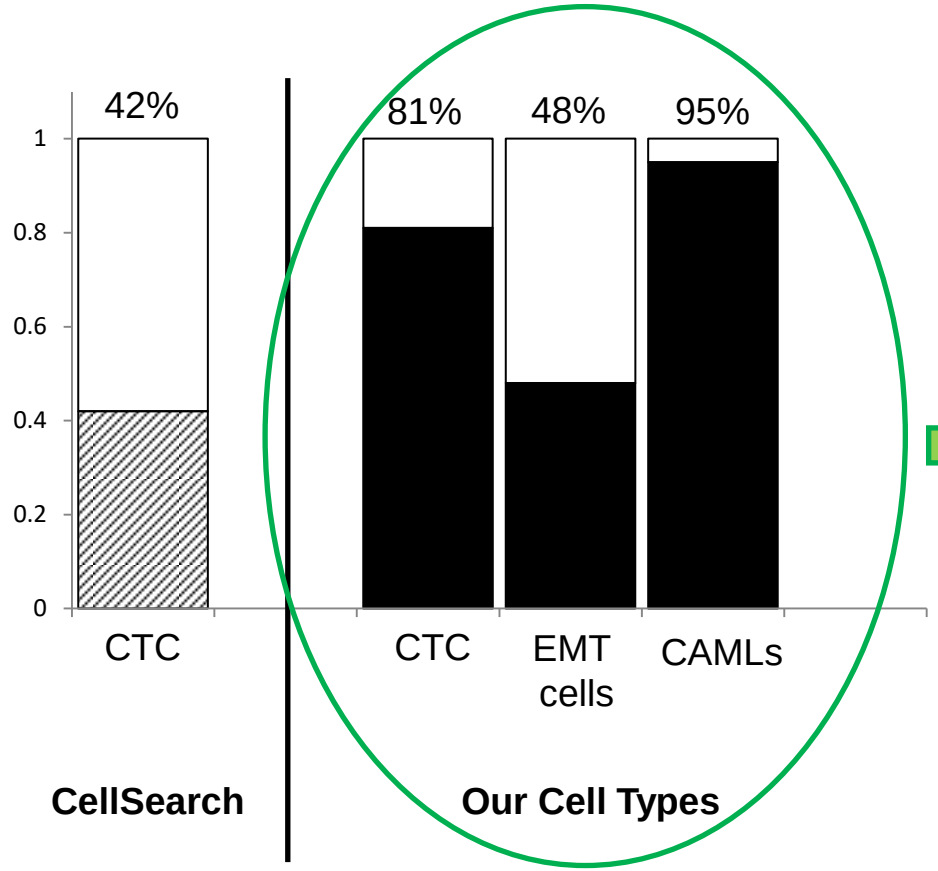
Cancer types

- 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%)

# We analyze all cancer-associated cells to maximize useable biomarkers

Presence of cell types in **Breast Cancer Patients**




95-100% Chance of identifying at useable biomarker

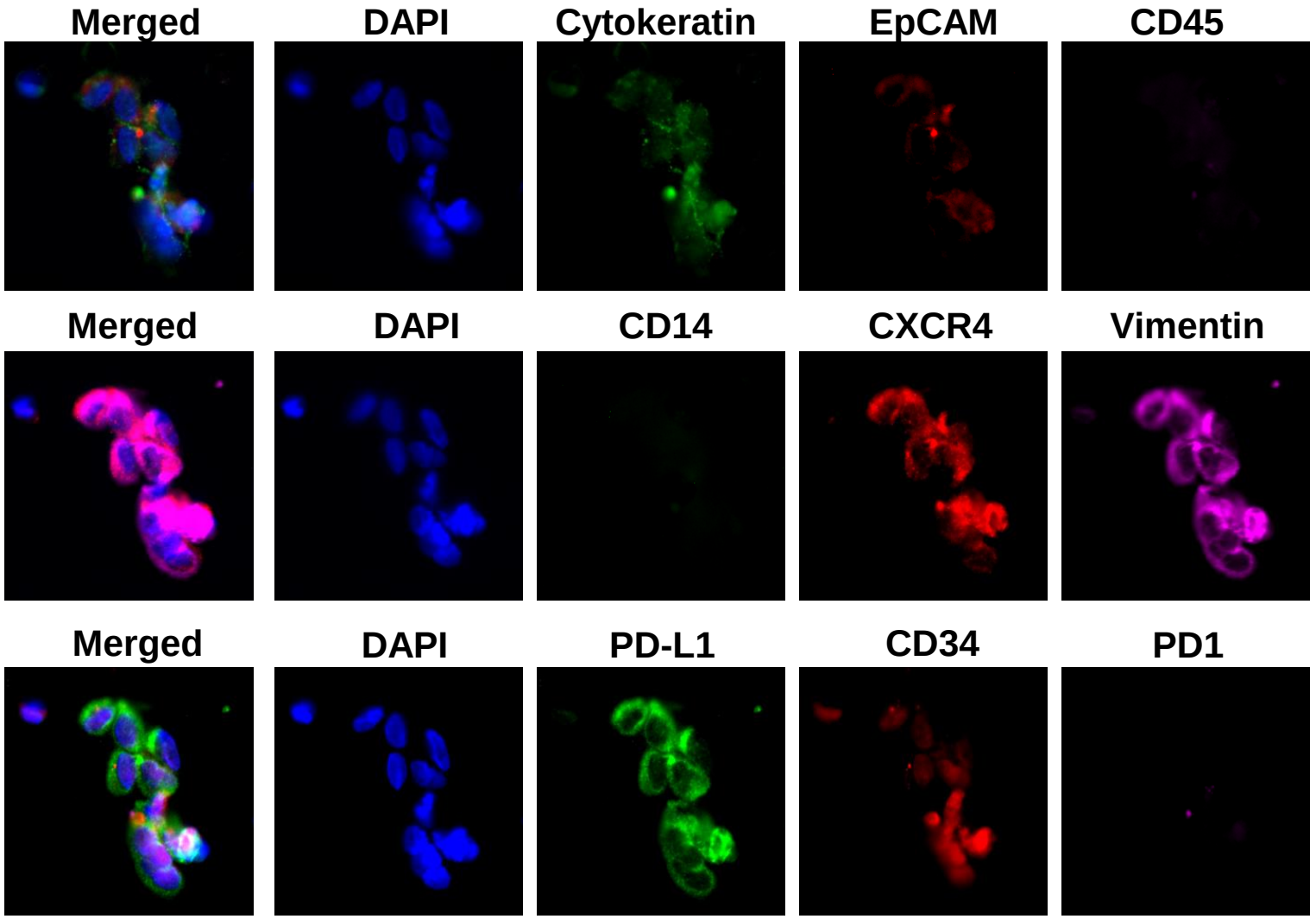
PNAS 2014 and Cytometry A 2014

# Clinical Utility
## CTCs and stromal cells

- **Companion diagnostics utilizing CAMLs**
  - Evaluate drug targets – not limited by availability of tissue
- **Monitor treatment response**
  - CAMLs and CTCs are independent indicators
  - Real-time measure of response
- **Clinical Trials**
  - Cohort selection
  - Real-time measure of response – even for immunotherapy
- **Cancer Screening**
  - At-risk populations: Lung, pancreatic, colorectal, etc.
  - Hard-to-detect cancers
  - Cancer recurrences

# Multi-analyte Subtyping

## Restaining Technique




Adams et al., Scientific Reports 2016

# Immunotherapy Diagnostic Needs

- **Companion diagnostics**
  - Currently based on tissue biopsy
  - Biopsy not able to guarantee inclusion of stromal infiltrated regions
  - Usually not repeatable
- **Monitor treatment**
  - Imaging cannot monitor treatment response because stromal infiltration causes the tumor to become larger

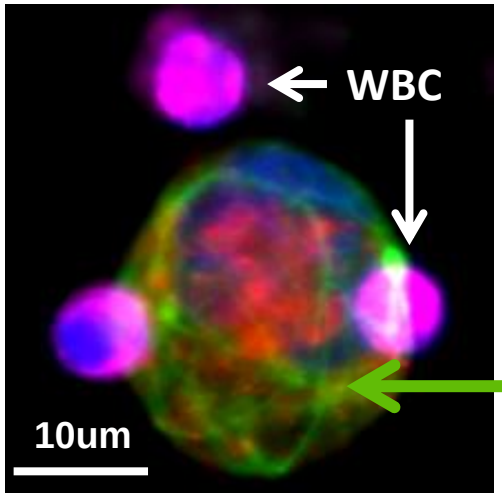
    (pseudo-progression)

# CellSieve™ Analysis of Immunotherapy

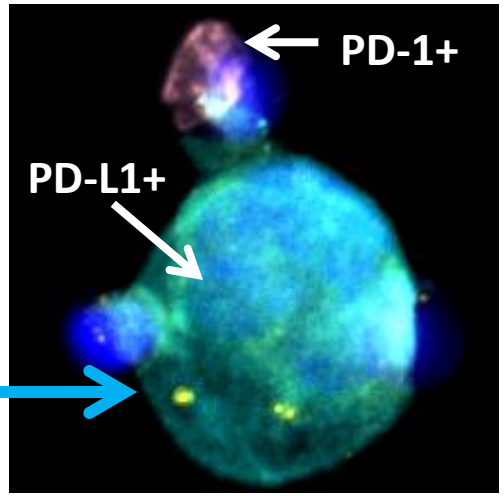
**Breast**

**CTC with bound white blood cells**

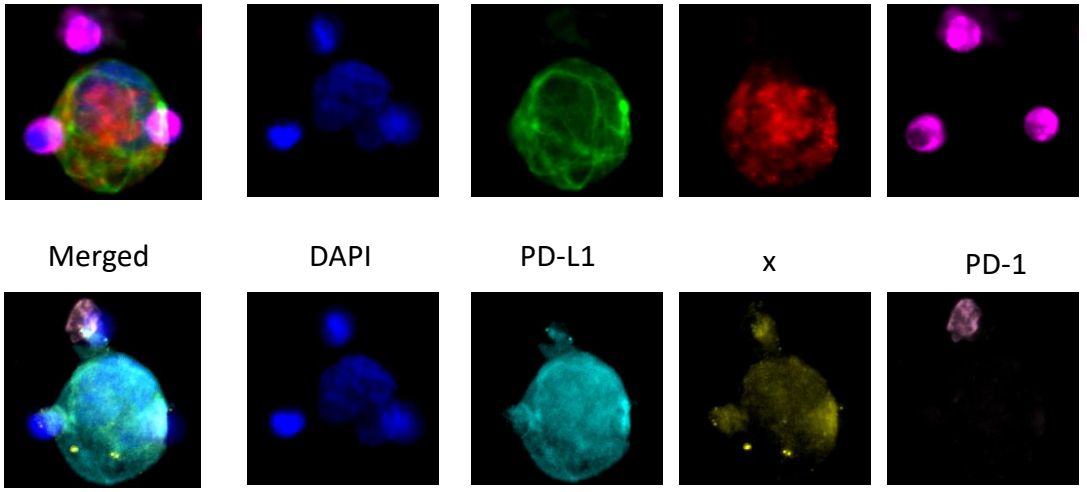
**Nucleus(dark blue)/CK(green)/ EpCAM(red)/CD45(violet)**




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




**CTC**

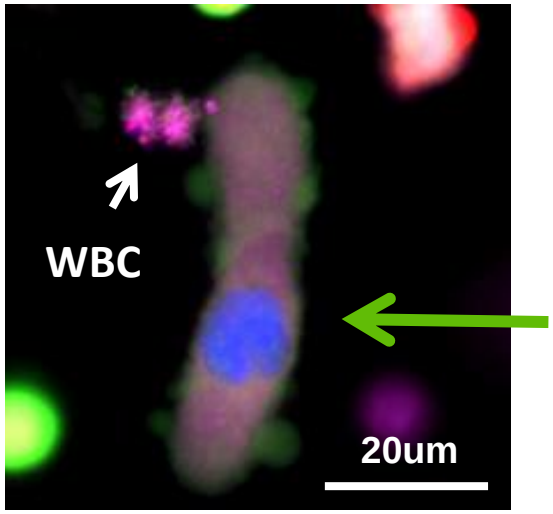


# CellSieve™ Analysis of Immunotherapy

**CAML**

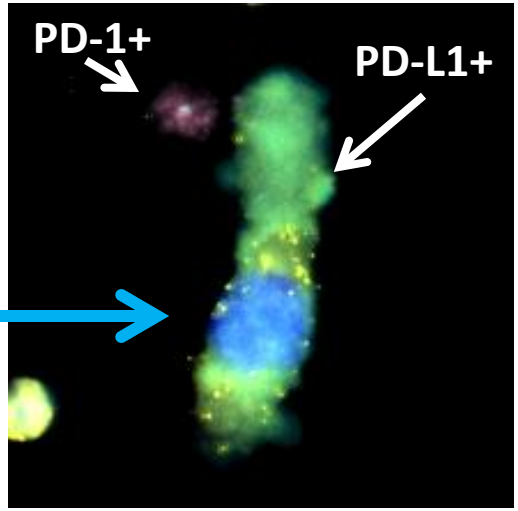
**Cytokeratin positive cell with bound white blood cell**

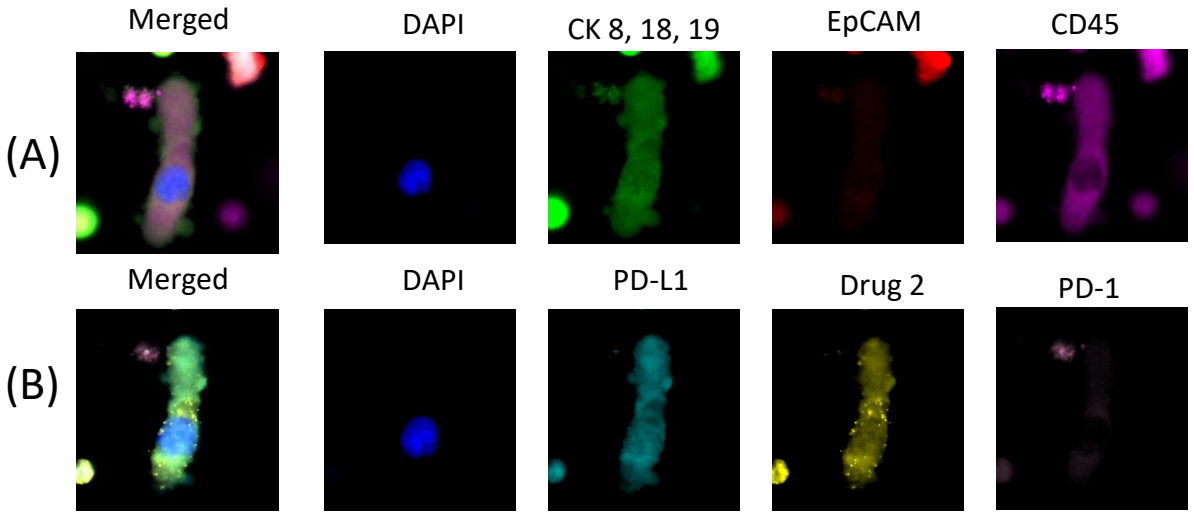
**Nucleus(dark blue)/CK(green)/ EpCAM(red)/CD45(violet)**




**CAML**

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



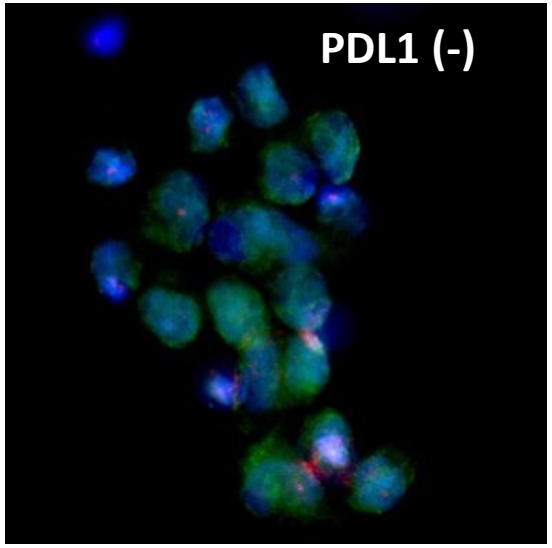
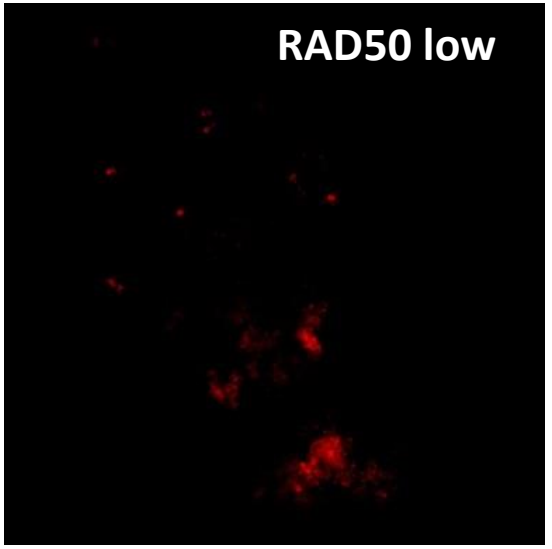
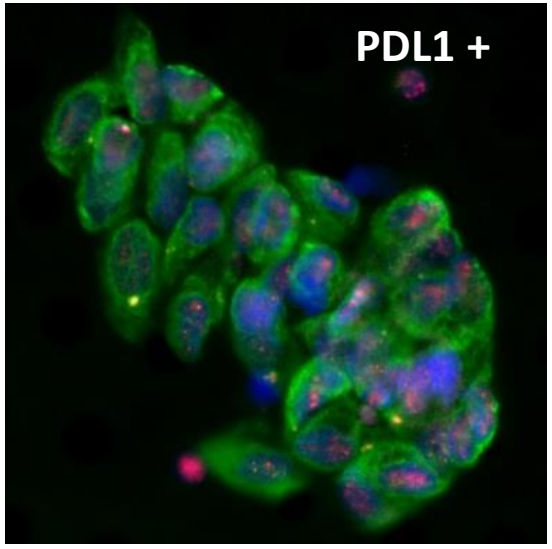
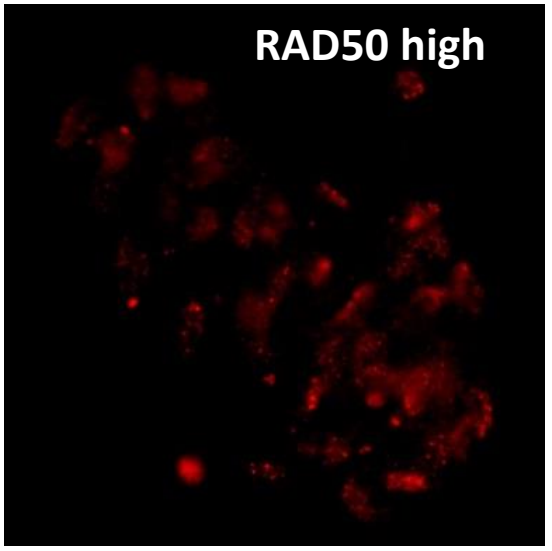



# CTCs and CStCs identify the upregulation or down regulation of immunotherapy in real time

- Oral Presentation WCLC 2015 - Sequential Assessment of DNA damage response and PD-L1 expression in circulating tumor cells of lung cancer patients during treatment with radiotherapy
- Poster Presentation AACR 2016 - Sequential tracking of PD-L1 expression and RAD50 induction in CTCs and circulating stromal cells of lung cancer patients during treatment with radiotherapy

# Effect of RAD50 and PDL1

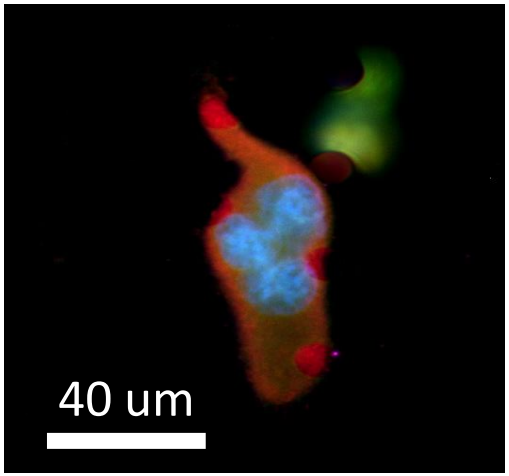
## NSCLC – before and after radiation therapy

| | Merge | RAD50 |
|---|---|---|
| **Before Radiation** |   |   |
| **Post Radiation** |   |   |

# CAMLs for Breast Cancer Screening

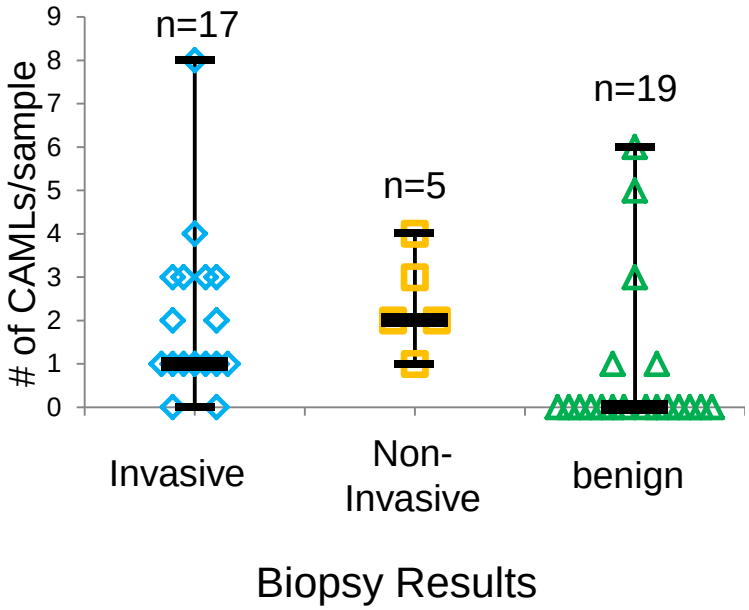
## Double blinded study

- **41 mammography positive patients**
  - Median age 52
- **Double blinded study**
  - Tissue biopsy
  - CAML blood testing
- **Blood test criteria – 1 or more CAML**
  - ≥ 30 µm
  - CD14 (+)
- **CAMLs differentiated benign vs malignant conditions**
  - Sensitivity (+ for cancer) – 88%
  - Specificity (no cancer ) – 74%

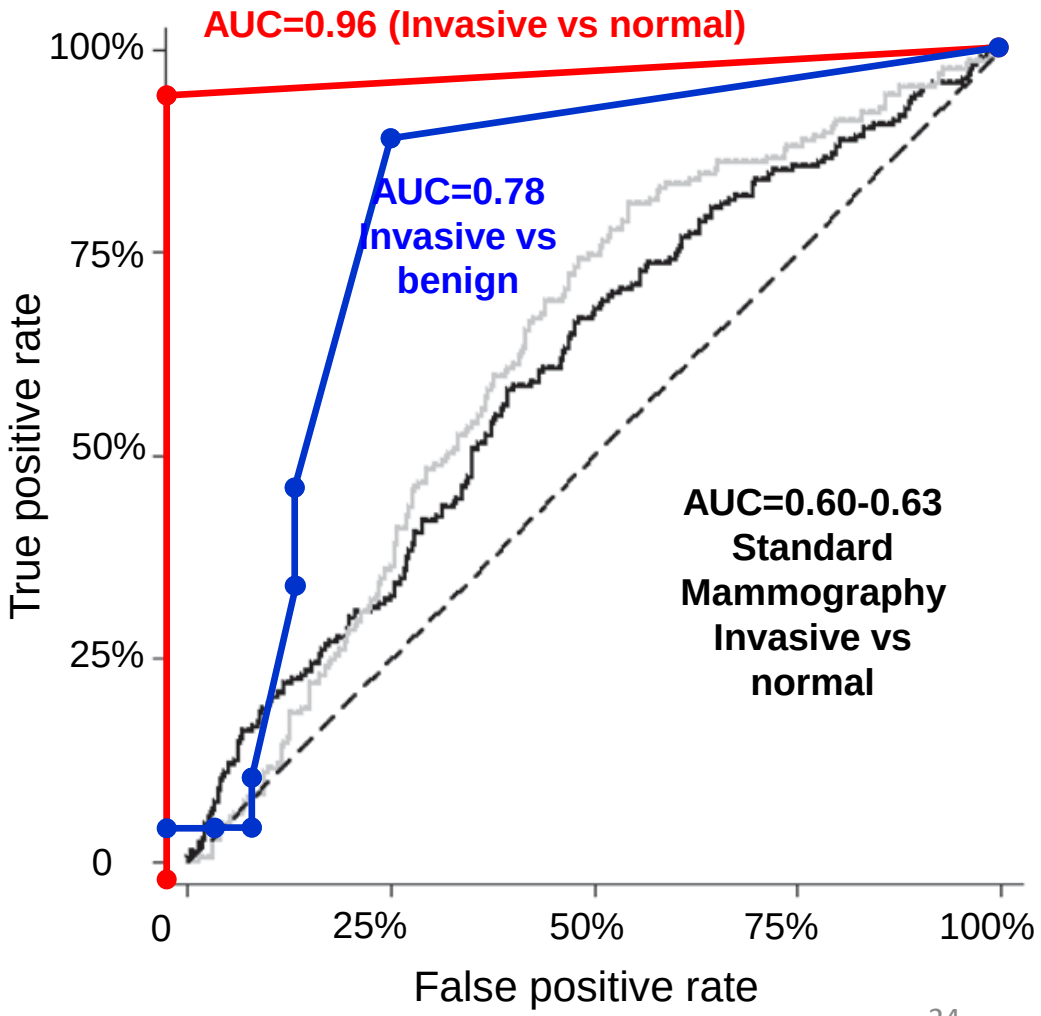



Adams, et al., Cancer Epidemiology, Biomarkers & Prevention 2016

# CAMLs for Breast Cancer Screening

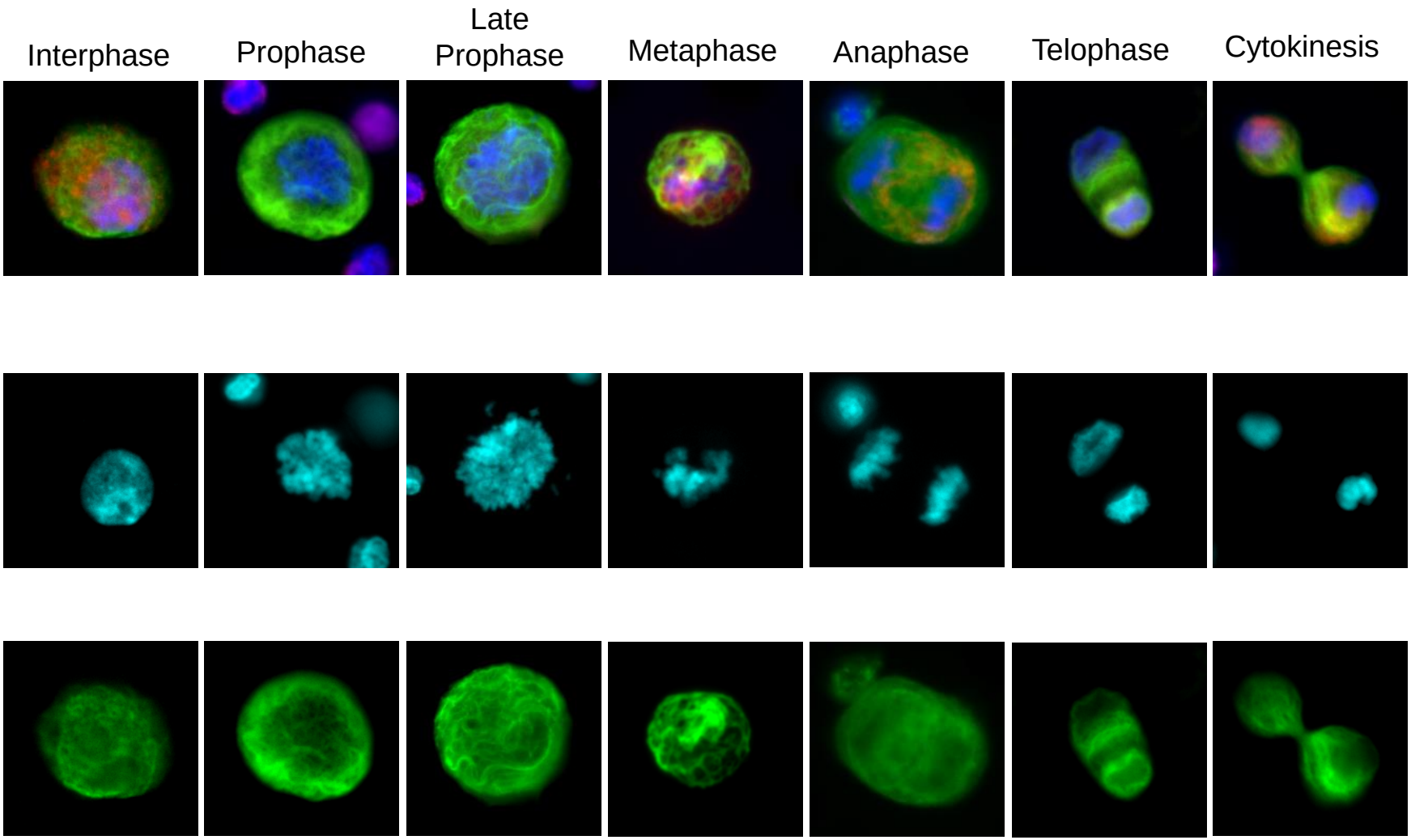



Superior to mammography



# CTCs in Division

## Cytokeratin (green), DAPI(blue)

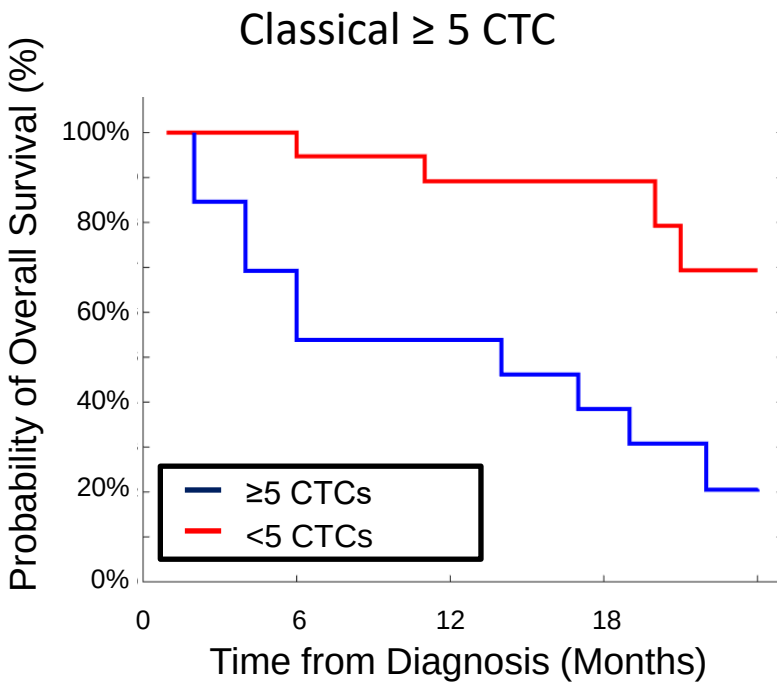



Adams, et al., Breast Cancer Research 2016

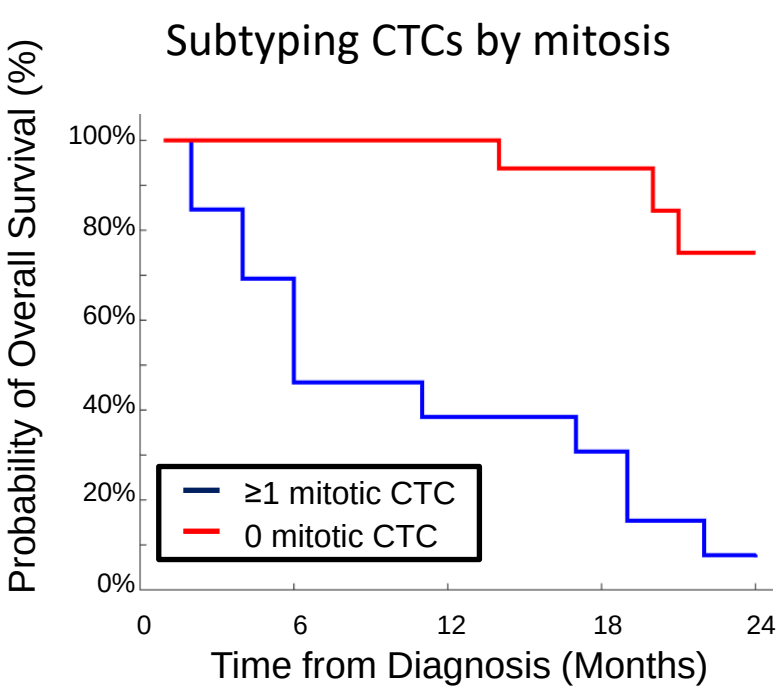
# CTCs subtyping by mitotic events

## Increased prognostic value than CTC count

Greater or less than 5 CTCs vs with and without a mitotic CTC event (n=36)




Hazard Ratio: 5.2




Hazard Ratio: 11.1

# Summary

- **CellSieve™ Liquid Cell Biopsy – isolates CTCs and stromal cells**
  - Applicable to many types of assays
  - Rapid, straightforward workflow
  - High sensitivity
  - High specificity
- **Very useful biomarker – CAML**
  - Found in all stages of cancer
  - Express cancer markers
  - Very easy to identify
  - More prevalent than CTCs
- **Same blood sample can be used for cfDNA**
- **Wide variety of assays and clinical utility**

# 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

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