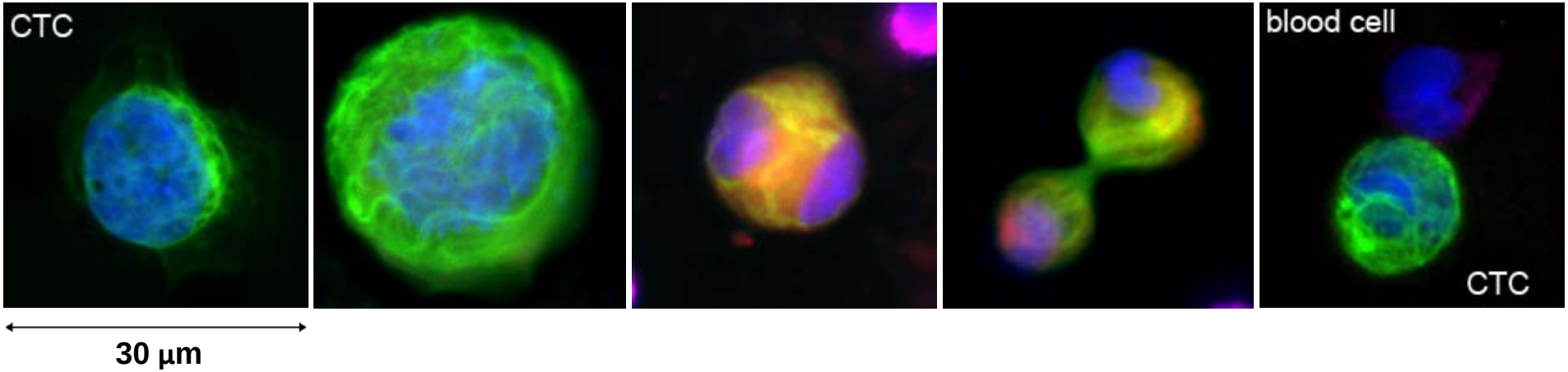
Filtration, Identification and Applications of CTCs and Circulating Cancer-Associated Cells



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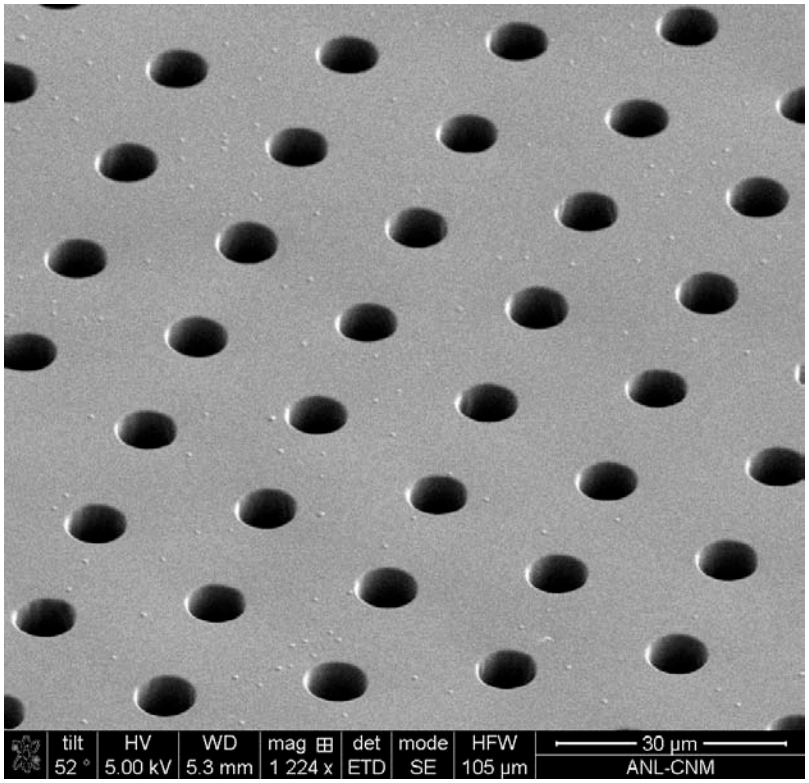
Outline

- CellSieve™ microfilter
- CTC
- Circulating giant cancer-associated cells

CellSieve™ Microfilter

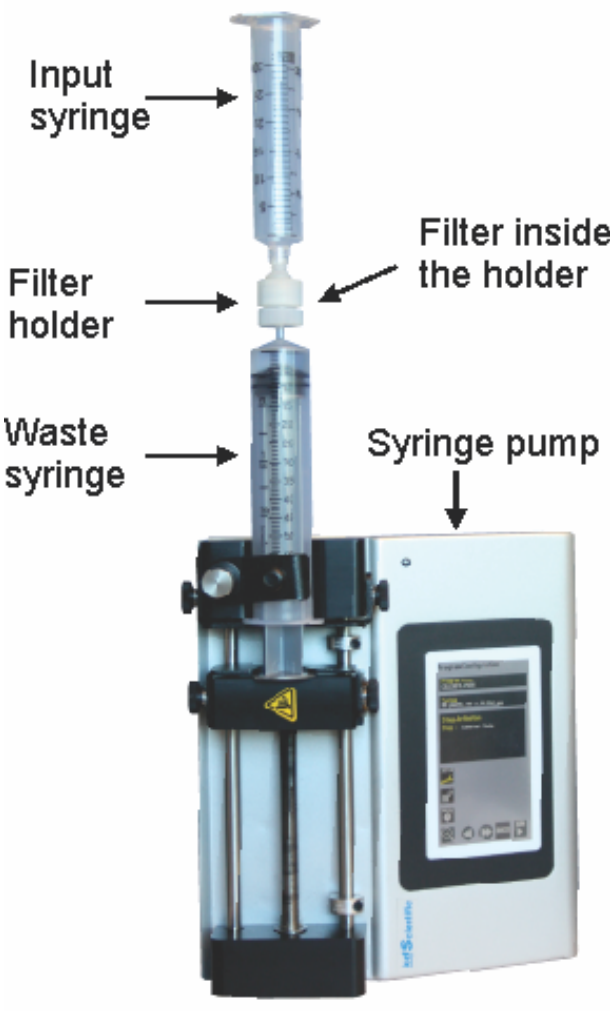
Independent of CTC surface markers

SEM Image



- Uniform 7µm pore size and distribution
- Non-fluorescent
- Lay flat on glass slide

Filtration & Assay System



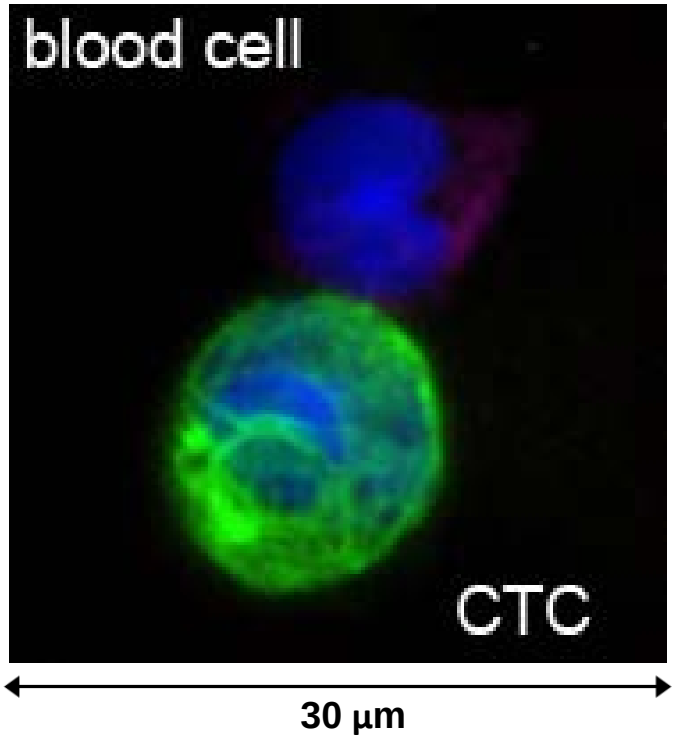
- Rapid
  - 3 min to filter 7.5 ml of blood
- Straightforward work flow
  - Entire enumeration assay can be performed in the filter holder
- Small and large sample size
- Consistent flow
- Gentle - no cell damage
- Low cost instrument

Platform Technology

- Assays of cells on the filter can be perform in the holder
- Cells can be back washed out
- **Single** cells can be picked off filter
- Directly lysis of cells on filter
- Sample types
  - Blood
  - Bone marrow
  - Urine
  - CSF

"True" CTCs

Criteria to improve specificity

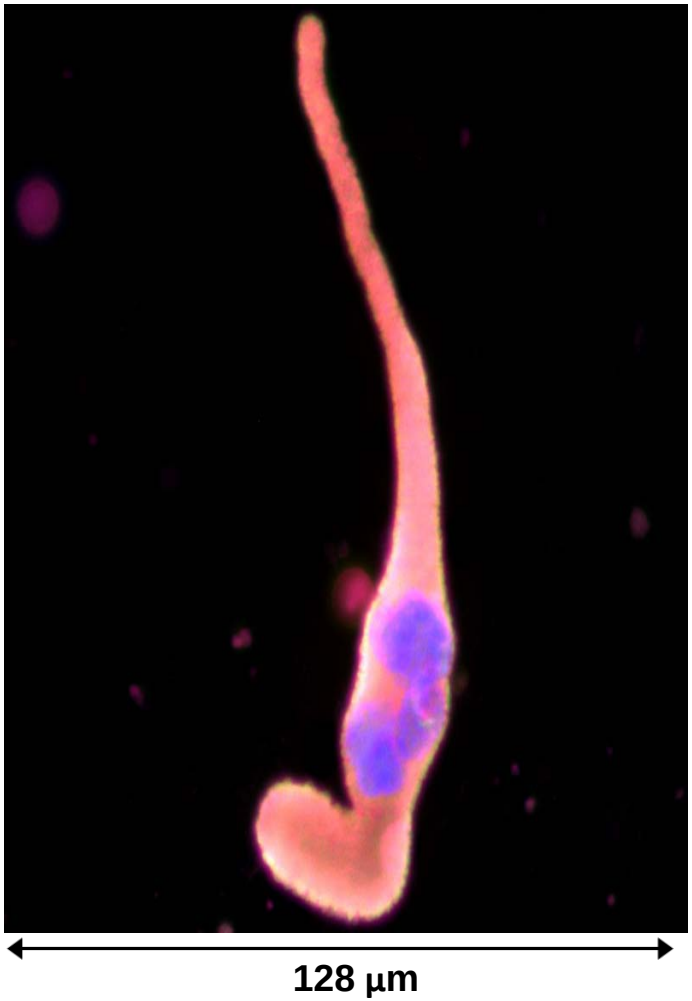


- DAPI positive
  - **and cancerous looking**
- To identify CTC
  - CK 8, 18, 19 (+)
  - **and filamentous pattern**
- To eliminate blood cells
  - CD45 (-)

Cancers of mesenchymal origin require other markers.

Circulating Cancer Associated Macrophage-like Cells (CAMLs)

Prostate

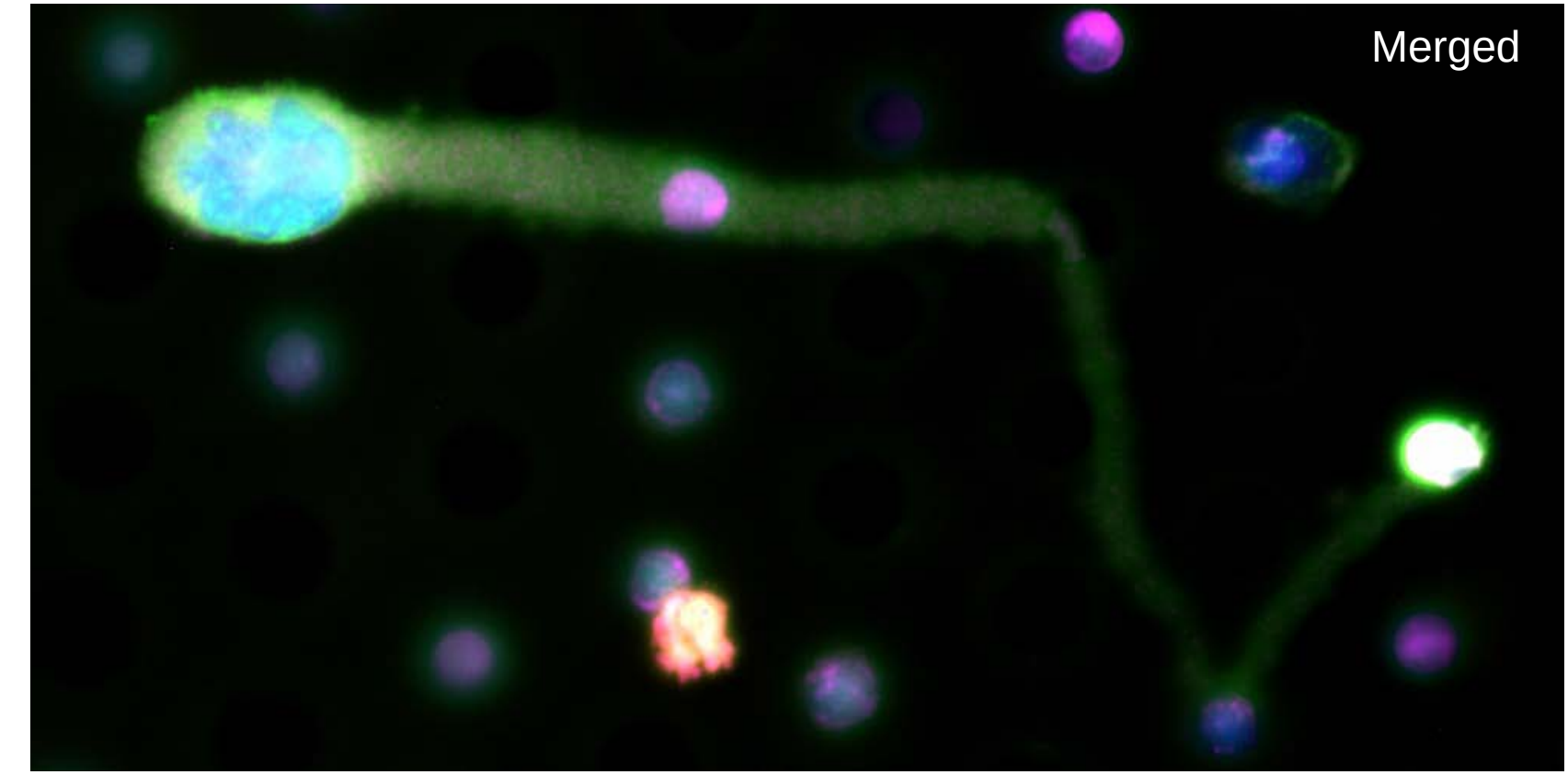


- Large atypical nucleus
- May express CK 8, 18 or 19 and EpCAM **and**

  CK and EpCAM are diffused
- Most of time CD45 positive
- Large: 20 - 300 µm
- Express CD11c, CD14
- Express endothelial markers CD146, TIE-2

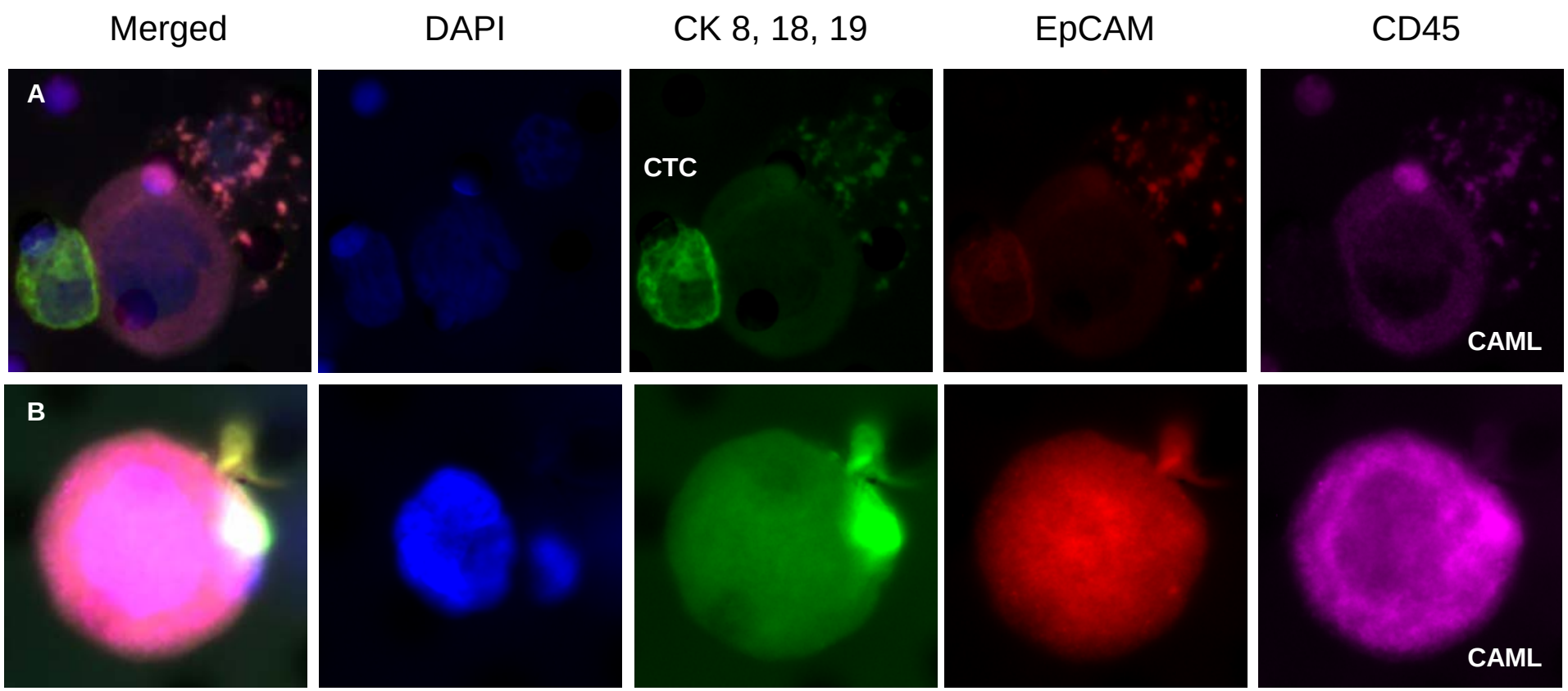
"In press" open access PNAS, under embargo

Breast Cancer Patient



160 µm

Interactions of CAMLs with CTCs

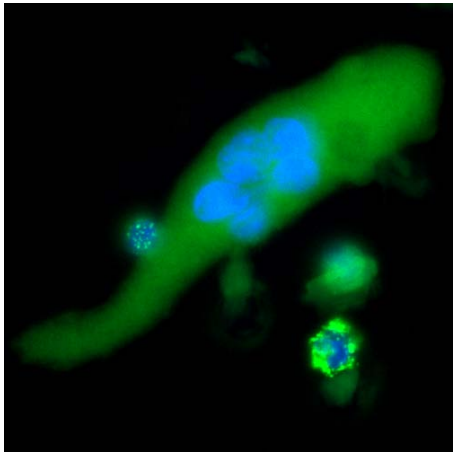


- CAMLs engulfing CTCs – explain presence of marks in CAMLs
- Found in ~10% of patient samples
- Short survival

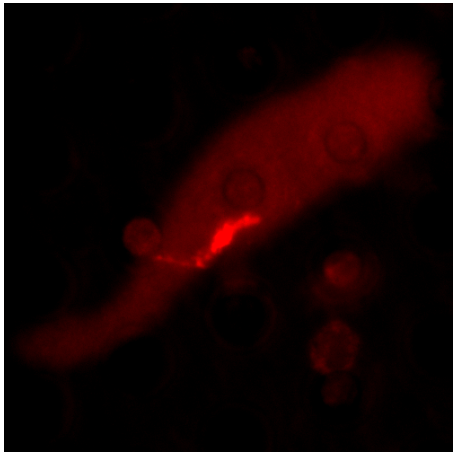
CAMLs with Ingested Markers

Prostate CAML

Merge



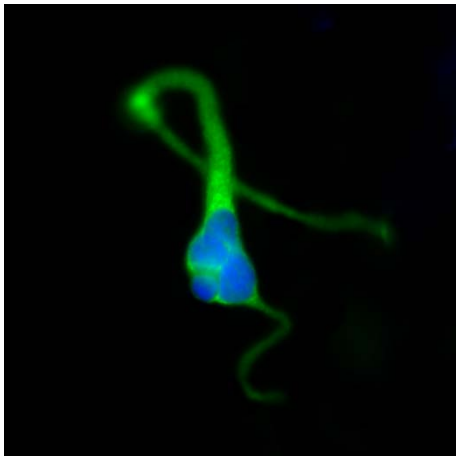
PSMA



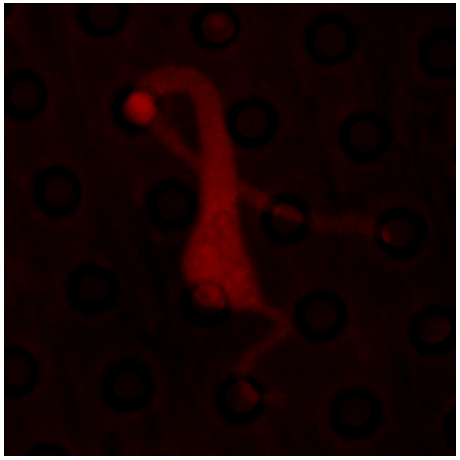
80 µm

Pancreatic CAML

Merge



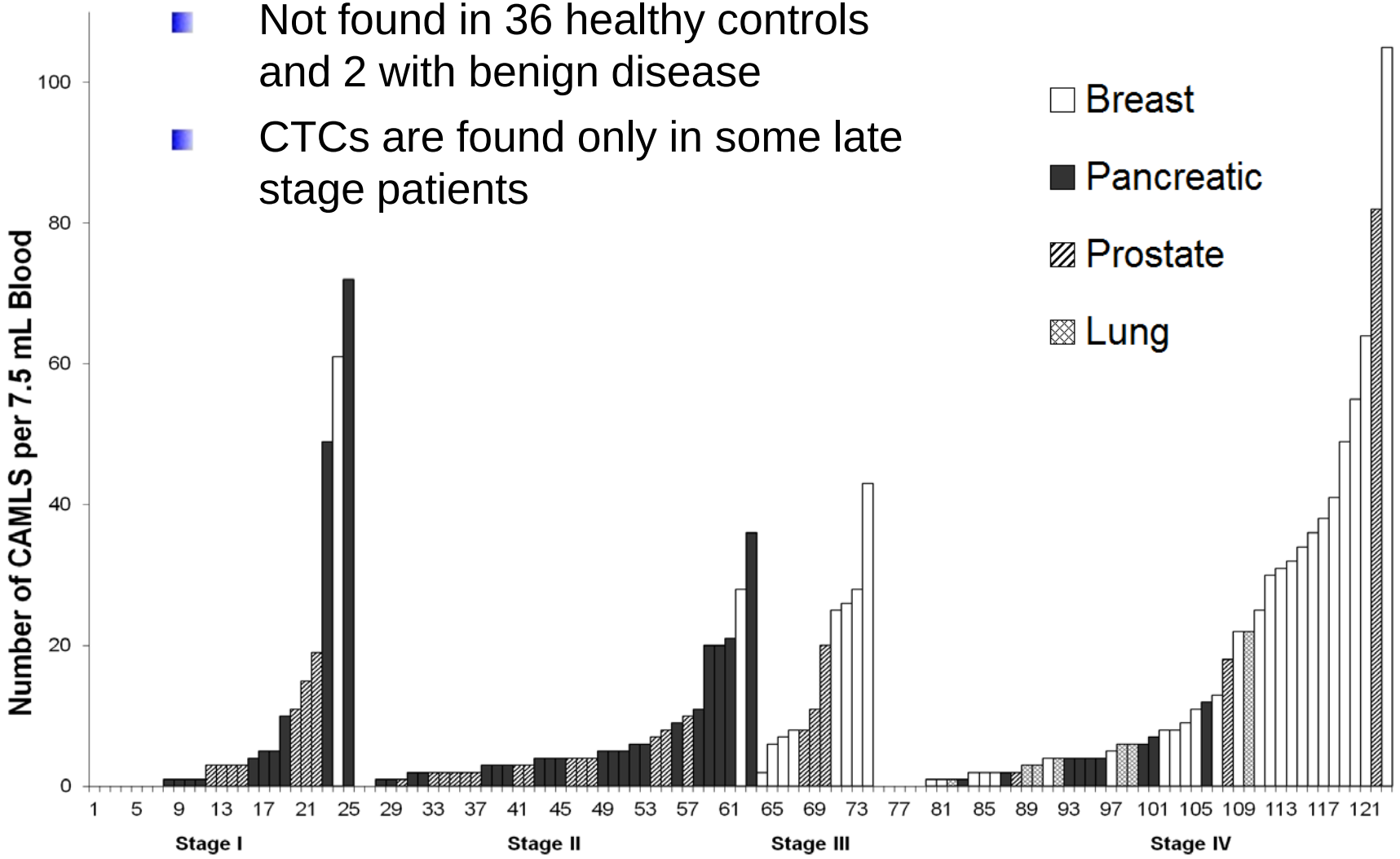
PDX-1



CAMLs More Prevalent than CTCs

Found even in stage I/II patients

- Not found in 36 healthy controls and 2 with benign disease
- CTCs are found only in some late stage patients

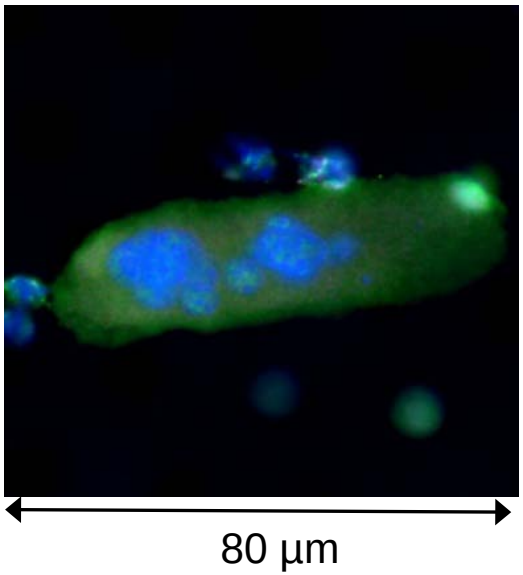


Clinical Utility of CAMLs

- Important to differentiate CTCs and CAMLs
  – They have different respond to therapy
- CAMLs more prevalent than CTCs
- May provide rapid indication of effectiveness of therapy together with CTCs
- Predict survival
- Potential biomarker for screening at risk patients
  - Lung cancer
  - Pancreatic cancer

Summary

- CellSieve™ microfilter
  - Applicable to all types of CTC assays
  - Rapid and straightforward work flow
  - High sensitivity
- Giant CAMLs
  - More prevalent than CTCs
  - Many potential clinical applications



Thank you

ALL THE PATIENTS

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Thank you

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