



# **Microfluidics for the Interrogation of Circulating Biomarkers in Cancer Patients**

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Arab-American Frontiers of Science, Engineering and Medicine  
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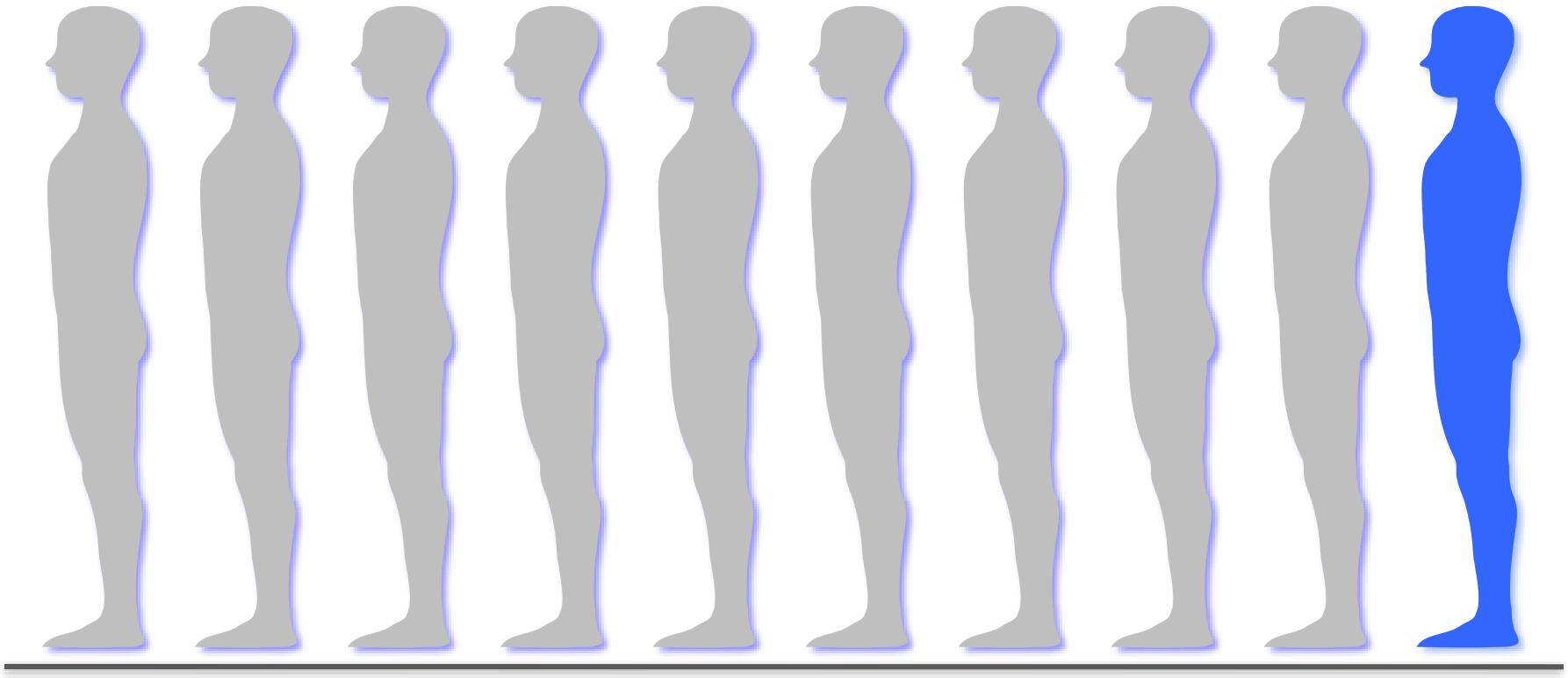
# Putting Cancer into Perspective

Number of deaths from cancer each **day** in the US:

**1,600 deaths / day (2016)**

Number of deaths from cancer each **day** in the world:

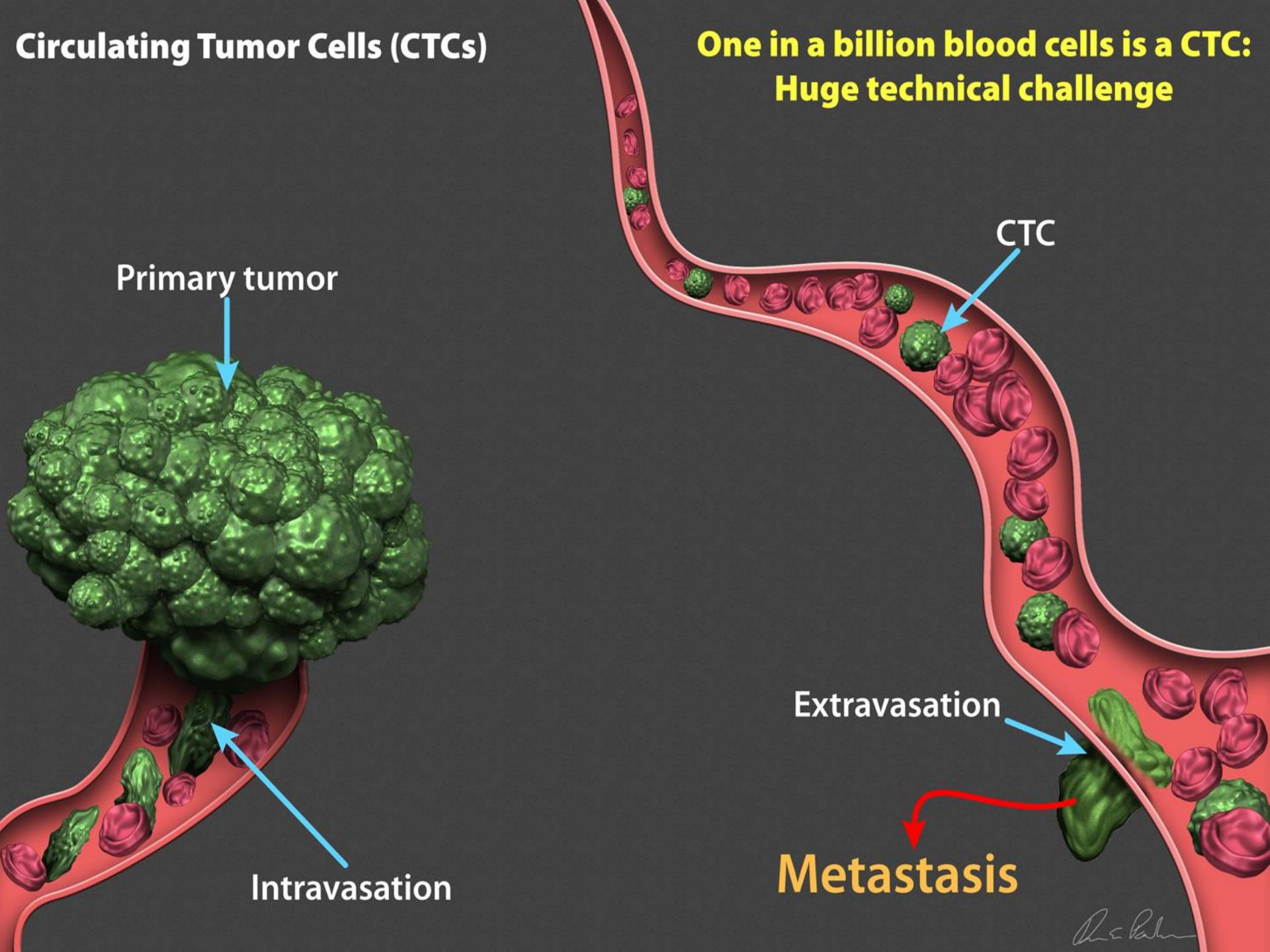
**24,000 deaths / day (2016)**



9 out of 10 deaths in cancer is due to  
**metastasis!**

## Circulating Tumor Cells (CTCs)

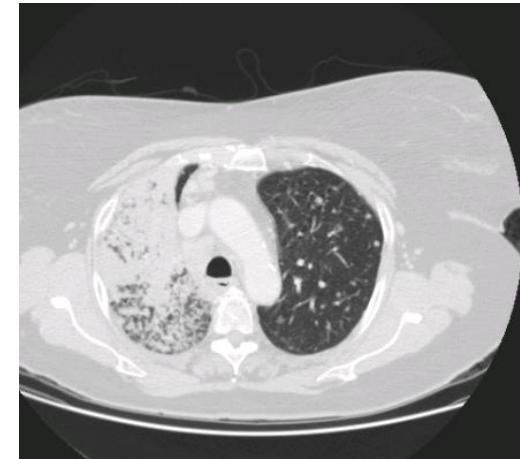
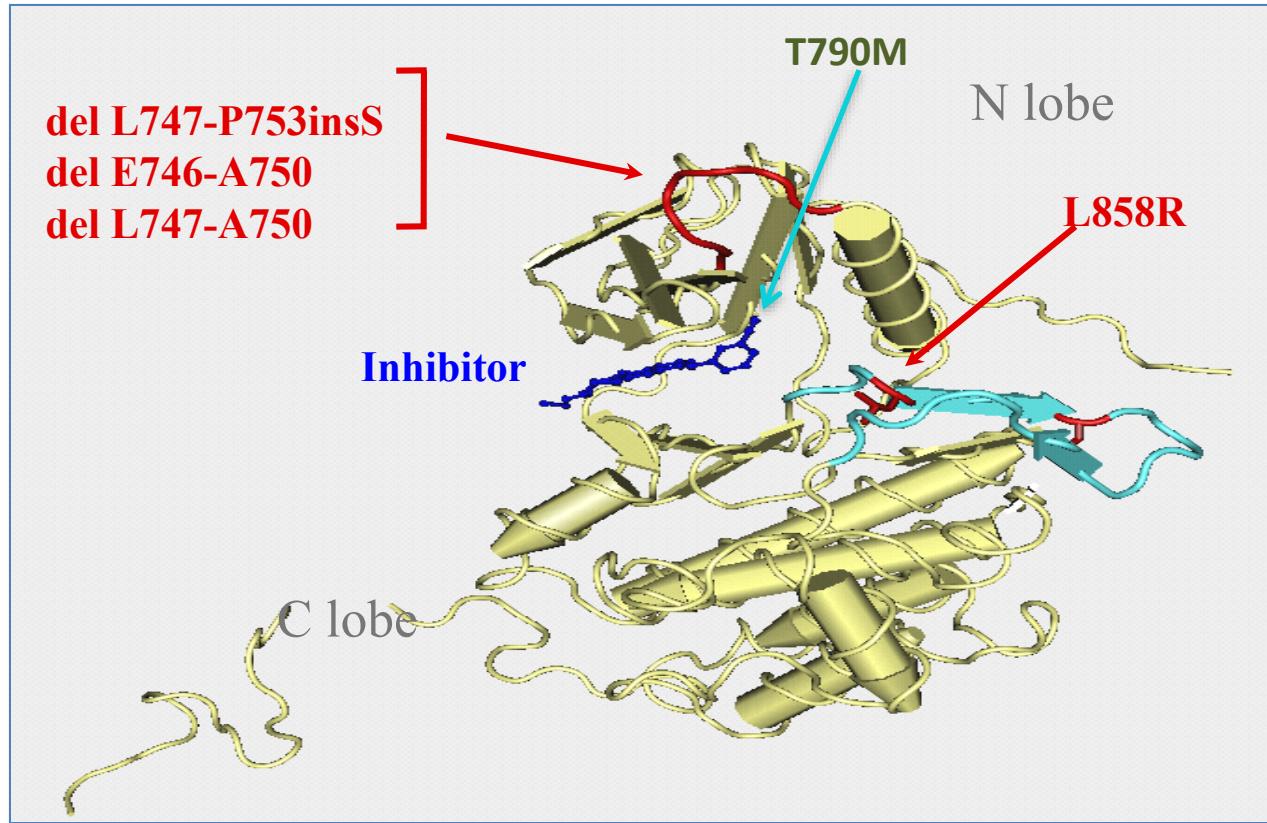
One in a billion blood cells is a CTC:  
Huge technical challenge



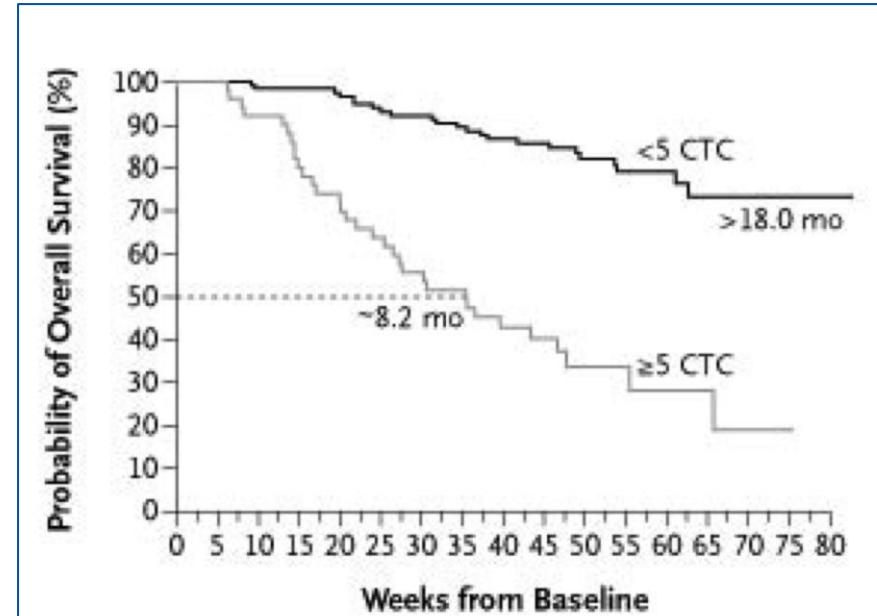
Paul Pahn

# “Liquid Biopsy” in Targeted Therapy

- Detection of sensitizing EGFR mutations
- Acquisition of drug resistance



2007: CTCs are found in ~20% of metastatic cancers using modified FACS and MACS, and filters



Cristofanilli et al., **NEJM** 2004

CTCs useful mostly for prognosis

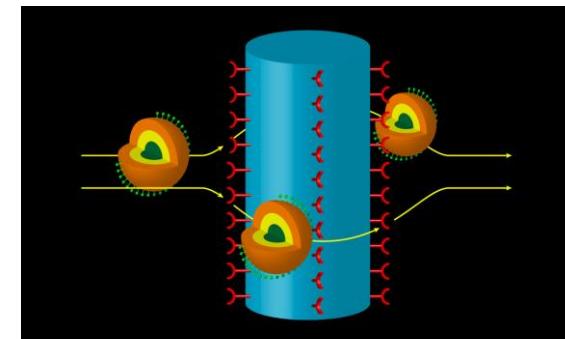
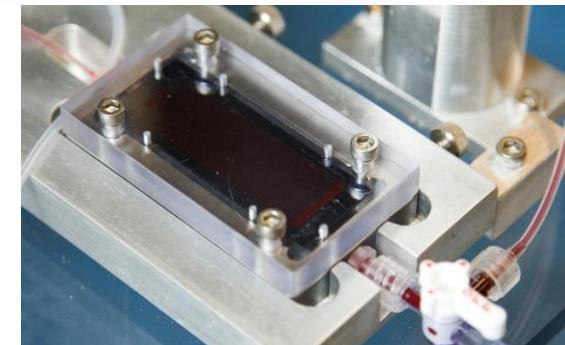
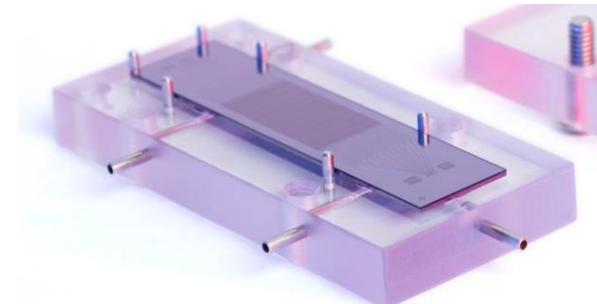
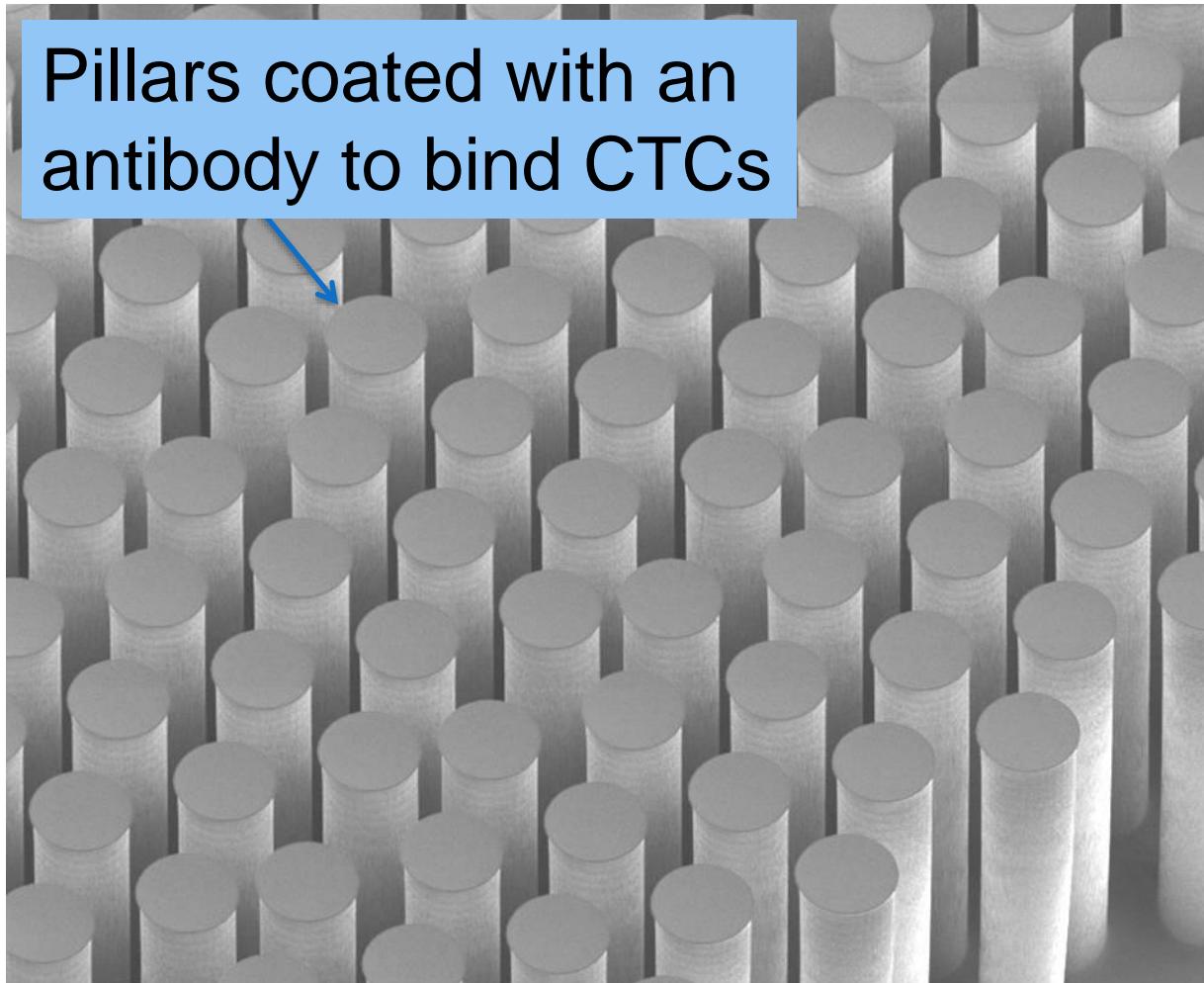
Why are the CTCs observed only in a small percentage of metastatic patients?

**Hypothesis:**

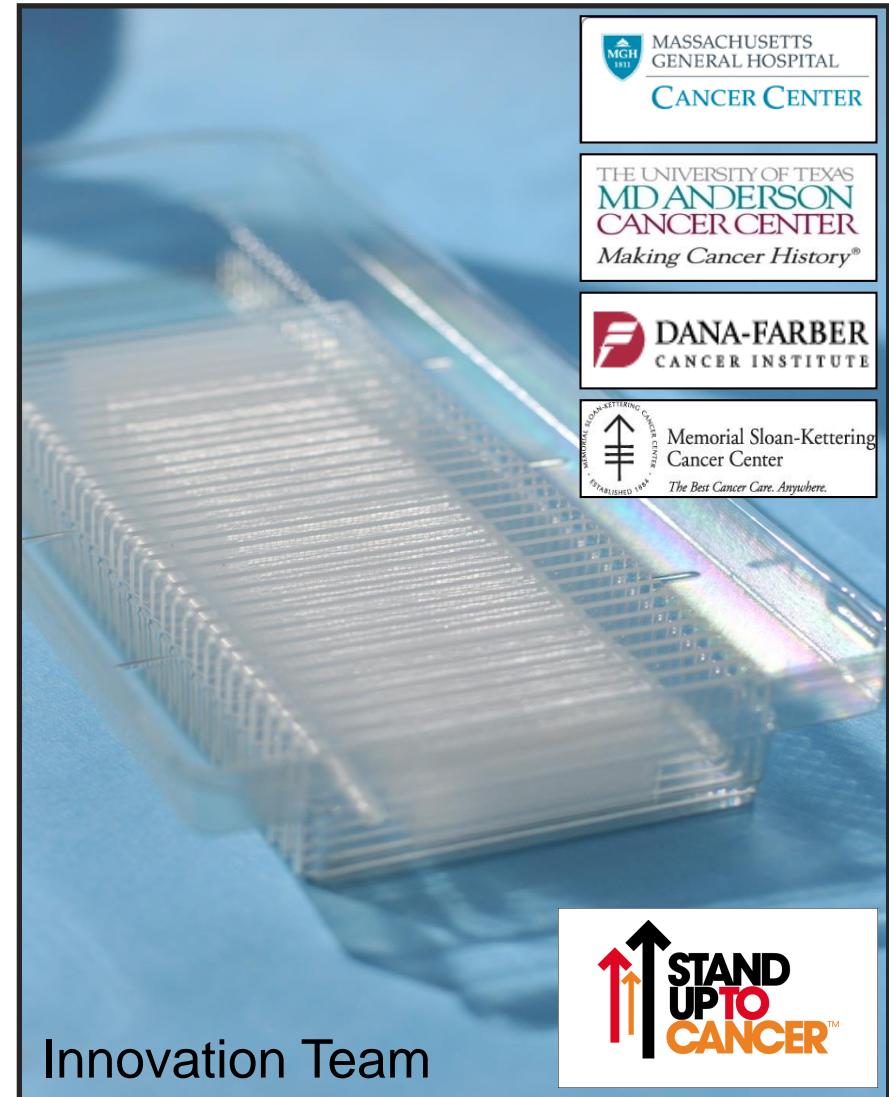
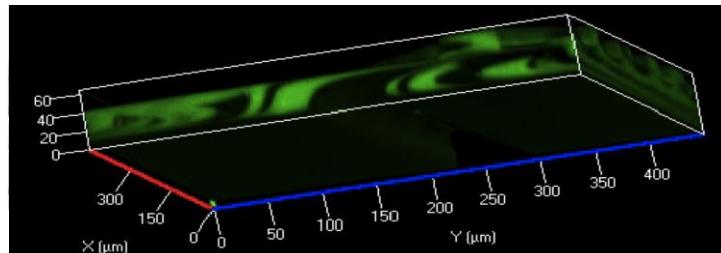
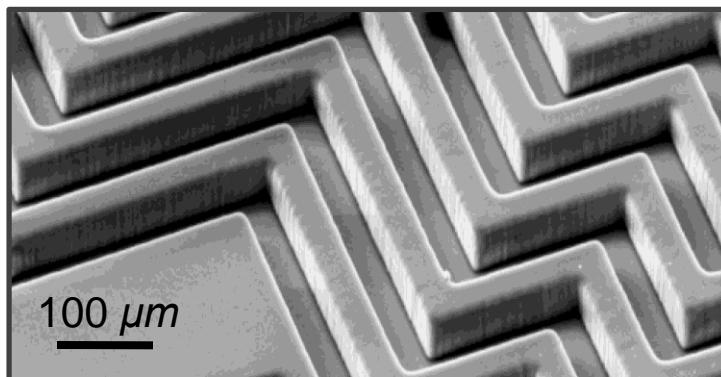
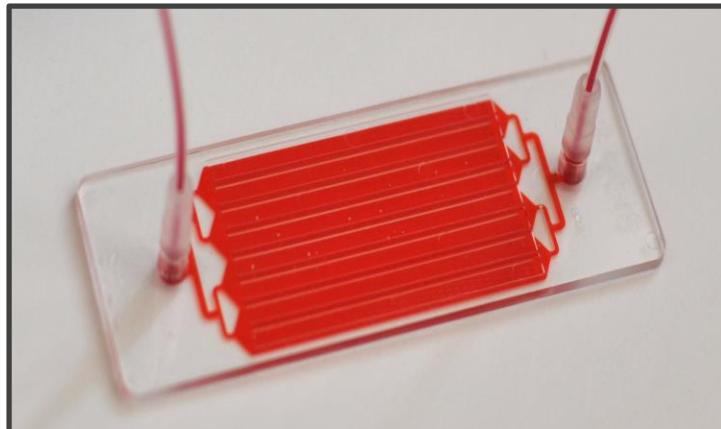
Biology vs. Technology

# 1<sup>st</sup> Generation Micropost CTC-Chip Validation Studies

Pillars coated with an antibody to bind CTCs



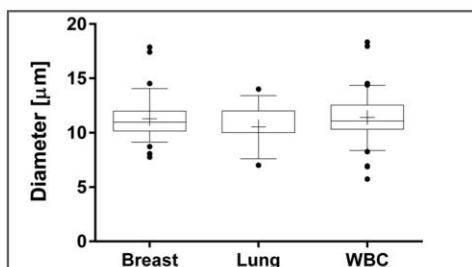
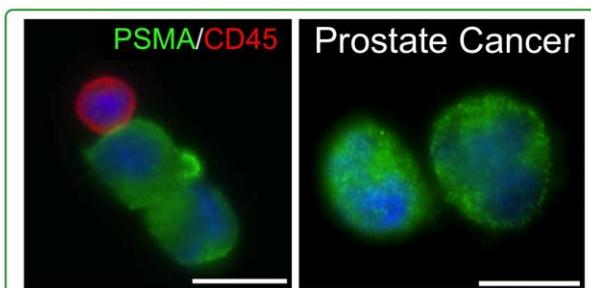
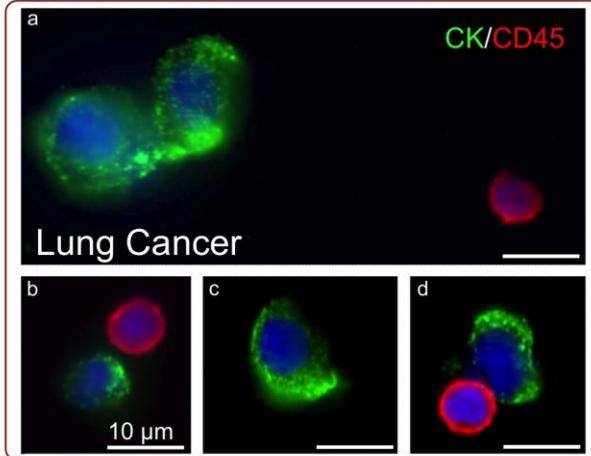
# 2<sup>nd</sup> Generation Herringbone CTC-Chip Scale-up and Dissemination



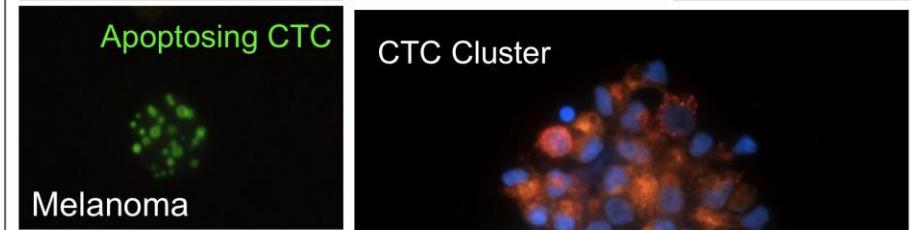
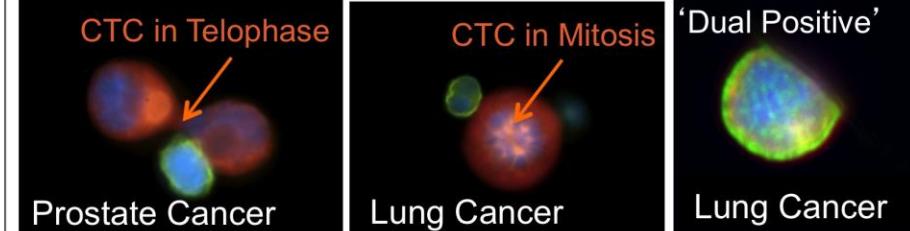
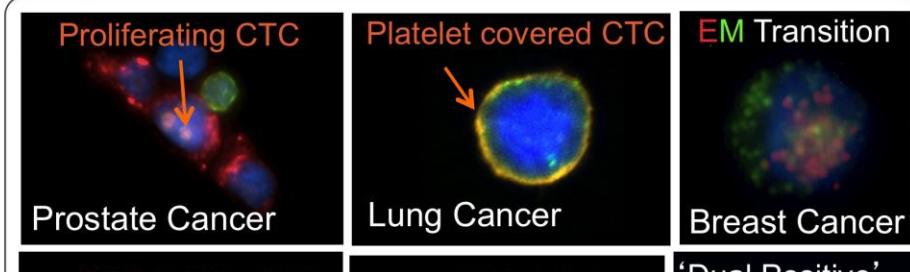
Innovation Team

# Patient CTCs come in different size, shape & phenotype

Size & Shape of CTCs



Phenotype of CTCs



20  $\mu$ m

# All cancers and all CTCs

***Avoid bias*** in CTC isolation and ***improve yield***, especially in non-epithelial cancers, or cancers undergoing EMT.



**Negative depletion:**

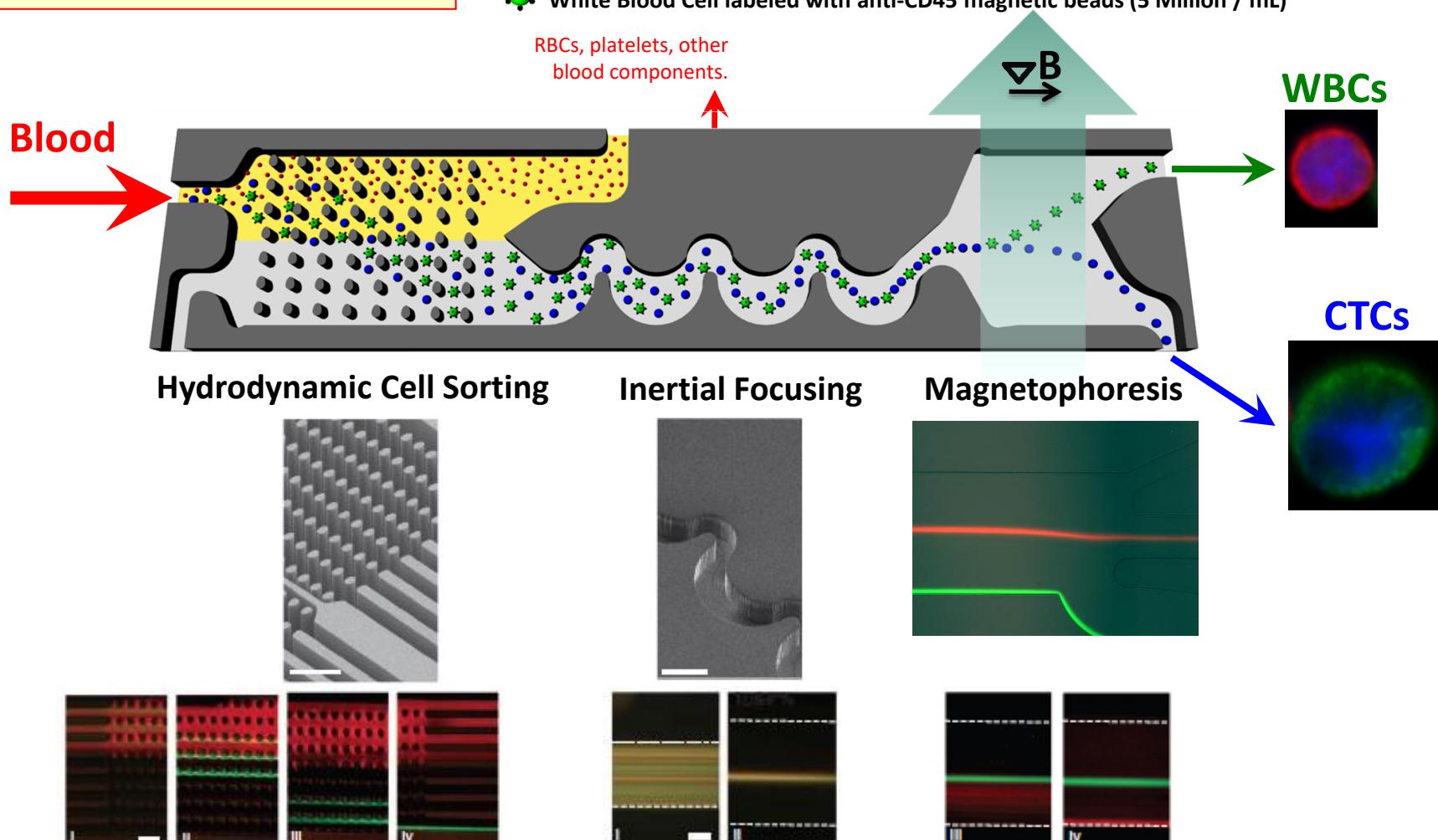
Removing the hay to uncover the needle...

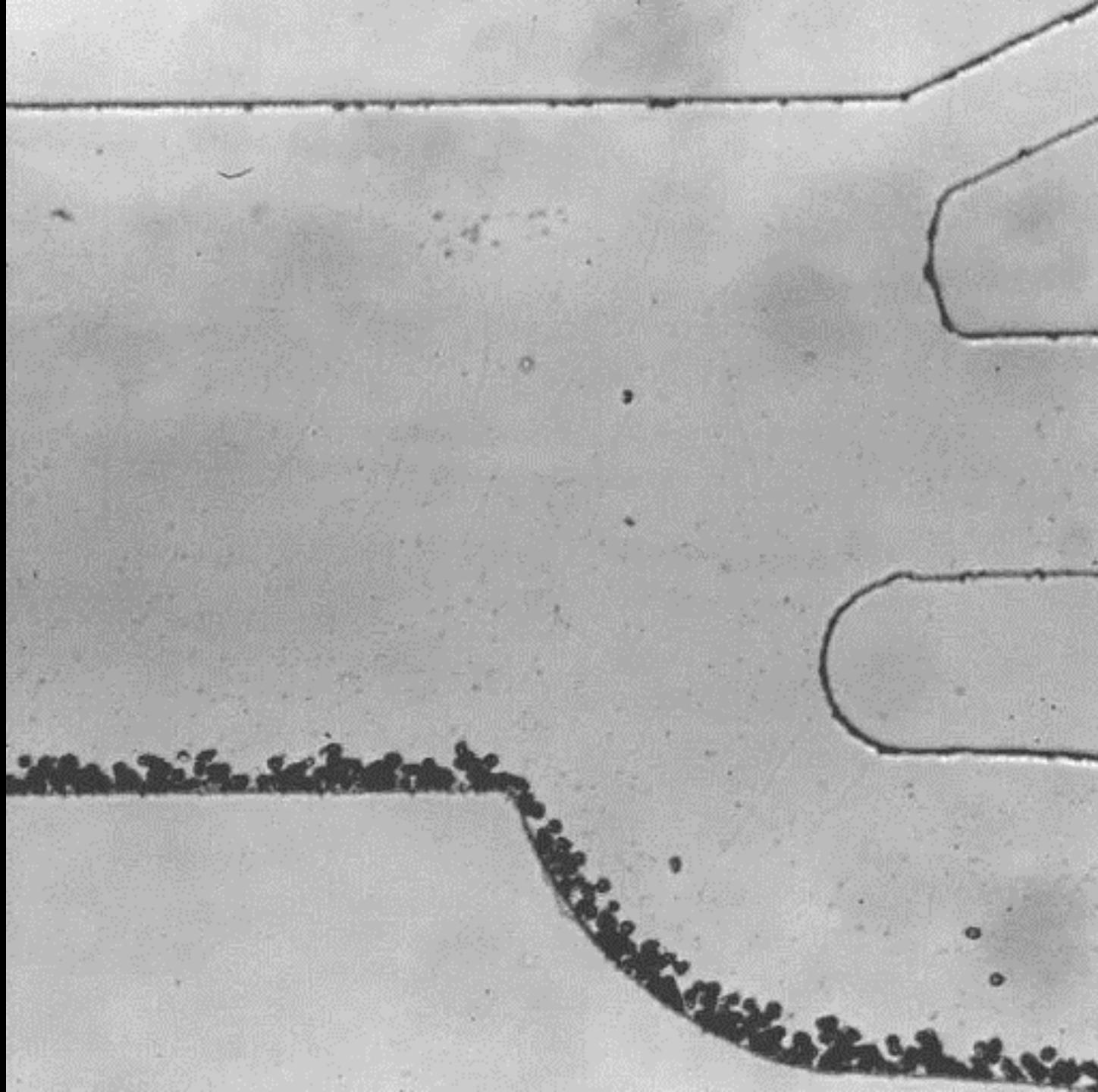


# CTC-iChip: *Negative Selection*

## Magnetic bead-tagged WBCs

- Red Blood Cell (8 Billion / mL)
- CTCs (1-100 / mL)
- White Blood Cell labeled with anti-CD45 magnetic beads (5 Million / mL)



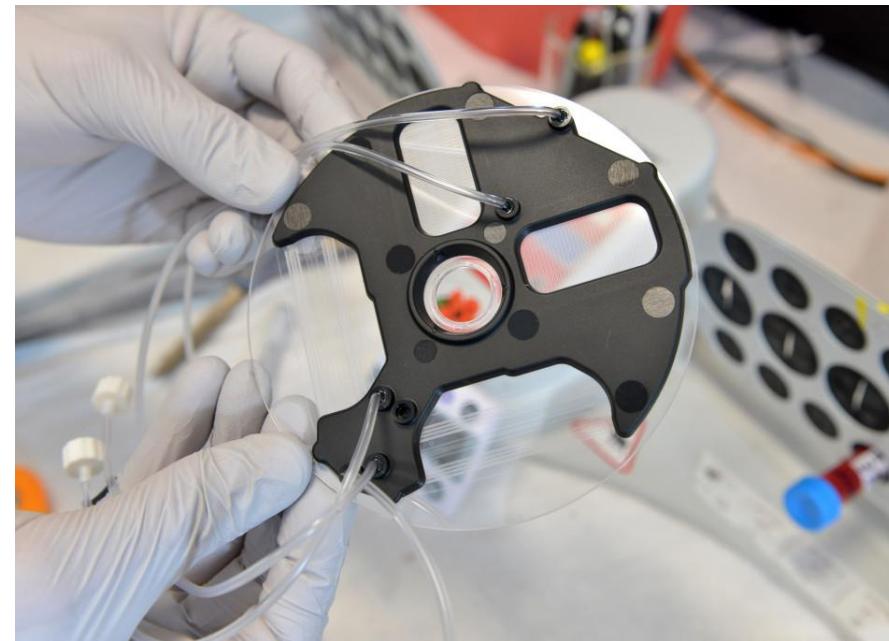


# “World-to-Chip” Coupling

Processor



CTC-iChip

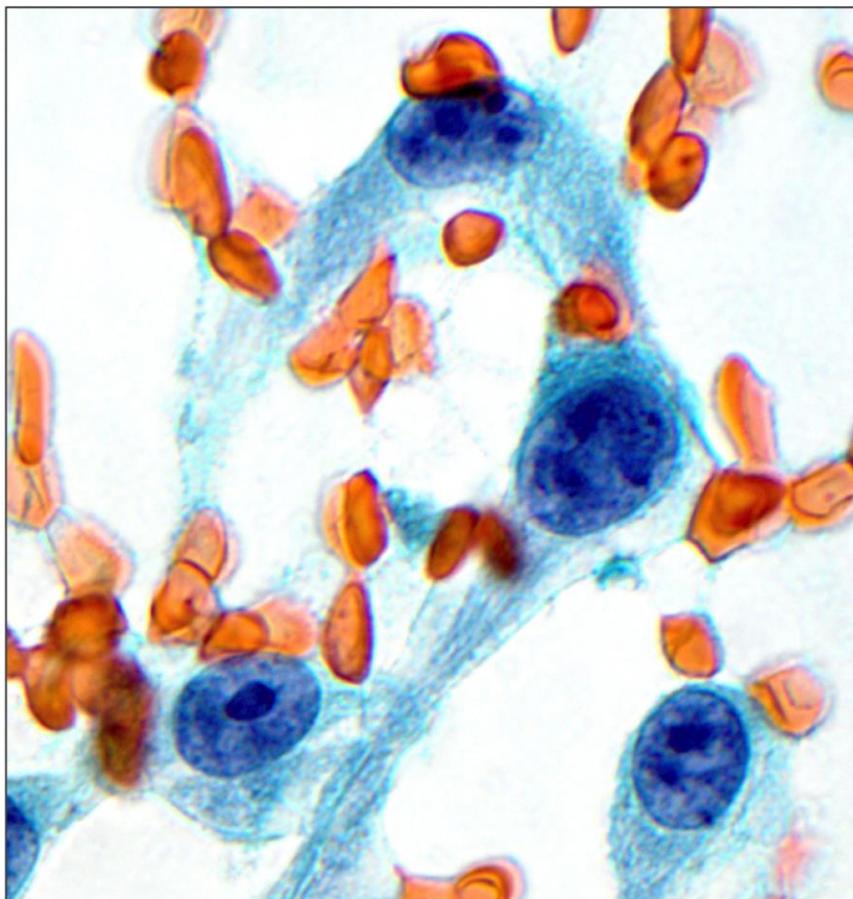


**Automated sample processing**  
Flowrate: 10-20 mL whole blood/hour  
20-30 million cells/second

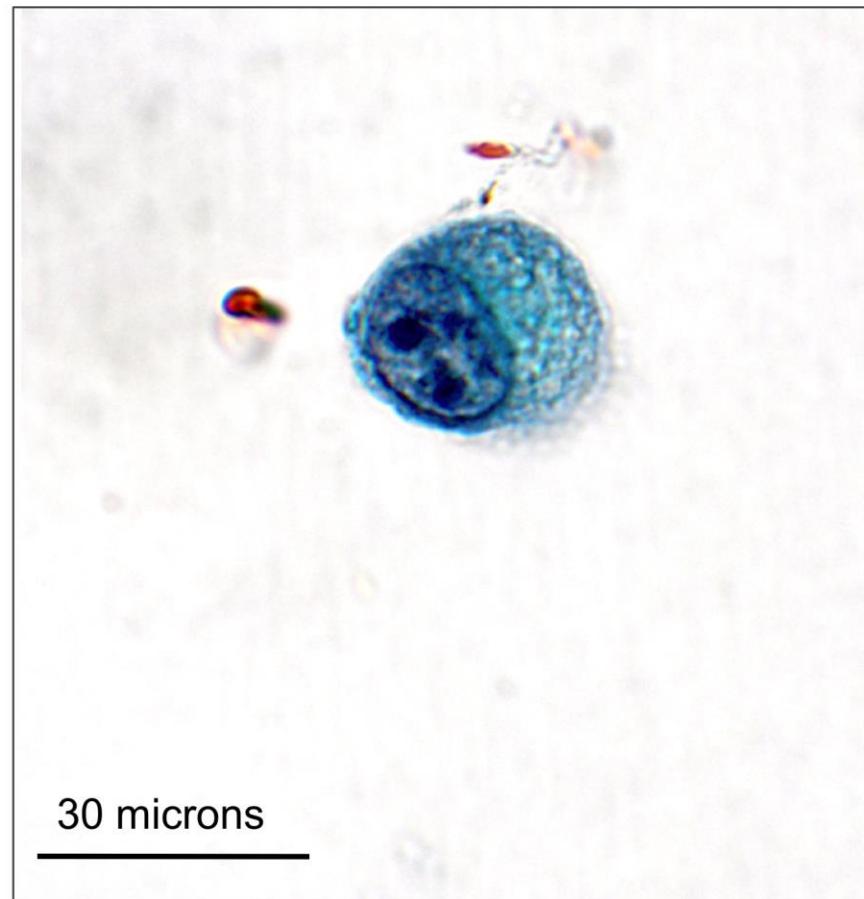


# CTC-iChip enables light microscopy

FNA

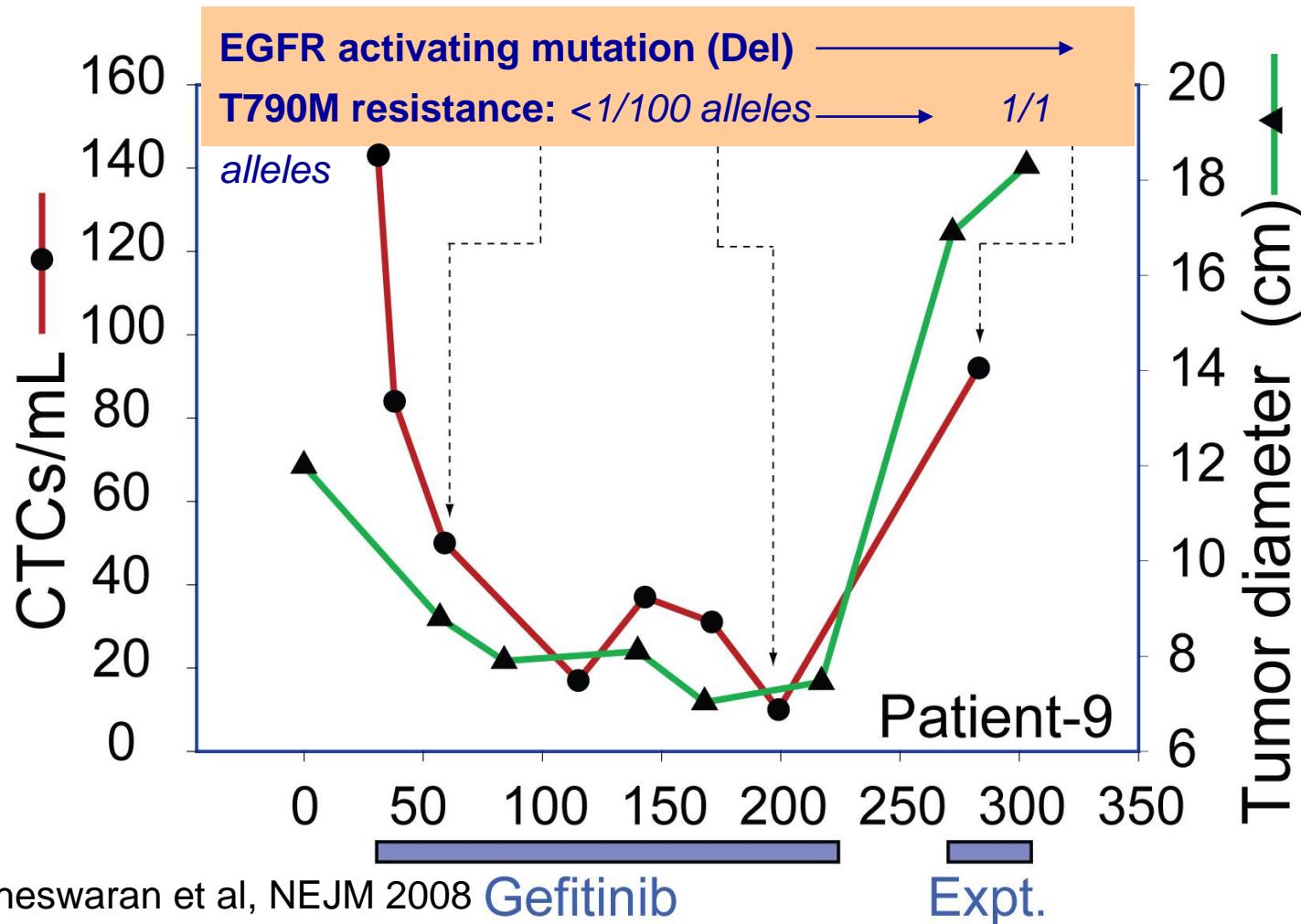


CTC



Papanicolaou staining of melanoma CTCs

# Lung Cancer: Serial Monitoring of EGFR Genotypes

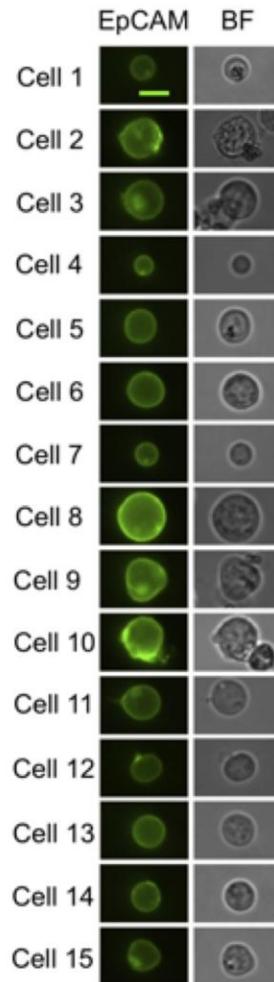
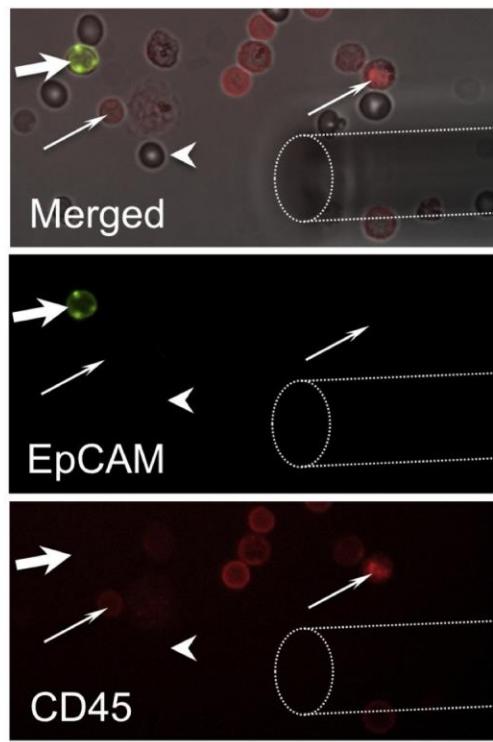


Maheswaran et al, NEJM 2008 **Gefitinib**

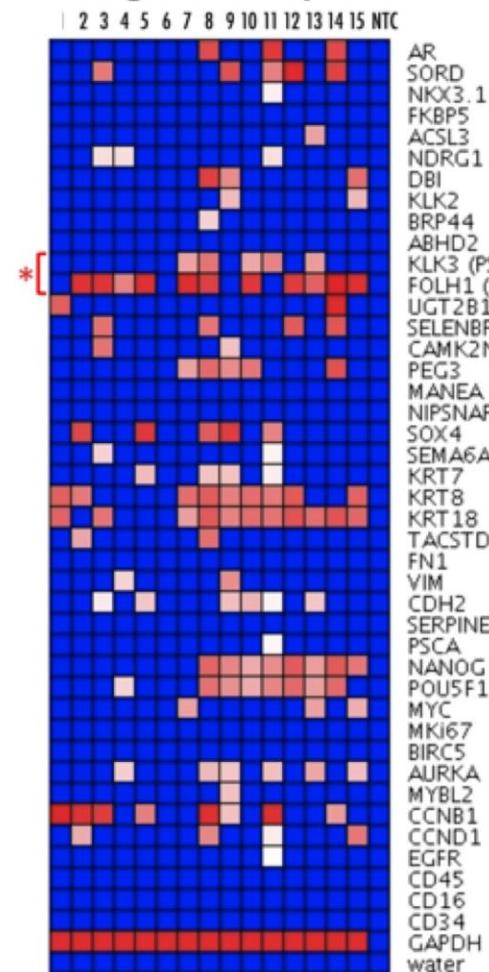
**Expt.**

# Measuring androgen signaling pathways in prostate cancer CTCs

## qRT-PCR (Fluidigm)



## Single Cell Expression



AR-on

PSA (AR-on)

PSMA (AR-off)

AR-off

Epithelial

Mesenchymal

Stem cell

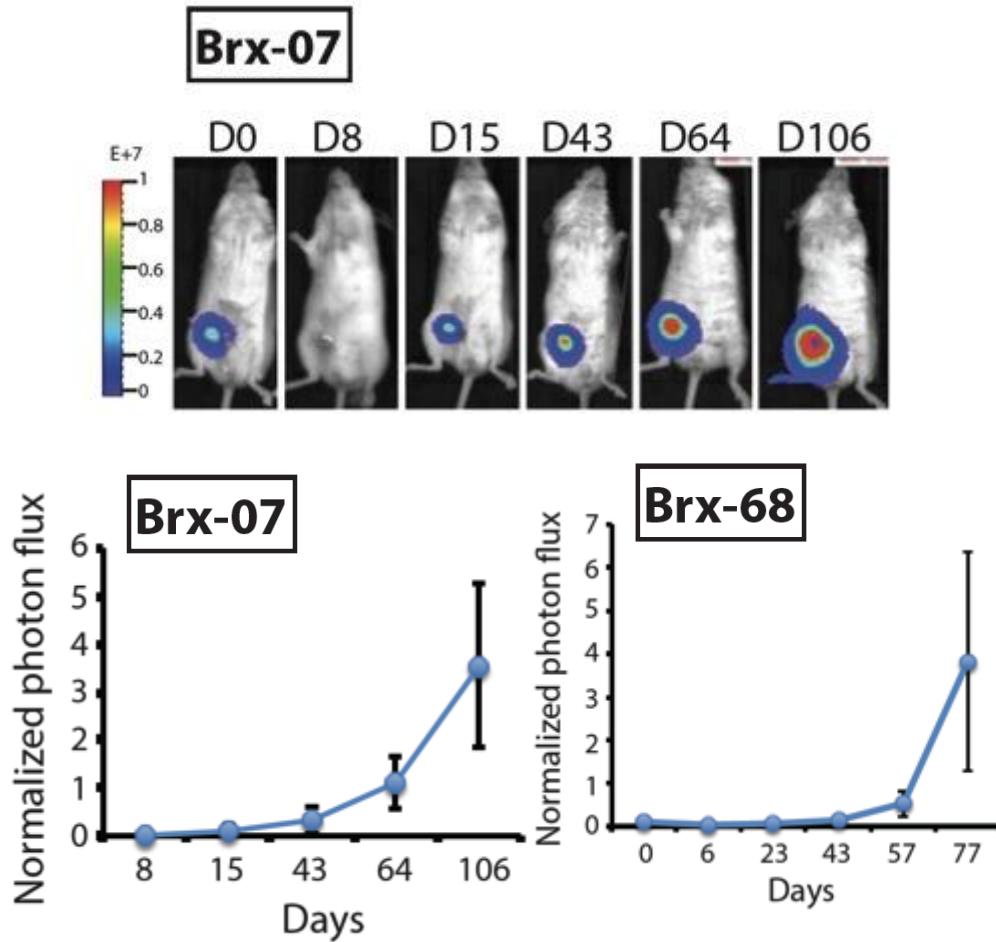
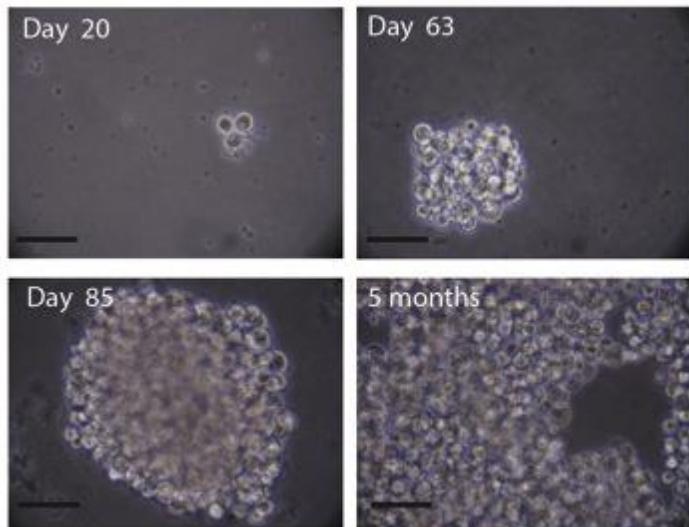
Proliferation

WBC

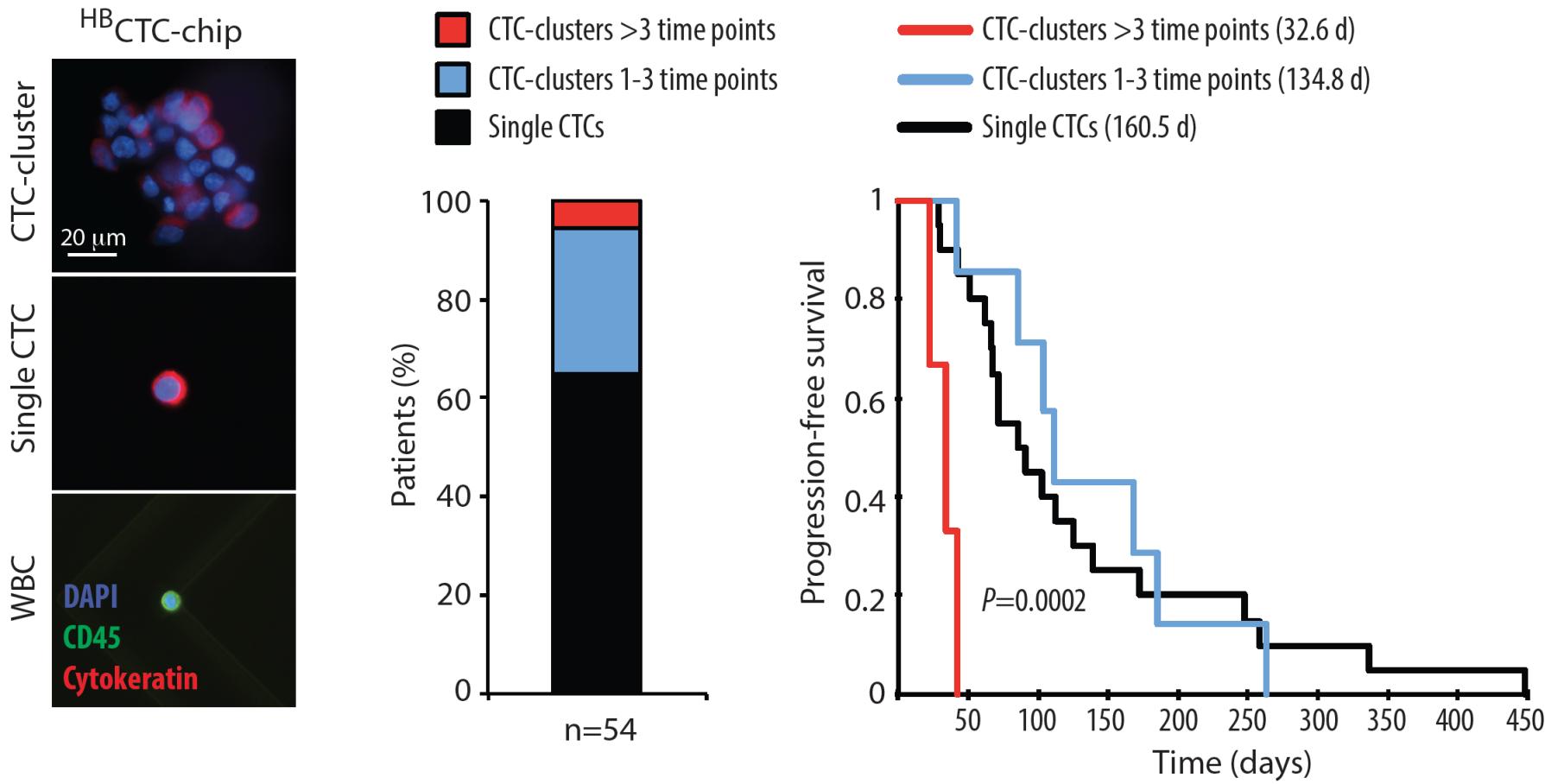
# i-Chip: Culture of patient CTCs

## Tumorigenesis in mouse models

*In vitro* expansion of CTCs  
from patients with  
hormone-positive breast  
cancer

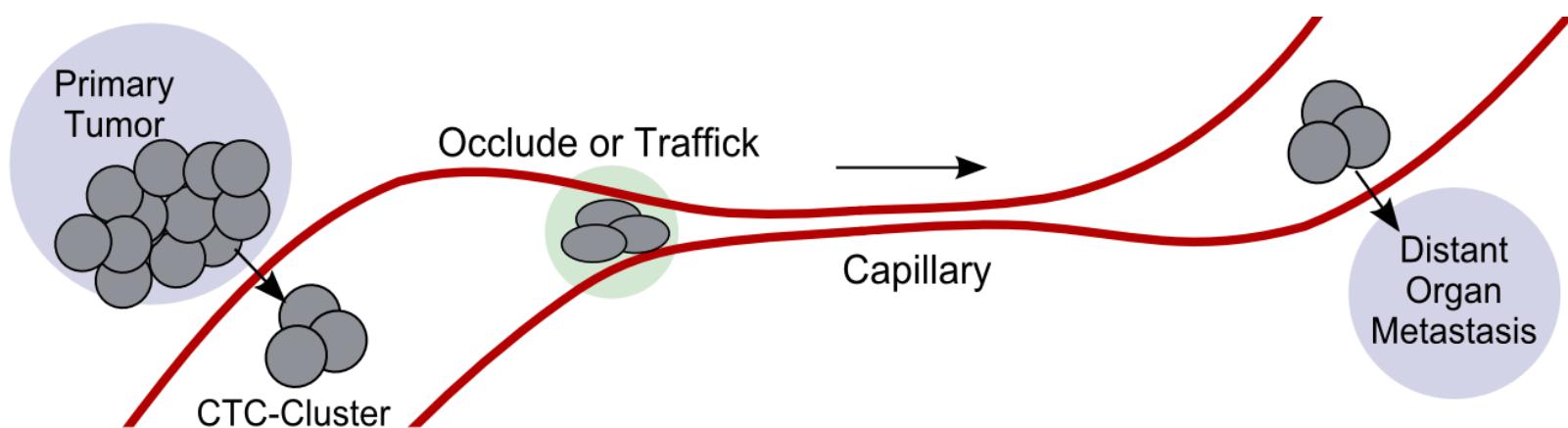


# Clinical response and CTC clusters

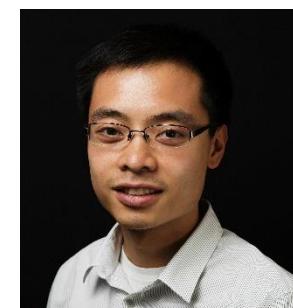


# CTC-Cluster Transit

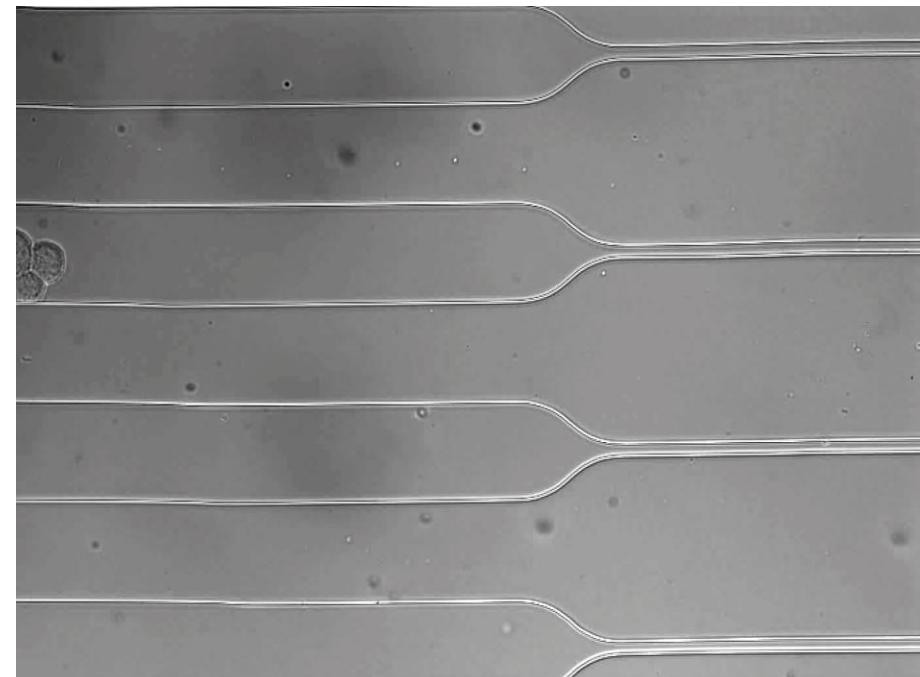
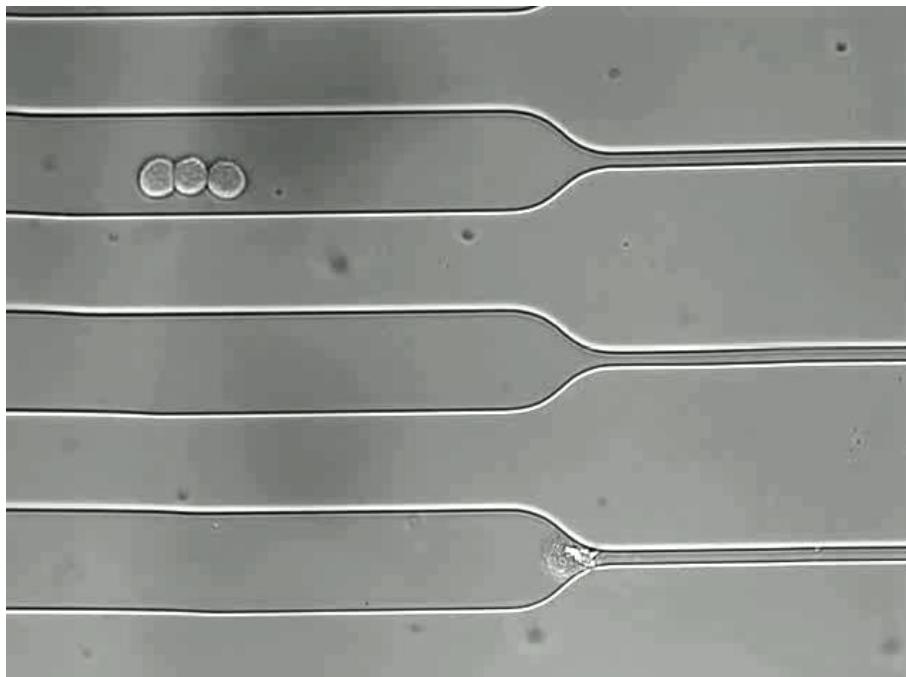
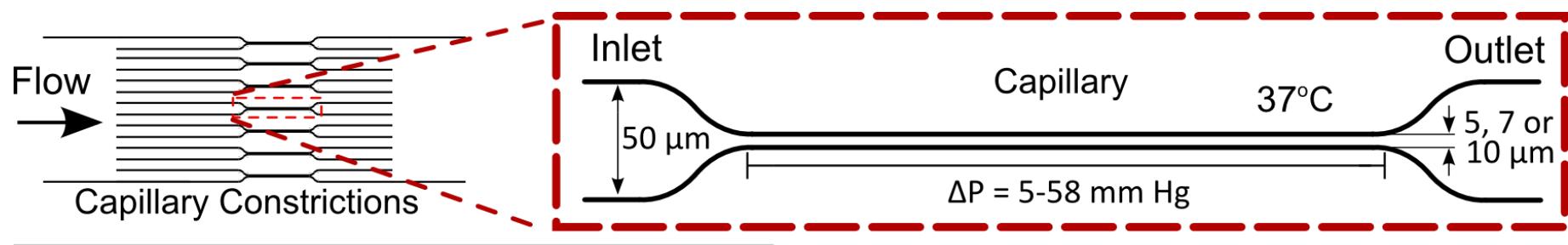
- CTC-Clusters (2-100+ cells) believed to occlude 5-10  $\mu\text{m}$  capillaries  $\rightarrow$  cannot contribute to dissemination of tumors to distant organs



Sam Au et al. (PNAS, 2016)

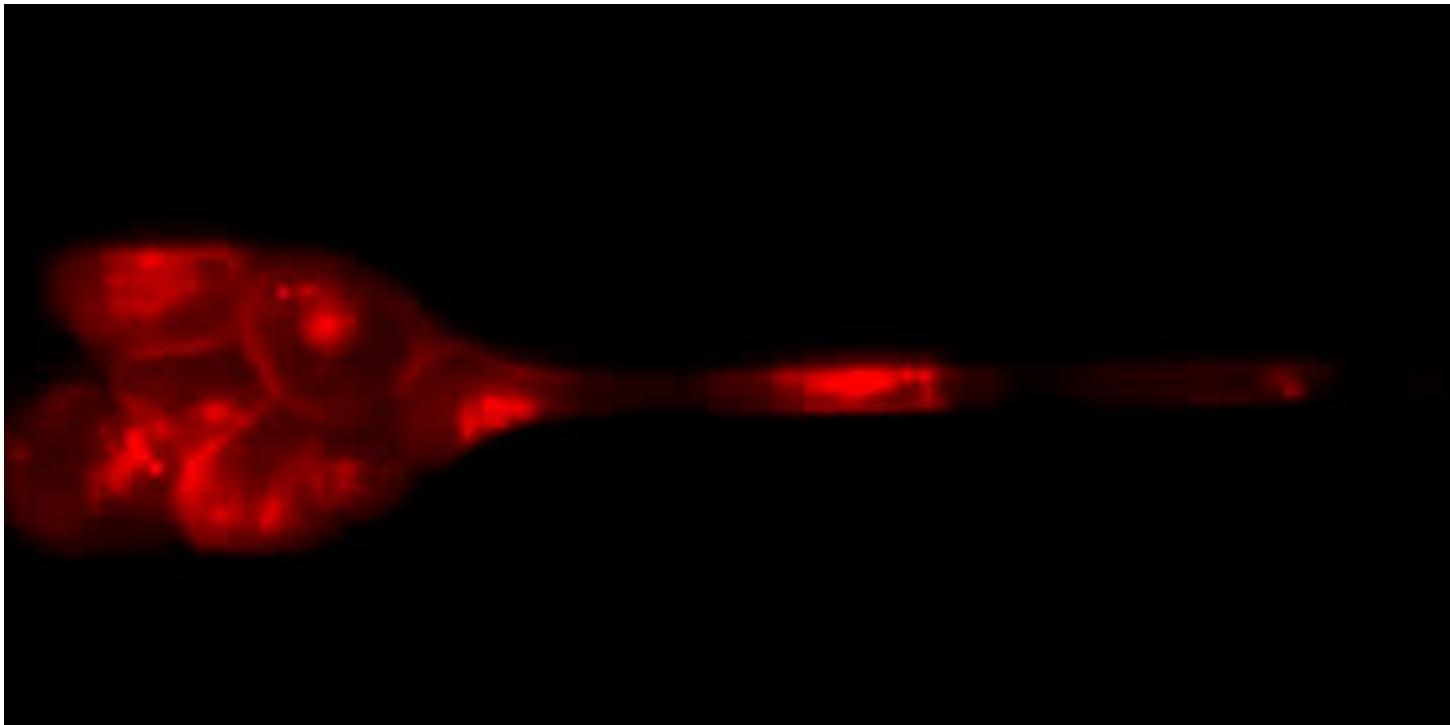


# Microfluidic Capillary Constrictions

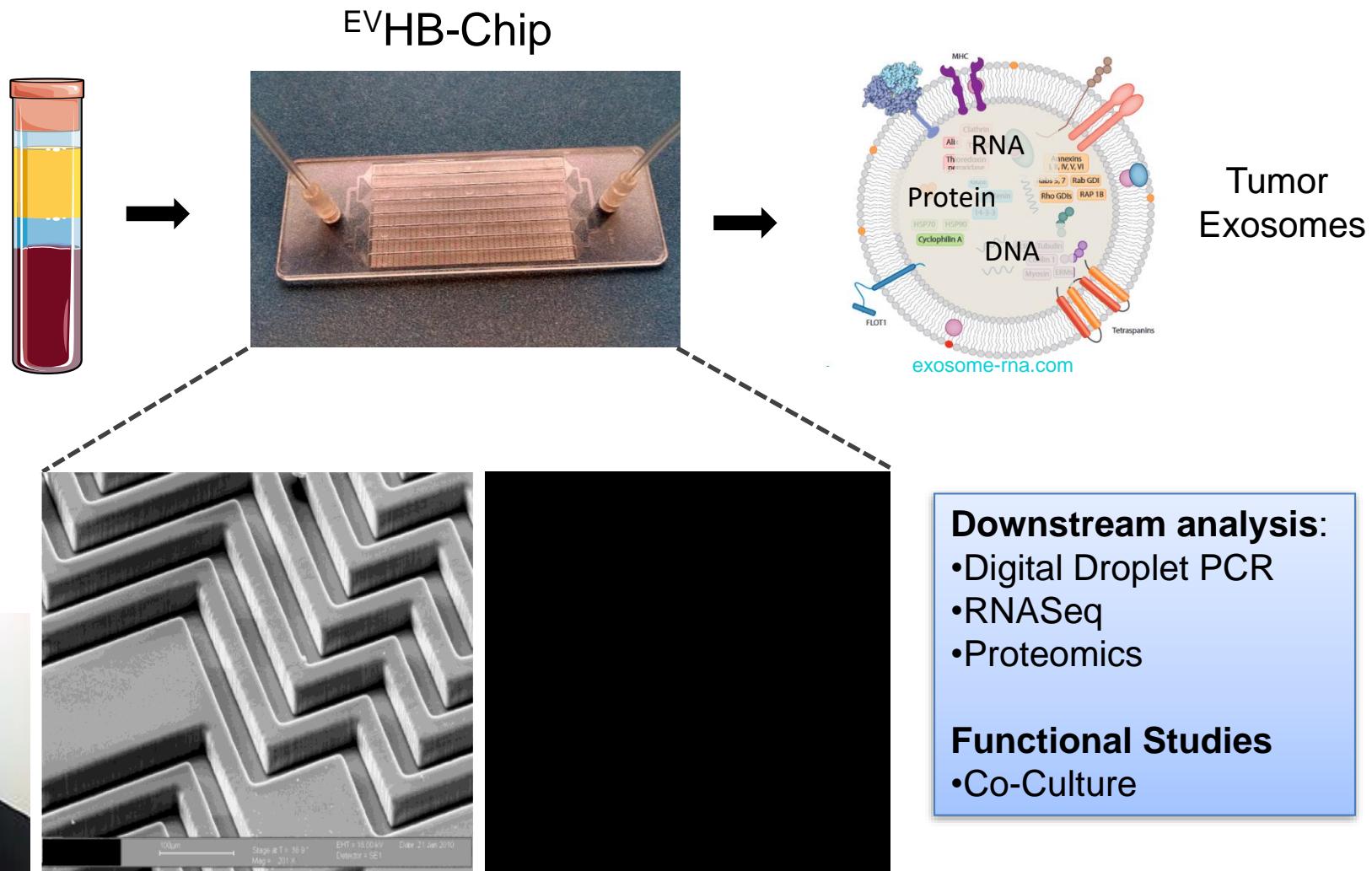


# Reorganization of Clusters at Entrance

- Cell membrane stain
- Complex organization dynamics



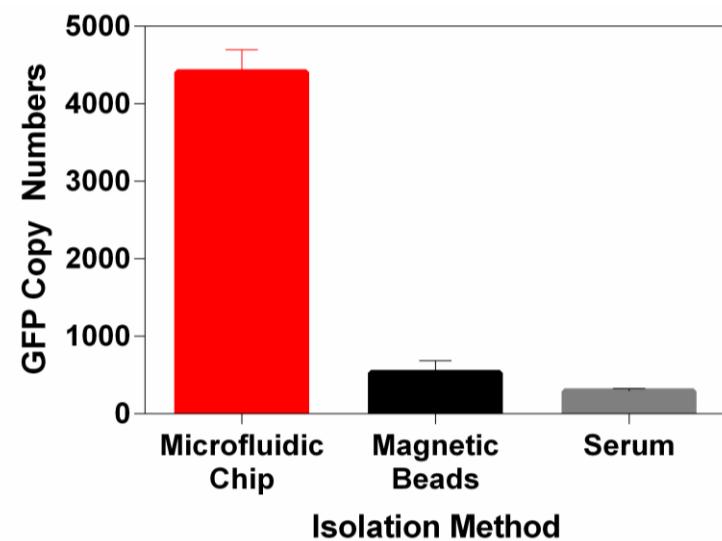
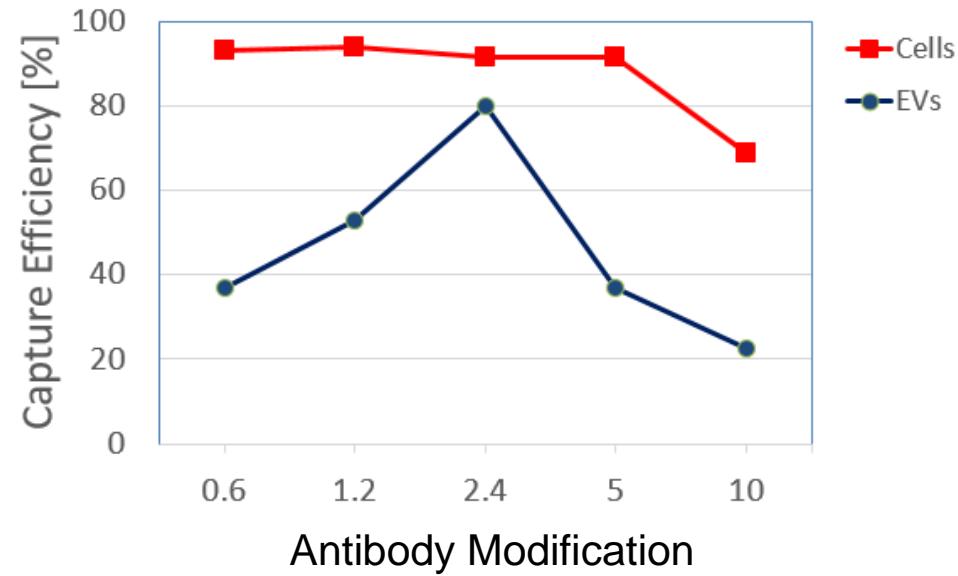
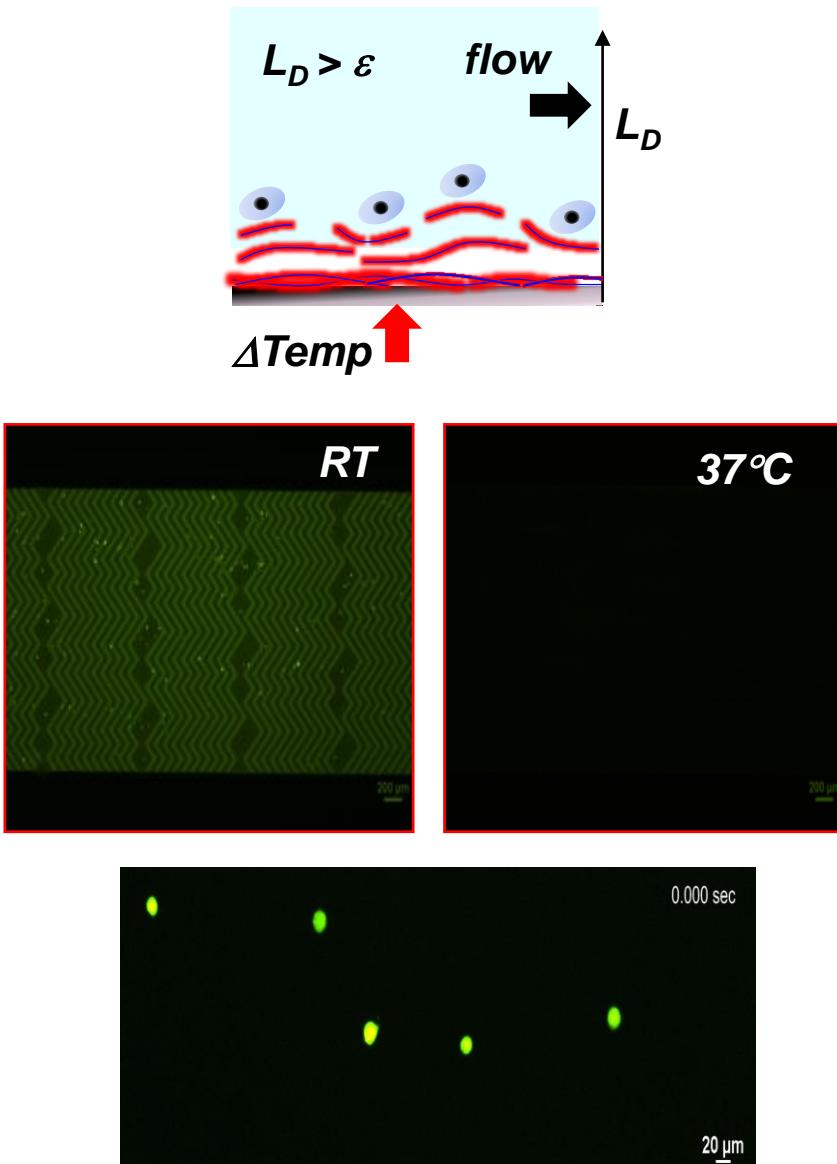
# Microfluidic Extracellular Vesicle Capture



Eduardo Reátegui (*Nat. Comm, Accepted*)

In collaboration with the  
Breakefield Laboratory

# Microfluidic Biomaterial Coating

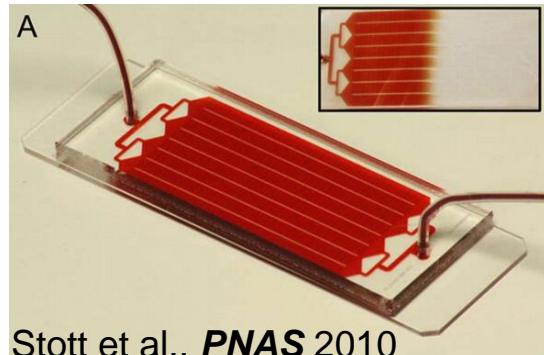


# CTCs and EVs in Precision Medicine

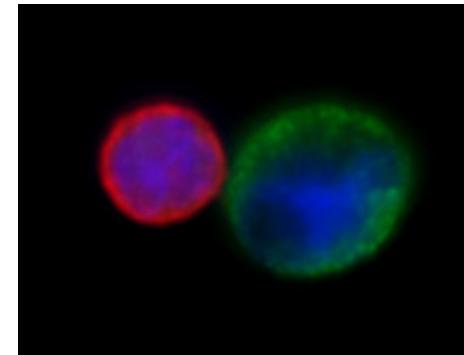
Blood cells  $\sim 10^{10}$   
CTCs 0~100



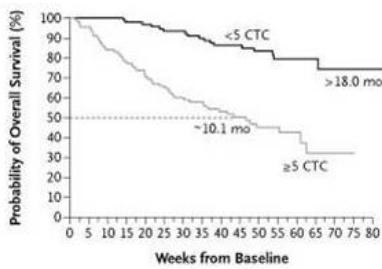
## Rare-Cell Isolation



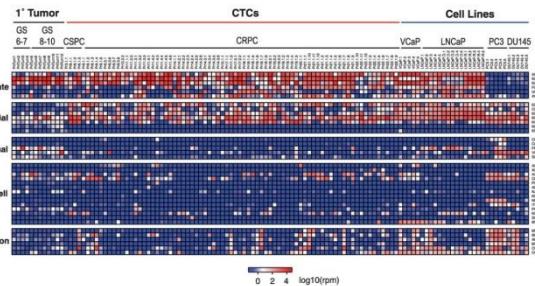
## CTC



### prognosis

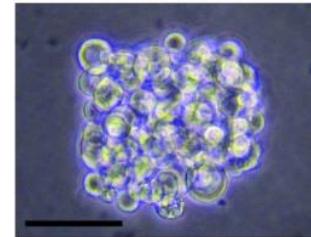


### expression profiling

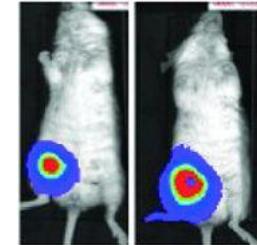


CTC count stable  
in fixed blood

### ex vivo culture

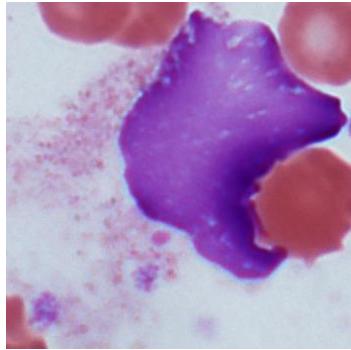


### personalized drug testing

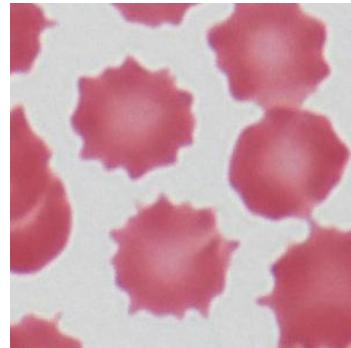


Require live cells!  
<4 hours after blood draw

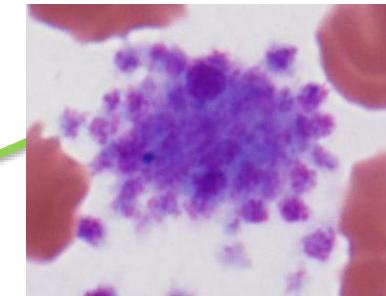
# Blood degradation damages target cells & cripples precise microfluidic technologies



Oxidative bursts  
Extracellular traps



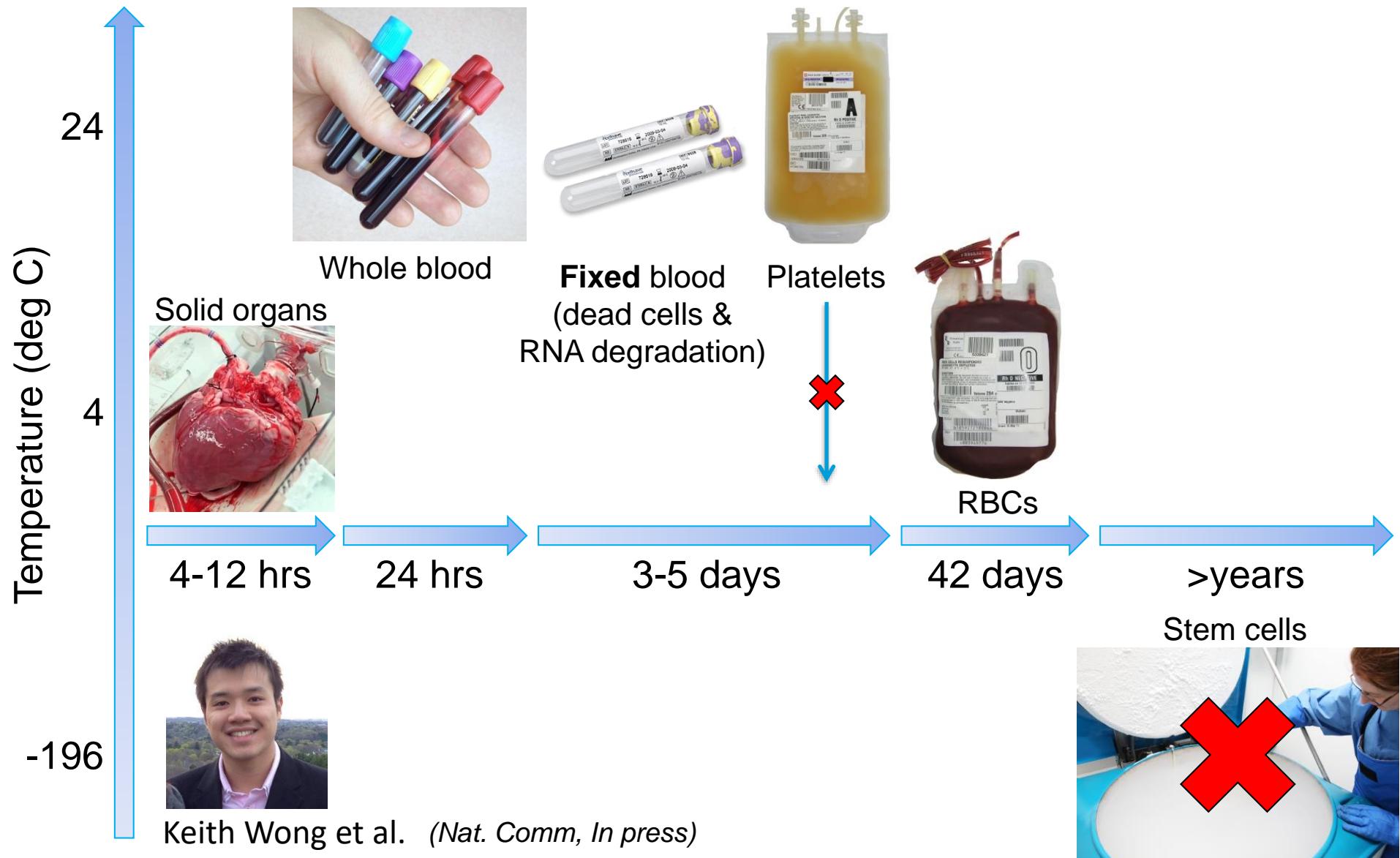
Hemolysis



Aggregation  
Clotting

Activation  
Cytokines

# Challenges of *whole* blood preservation



# Microfluidics for Liquid Biopsy: An Enabling Technology for Cancer Care

