



Lessons from Capture of Circulating Tumor Cells

Mehmet Toner



Liquid Biopsies: Complementary technologies

Event	Cancer screening	Localized cancer	Metastatic cancer	Refractory cancer
Treatment Strategy	Early intervention	Risk of dissemination and detection of recurrence	Treatment selection and monitoring response	Mechanism of resistance and new treatment

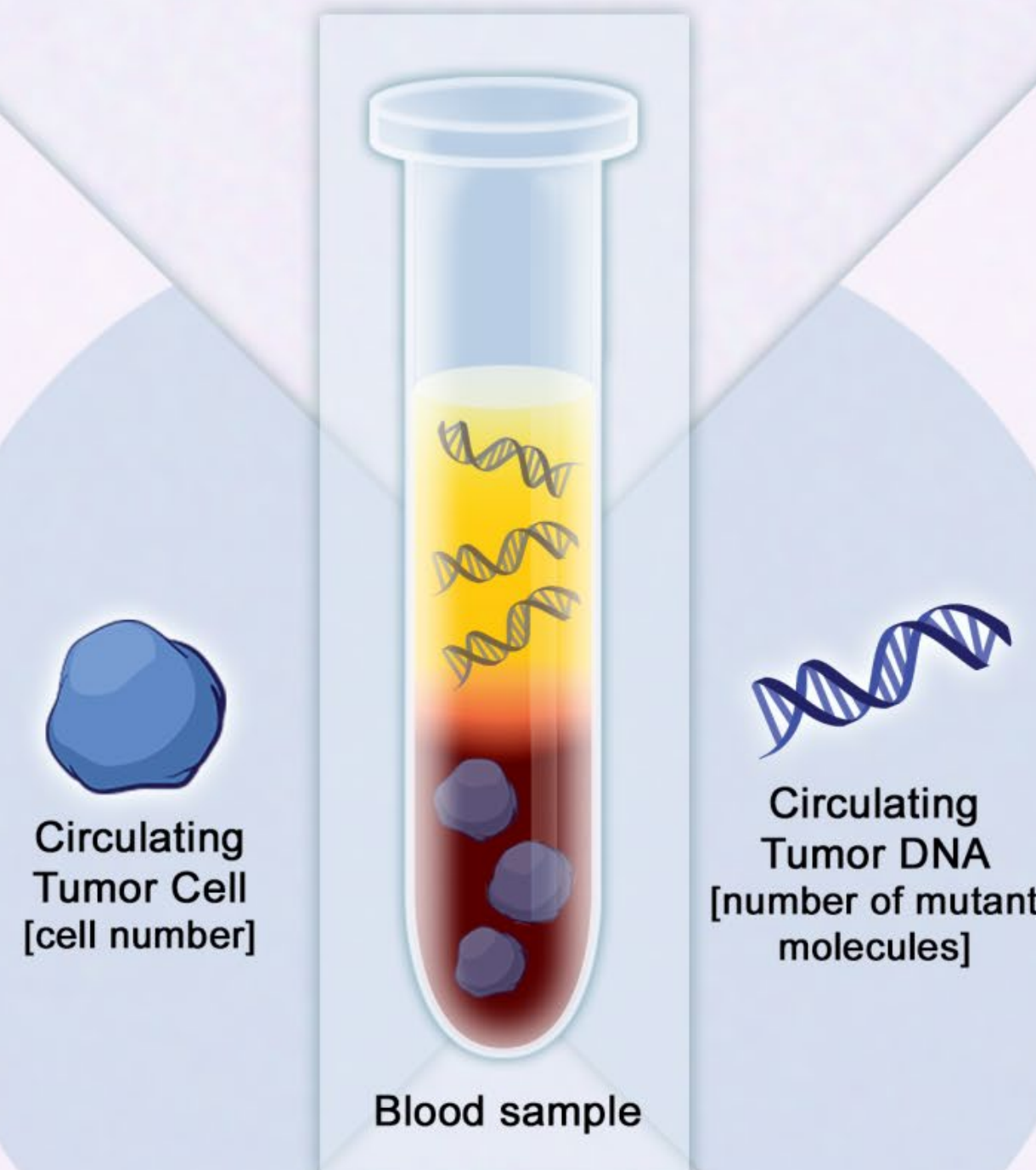
Circulating Tumor Cells (CTCs)

Applications:

- Monitoring progression (*mutations, signaling, EMT*)
- Individualized drug testing (*ex vivo culturing/analysis*)
- Detecting recurrence and early cancer detection?

Limitations:

- Very rare cells (*1-5 CTCs in 20 ml blood*)
- Complex technologies



Plasma DNA (ctDNA)

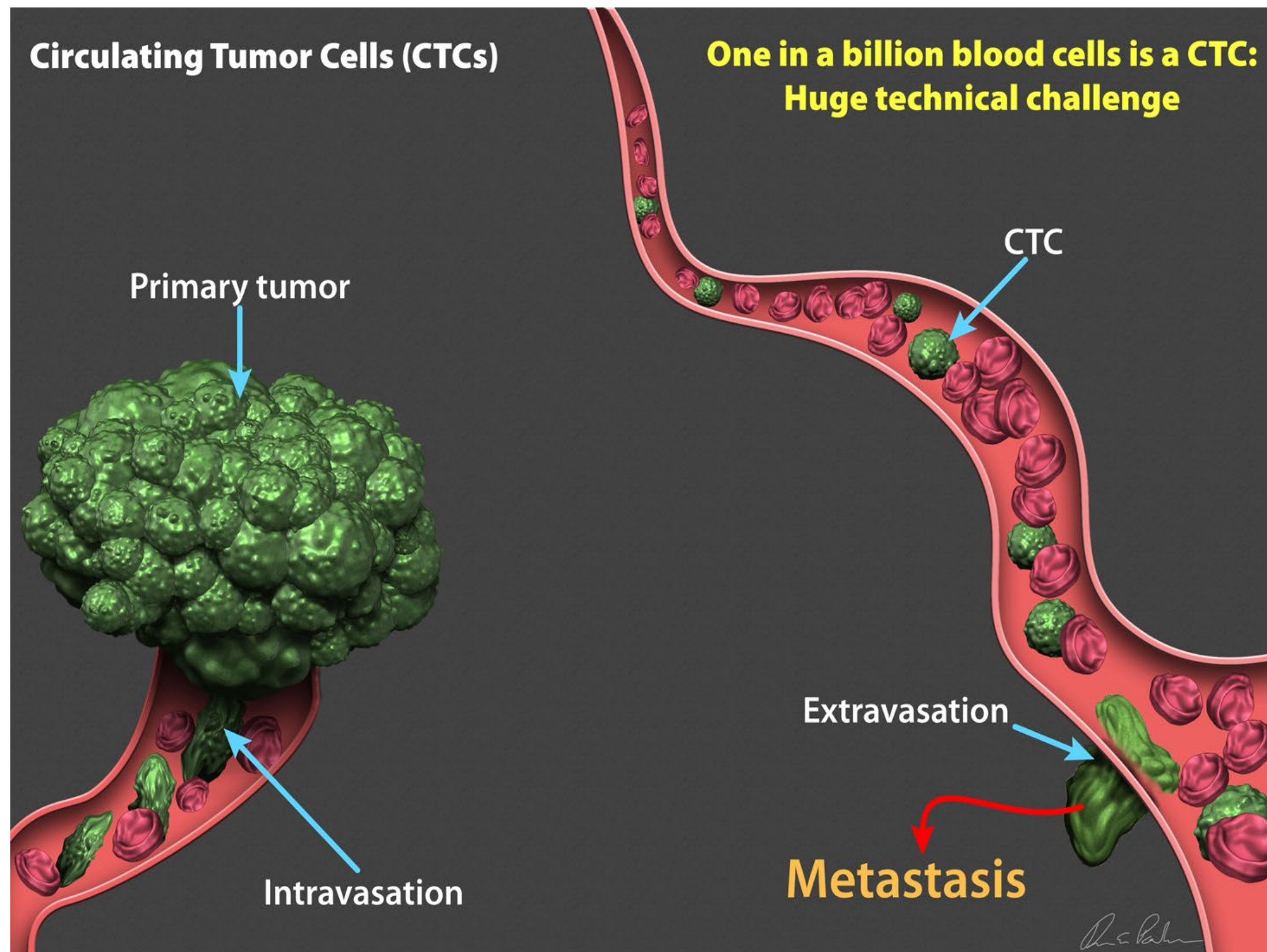
Applications:

- Acquired resistance mutation
- Monitoring recurrence ?
- Early cancer detection?

Limitations:

- Only DNA markers
- Tumor DNA is small fraction
- Tissue of origin?
- Aging blood cells also acquire mutations

Tumor Cell-based Liquid Biopsy (TCLB)



True Noninvasive Biopsy

Advantages:

- “*Real-time*” window into ever-evolving heterogeneous molecular landscape of cancer
- All the analytes required for both a definitive diagnosis and the identification of the origin of tumor

Need for:

- Sample large blood volume
- Robust, high throughput clinical grade device
- Proof of principle in real time clinic settings



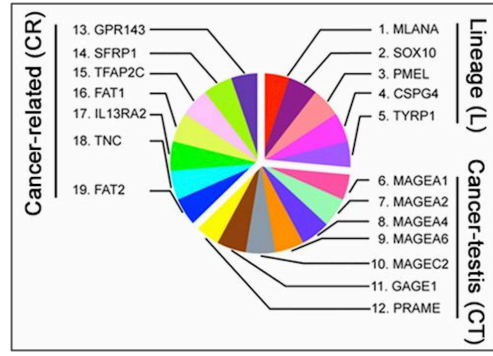
CTC-Chip

- **Positive selection**
- **Negative depletion**
- **Size-based sorting**

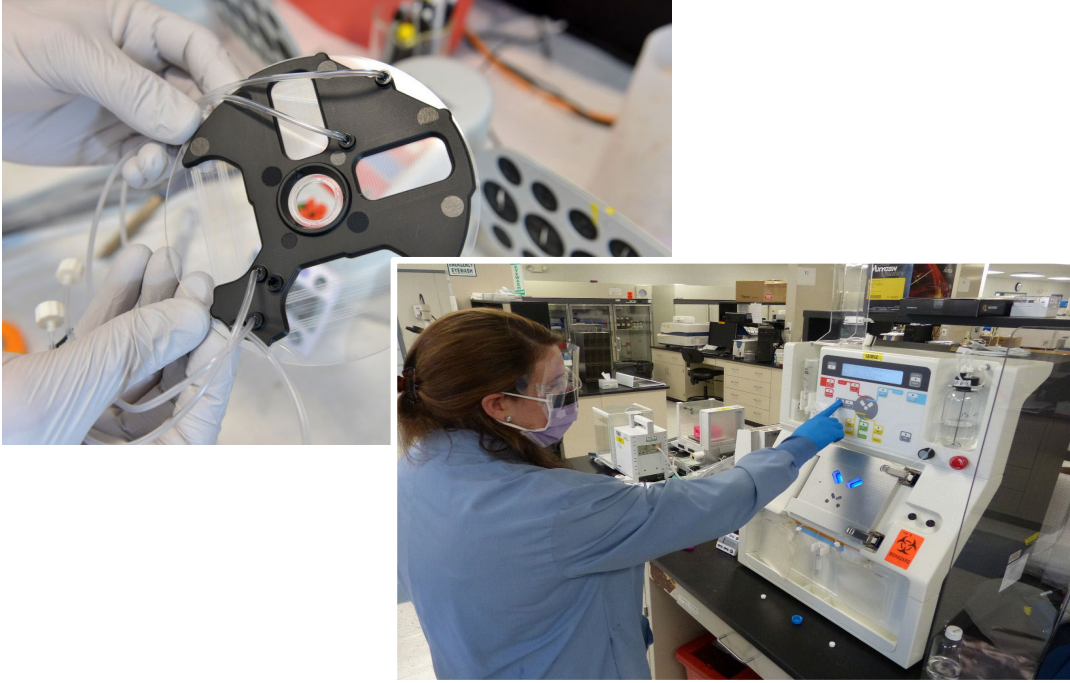
~10-20 mL
(~0.4% of 5L blood volume)



CTCs



Nagrath et al., Nature (2007)
Ozkumur et al., Sci Trans Med (2013)

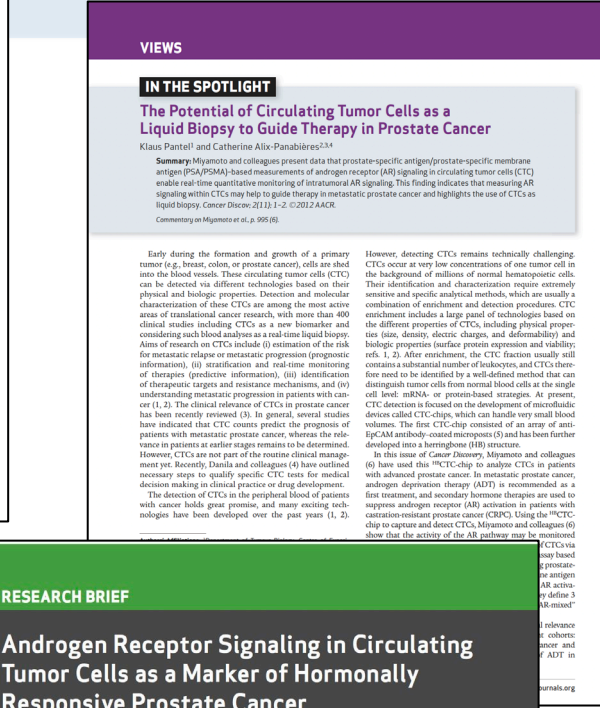
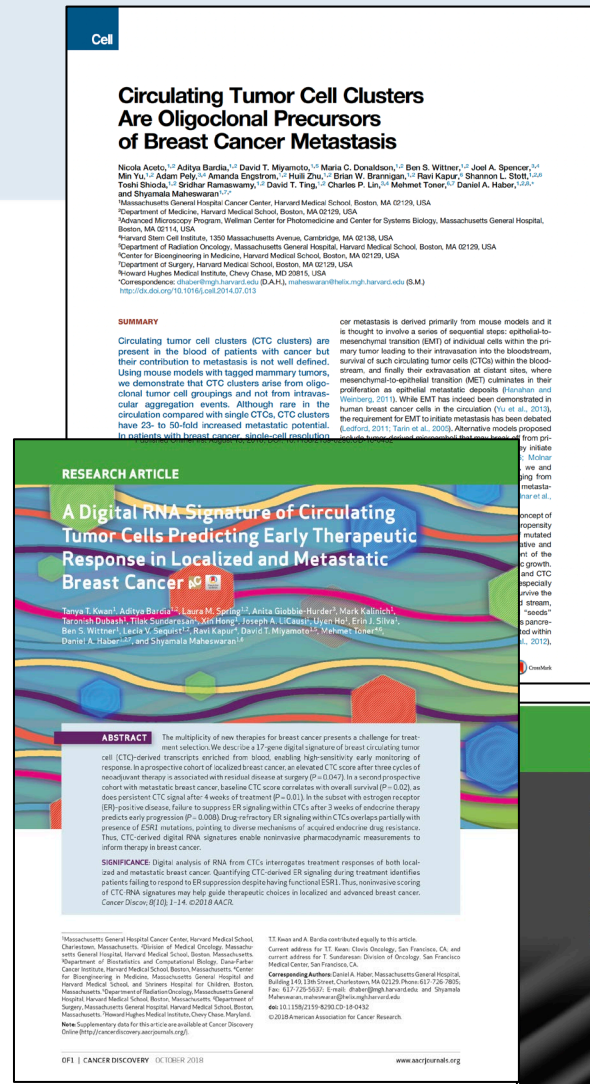


A 3D model of a cell. The nucleus is a large, blue, spherical structure with a darker blue, textured interior. A red, bean-shaped mitochondrion with internal folds is attached to the surface of the nucleus. The entire cell is surrounded by a thin, translucent blue membrane.

Figure 1 displays four panels of time-lapse microscopy images showing the development of a cell cluster over time. The panels are labeled: Day 20, Day 63, Day 85, and 5 months. Each panel includes a scale bar in the bottom left corner.

Single cell analytics

Molecular signature
(*CTC Score*)

[illegible]

developed a molecular signature of melanoma circulating tumor cells (CTCs) to quantify early tumor response using blood-based detection of CTCs. The authors used a novel, high-throughput, microfluidic-based assay to microfluidically enriched CTCs directly into a fluorescently colored oil of a patient treated with the anti-PD-1 immunotherapy, a desmolein (in percent covered by a 42-fold therapy) compared to a control immunotherapy (desmolein + anti-CTLA-4). The authors reported a mean CTC count of 1.0 (range 0.0 to 1.0) per 100,000 cells in the control group and 0.0 (range 0.0 to 0.0) per 100,000 cells in the treatment group. The authors reported a mean CTC count of 1.0 (range 0.0 to 1.0) per 100,000 cells in the control group and 0.0 (range 0.0 to 0.0) per 100,000 cells in the treatment group. The authors reported a mean CTC count of 1.0 (range 0.0 to 1.0) per 100,000 cells in the control group and 0.0 (range 0.0 to 0.0) per 100,000 cells in the treatment group.

LETTER

RNA sequencing of pancreatic cells implicates WNT signaling

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Considerable human cells (CTCs) that reside at low concentrations in the pancreas and secrete CTCs have been proposed to contribute to pancreatic cancer. However, the biology of these cells is poorly understood. To address this, we used single-cell RNA sequencing to identify the gene expression profiles of CTCs and their precursors. We found that CTCs and their precursors express a distinct set of genes, including Wnt signaling components. These findings suggest that CTCs and their precursors may play a role in pancreatic cancer development.

Understanding the biology of pancreatic cancer is a major goal of cancer research. One of the most pressing questions is how pancreatic cancer develops. A leading hypothesis is that pancreatic cancer arises from a population of cells called CTCs (cancer-associated cells) that reside at low concentrations in the pancreas and secrete CTCs. However, the biology of these cells is poorly understood. To address this, we used single-cell RNA sequencing to identify the gene expression profiles of CTCs and their precursors. We found that CTCs and their precursors express a distinct set of genes, including Wnt signaling components. These findings suggest that CTCs and their precursors may play a role in pancreatic cancer development.

Single-cell RNA sequencing (scRNA-seq) is a powerful tool for studying gene expression at the single-cell level. It has been used to identify new cell types and states, and to study the heterogeneity of cell populations. In this study, we used scRNA-seq to identify the gene expression profiles of CTCs and their precursors. We found that CTCs and their precursors express a distinct set of genes, including Wnt signaling components. These findings suggest that CTCs and their precursors may play a role in pancreatic cancer development.

Wnt signaling is a key pathway in many biological processes, including cell proliferation, differentiation, and migration. It is also involved in cancer development. In this study, we found that CTCs and their precursors express Wnt signaling components, suggesting that Wnt signaling may play a role in their biology. This finding is consistent with the hypothesis that CTCs and their precursors are involved in pancreatic cancer development.

Our findings suggest that CTCs and their precursors may play a role in pancreatic cancer development. This finding is consistent with the hypothesis that CTCs and their precursors are involved in pancreatic cancer development. We will continue to study the biology of these cells to better understand their role in pancreatic cancer.

Key words: single-cell RNA sequencing, CTCs, Wnt signaling, pancreatic cancer.

Abbreviations: CTCs, cancer-associated cells; scRNA-seq, single-cell RNA sequencing; Wnt, wingless-type; Wnt signaling, Wnt signaling pathway.

Conflict of interest statement: No conflict declared.

Received: 10/10/2018; **Accepted:** 10/10/2018; **Published:** 10/10/2018.

Supplementary information: Supplementary information is available for this article. See the article text for details.

Correspondence: Andrew S. Belmont, Department of Cell Biology, Princeton University, Princeton, NJ 08542. E-mail: abelmont@princeton.edu

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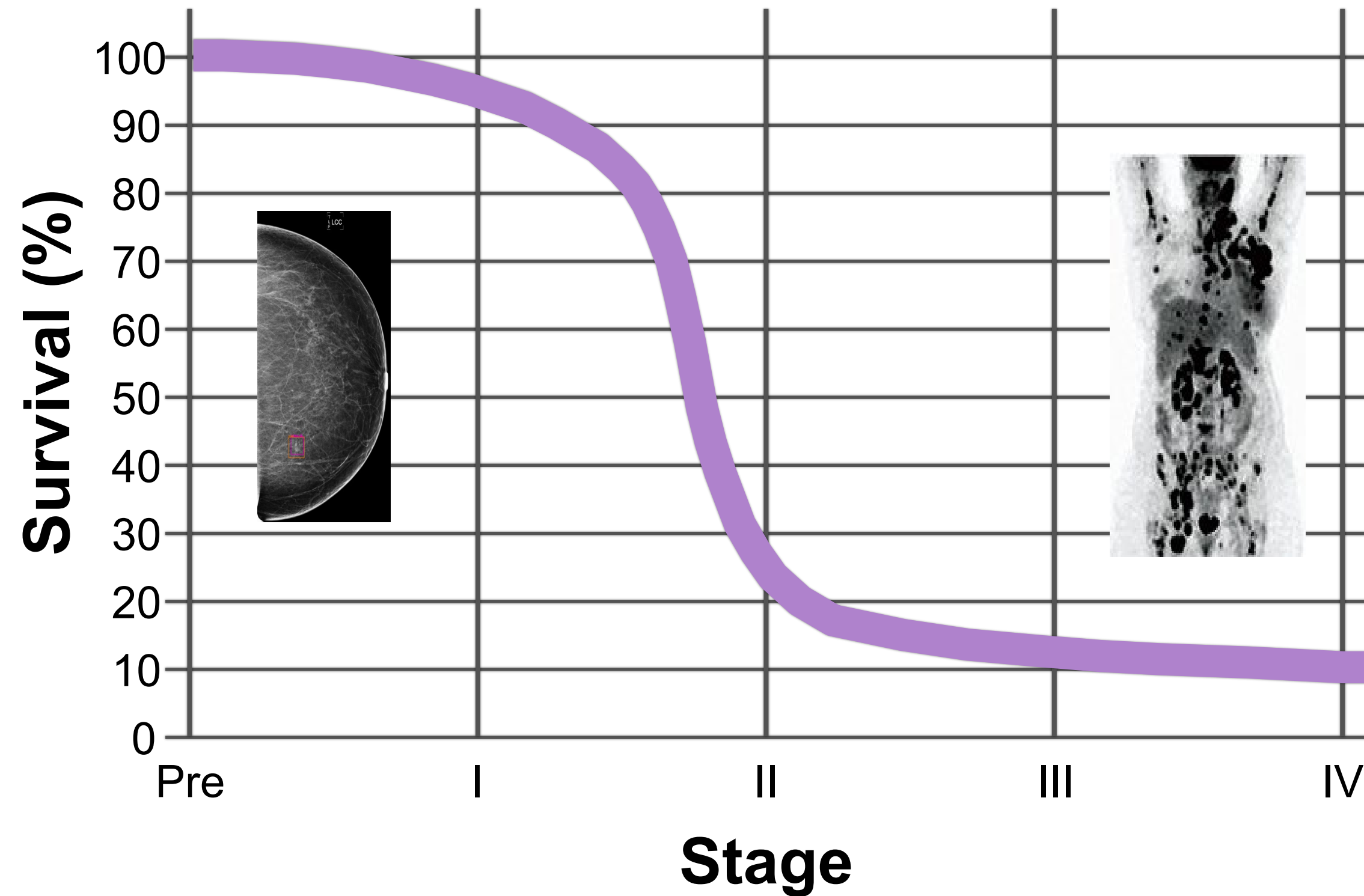
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CCP-associated C₂ domain (Fig. 3). The high contrast between the two had resulted in a 1.5-fold increase in the number of C₂ domains per CCP. This increase was not due to a change in the size of the C₂ domain, as the CCPs associated with the 1.5-fold increase in C₂ domains were purified from the same culture conditions and were of the same size (data not shown). The CCPs associated with the 1.5-fold increase in C₂ domains were purified from the same culture conditions and were of the same size (data not shown). The CCPs associated with the 1.5-fold increase in C₂ domains were purified from the same culture conditions and were of the same size (data not shown).

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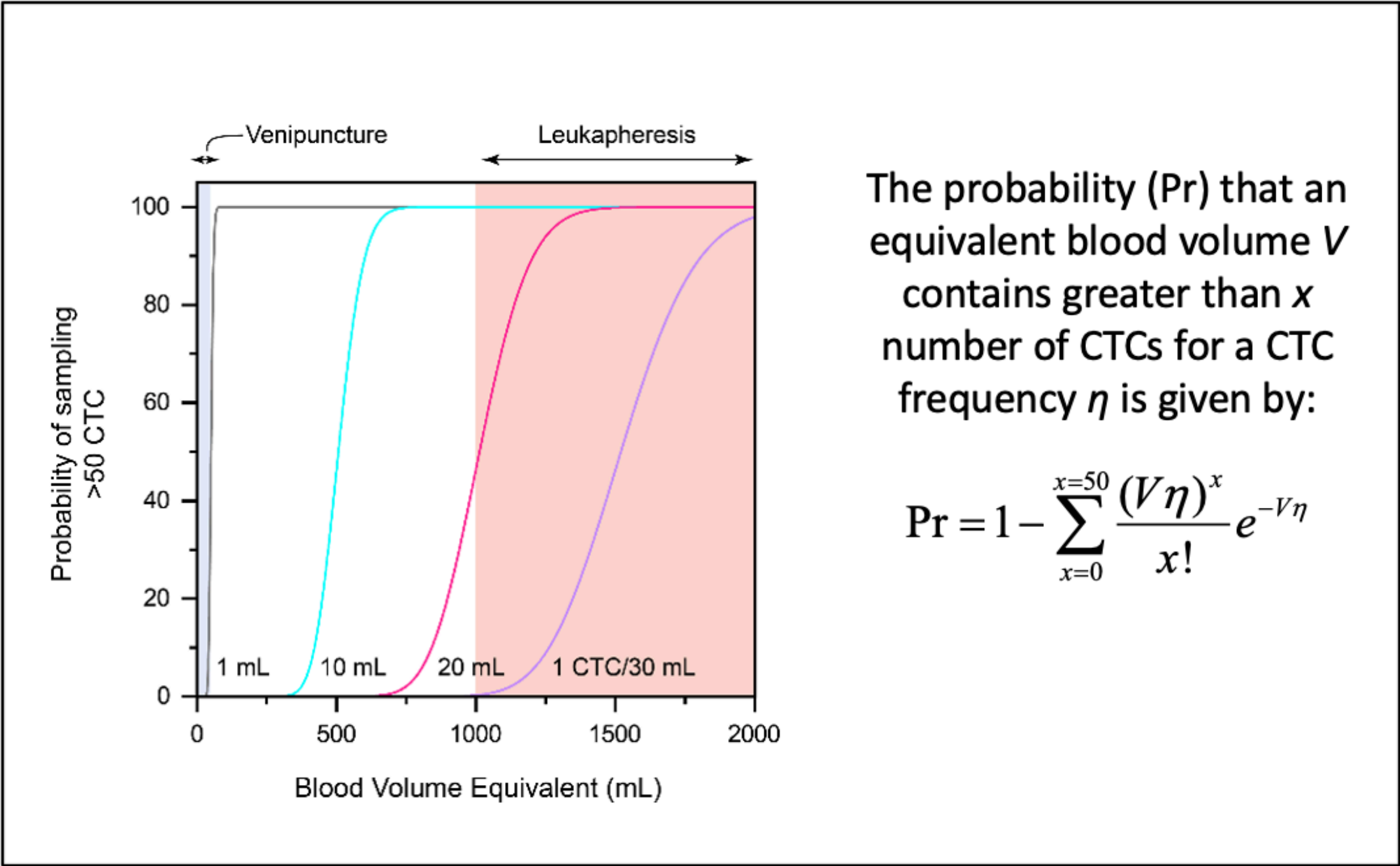
Late-stage therapy is highly ineffective



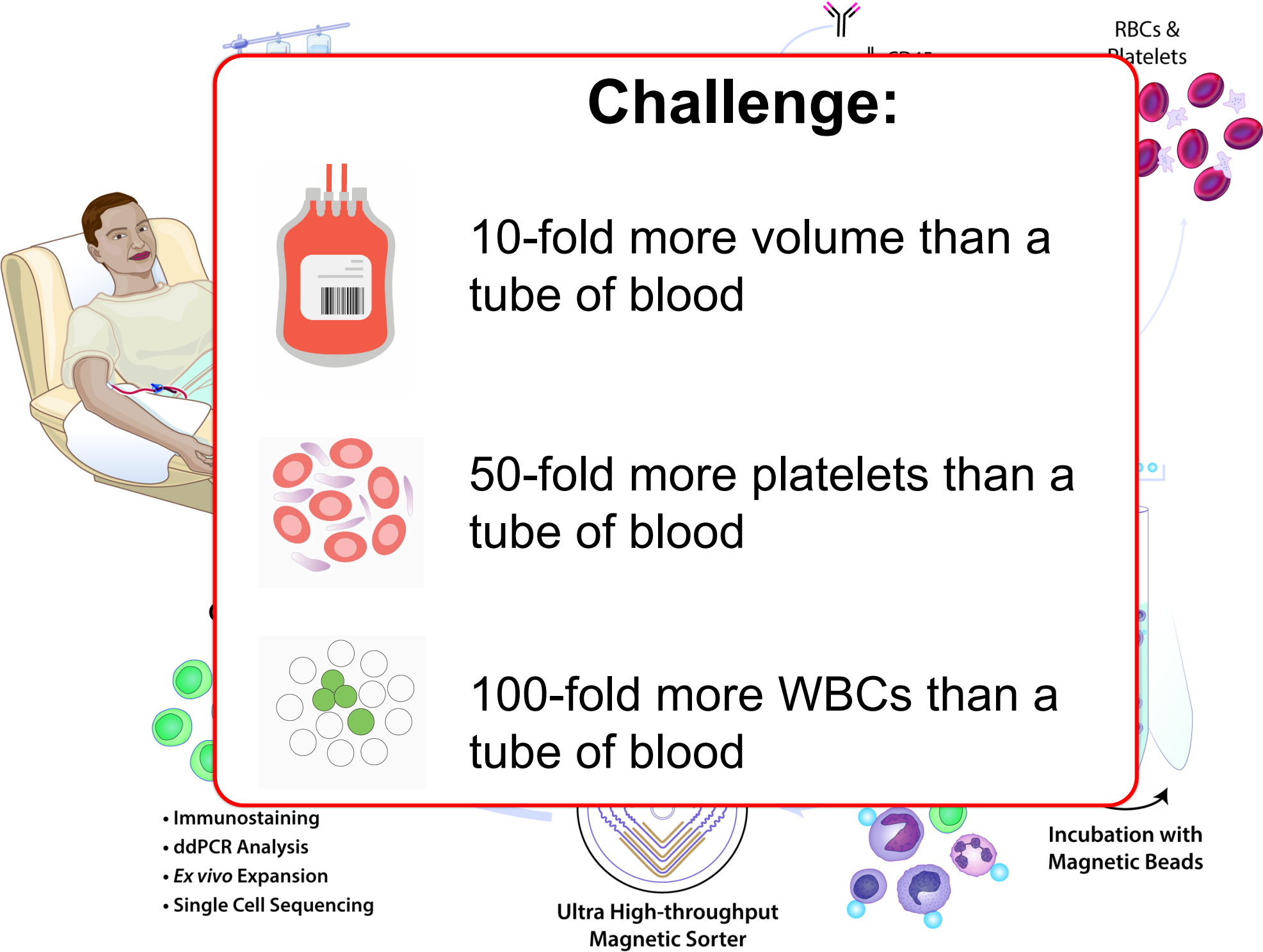
Changing landscape of cancer screening:

- Only a tiny fraction of nodules identified in screening are cancerous
- Increasing use of MRI and CT (especially Low Dose CT) scans led to an explosion of ‘incidentalomas’
- ctDNA testing for cancer-associated DNA methylation has a false positive rate of 62%

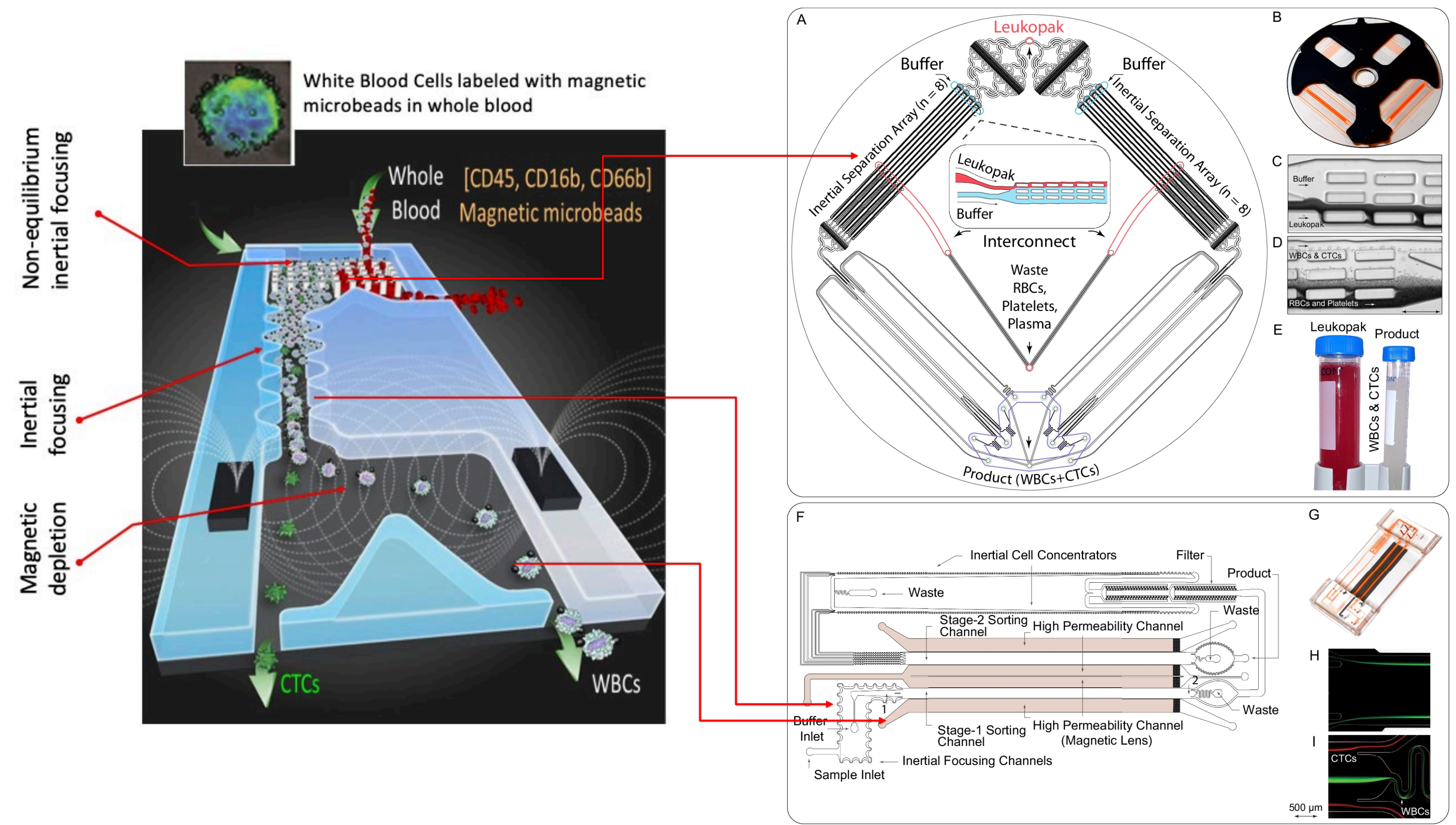
How much blood volume needed for a robust CTC assay?



Tumor Cell-based Liquid Biopsy using leukopaks



CTC-Chip: Multiphysics negative depletion of blood cells



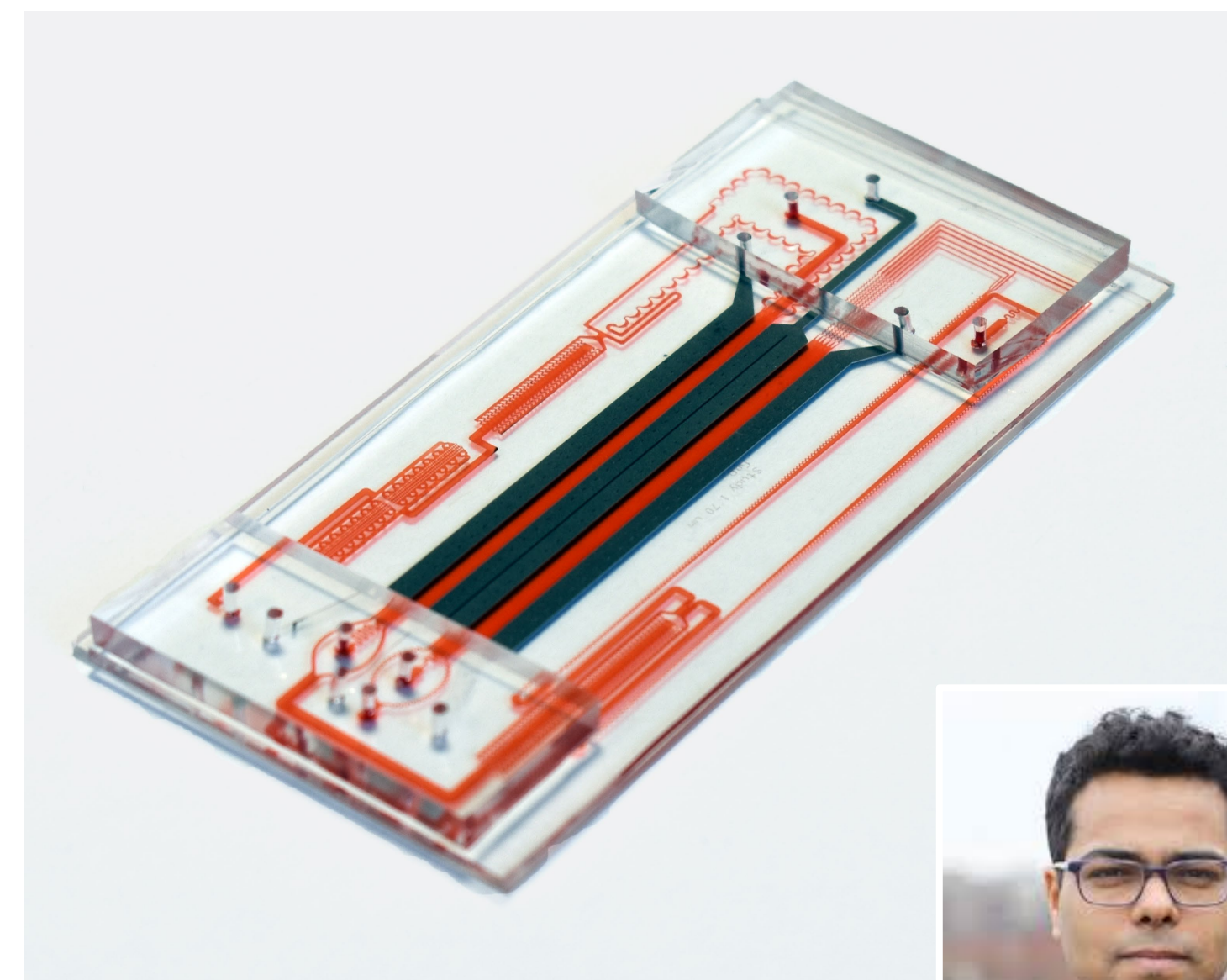
Ultrahigh-throughput magnetic sorting of large blood volumes for epitope-agnostic isolation of circulating tumor cells

Avanish Mishra^{a,b,c,d,1}, Taronish D. Dubash^{c,d,1}, Jon F. Edd^{a,b,c}, Michelle K. Jewett^{a,b,c}, Suhaas G. Garre^{a,b,c}, Nezihi Murat Karabacak^{a,b,e}, Daniel C. Rabe^{a,b,c,d}, Baris R. Mutlu^{a,b,d}, John R. Walsh^{a,b,c}, Ravi Kapur^f, Shannon L. Stott^{a,b,c,d}, Shyamala Maheswaran^{c,d}, Daniel A. Haber^{c,d,g,2}, and Mehmet Toner^{a,b,d,e,2}

^aBioMEMS Resource Center, Charlestown, MA 02129; ^bCenter for Engineering in Medicine and Surgery, Massachusetts General Hospital, Boston, MA 02114; ^cCancer Center, Massachusetts General Hospital, Boston, MA 02114; ^dHarvard Medical School, Boston, MA 02115; ^eShriners Hospitals for Children, Boston, MA 02114; ^fMicroMedicine, Inc., Waltham, MA 02451; and ^gHoward Hughes Medical Institute, Bethesda, MD 20815

Contributed by Daniel A. Haber, May 13, 2020 (sent for review April 10, 2020; reviewed by James J. Collins and John A. Rogers)

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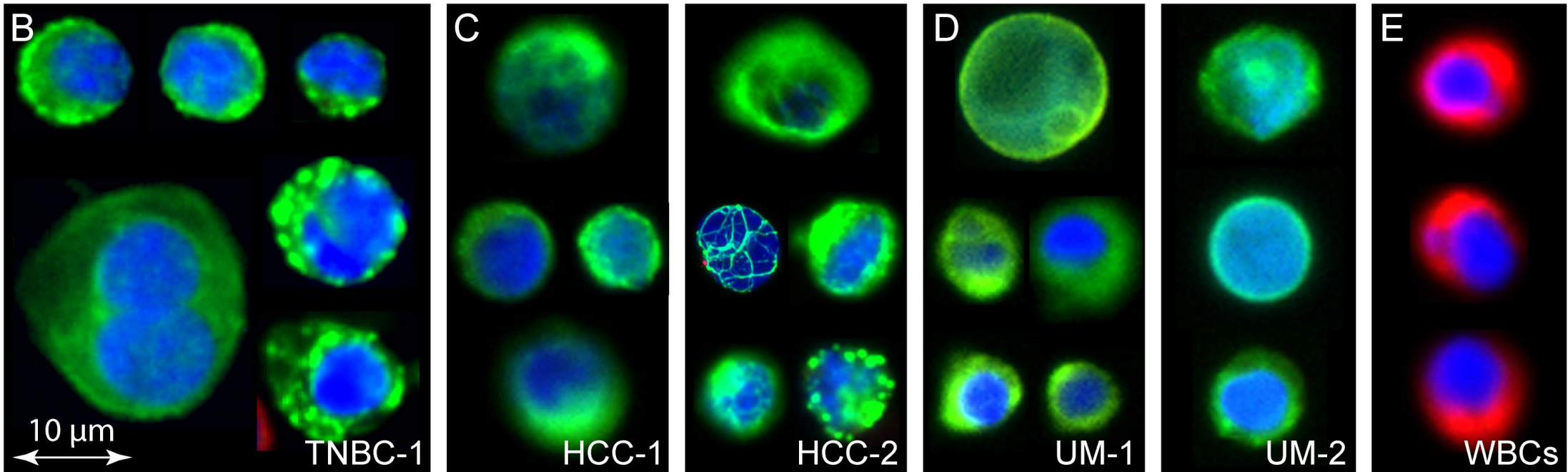


Avanish Mishra

1. Process large volumes at a single cell resolution: 300 million cells per min at 1 CTC in 40 mL blood sensitivity
2. Apply in **unbiased** way to all cancers, without having to know which cancer ahead of time:
“Negative Depletion” of normal blood cells vs **“Positive Selection”** or **“Size Selection”** of tumor cells
2. Tumor cells are **“untouched and undamaged”** and hence they can be studied and cultured for analysis

Clinical results from cancer patients

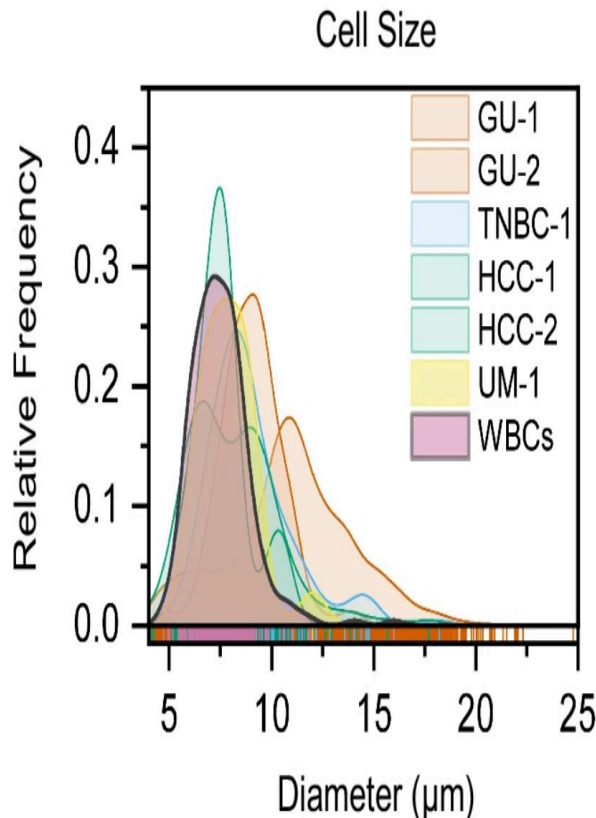
Tumor type	Total Blood Volume Processed (mL)	Number of Isolated CTCs
Prostate (GU-1)	6379	58,125
Prostate (GU-2)	5732	1,276
Breast (TNBC-1)	5991	4,580
Liver (HCC-1)	4565	4,109
Liver (HCC-2)	7176	1,490
Uveal Melanoma (UM-1)	6523	720
Uveal Melanoma (UM-2)	4345	100



Green - Tumor Markers
Blue - Nucleus
Red – Exclusion markers

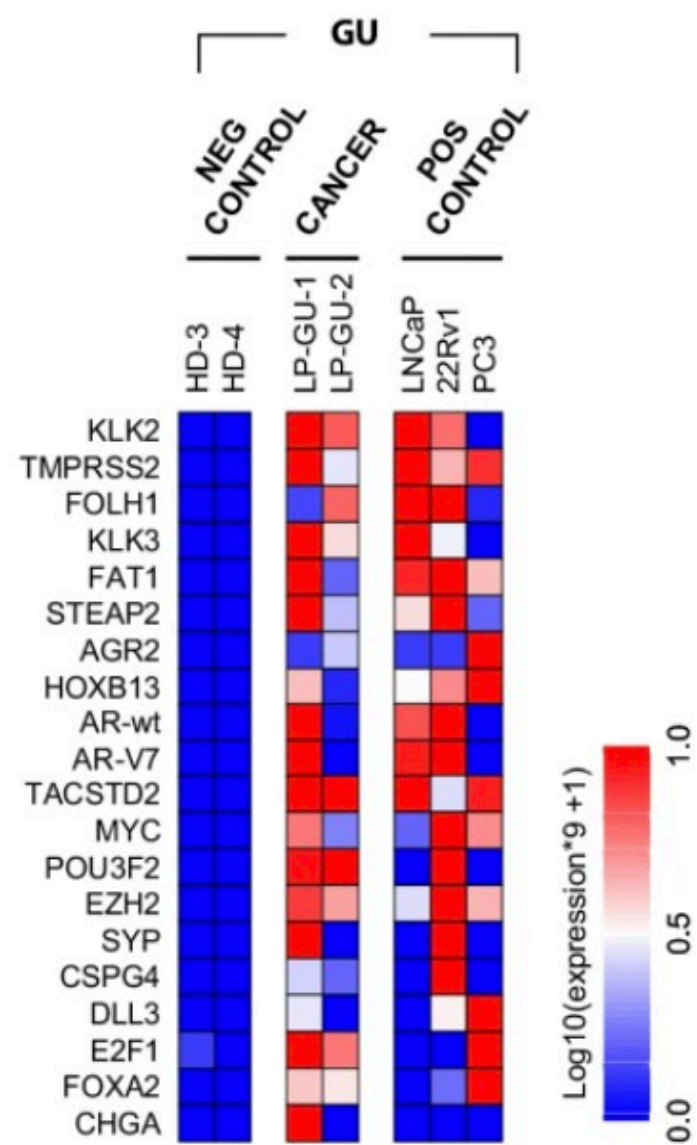
GU: EpCAM/C11/CK19
TNBC: EpCAM/C11/CK19
HCC: EpCAM/ C11/CK19/ASGR1/GPC3
UM: Sox10/Melan-A/NG2

- 99% of WBCs overlap with 67% of CTCs with size ranging from 4 to 25 microns
 - Intra-patient variation in tumor marker expression range from 2 to 20-fold



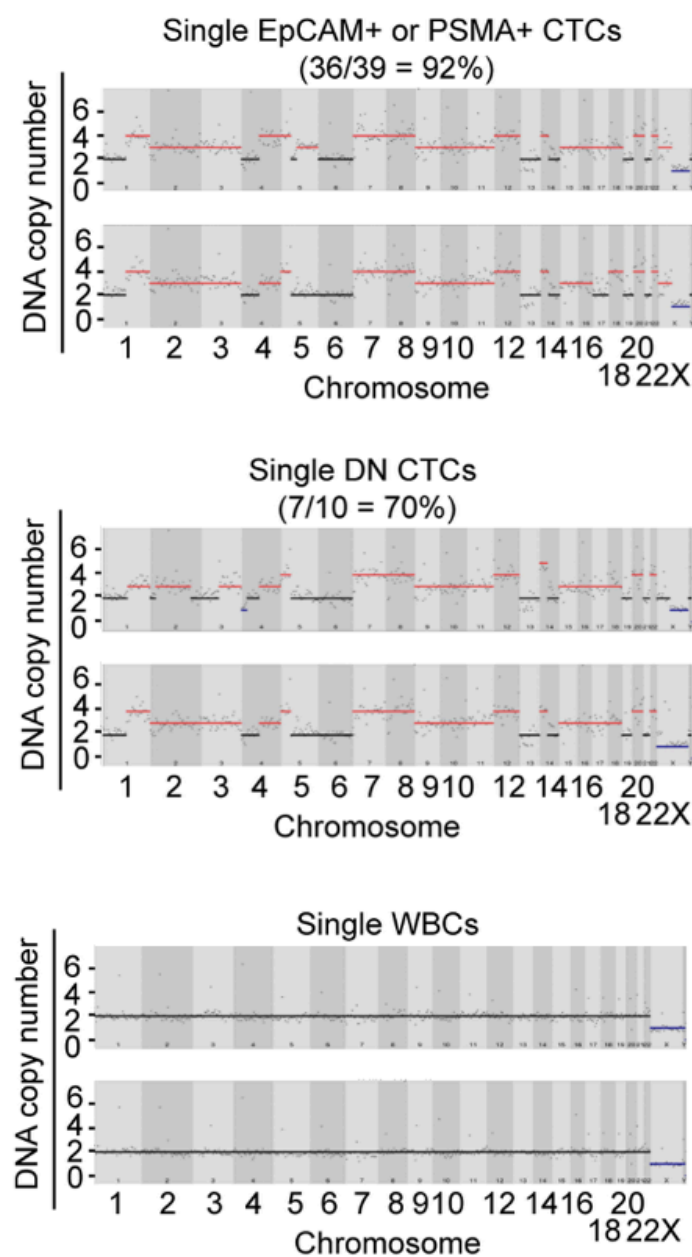
Clinical results from cancer patients

Tumor origin:
Cell lineage-based
RNA signature



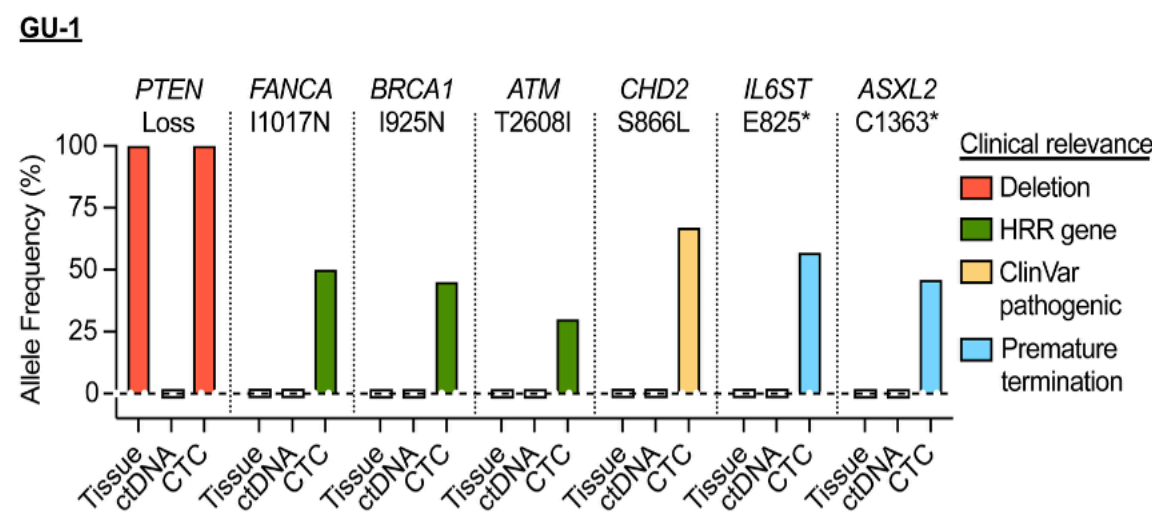
ddPCR confirms the presence of the expected tumor type with high specificity

Single-cell DNA (CNV) analysis providing bonafide proof of cancer



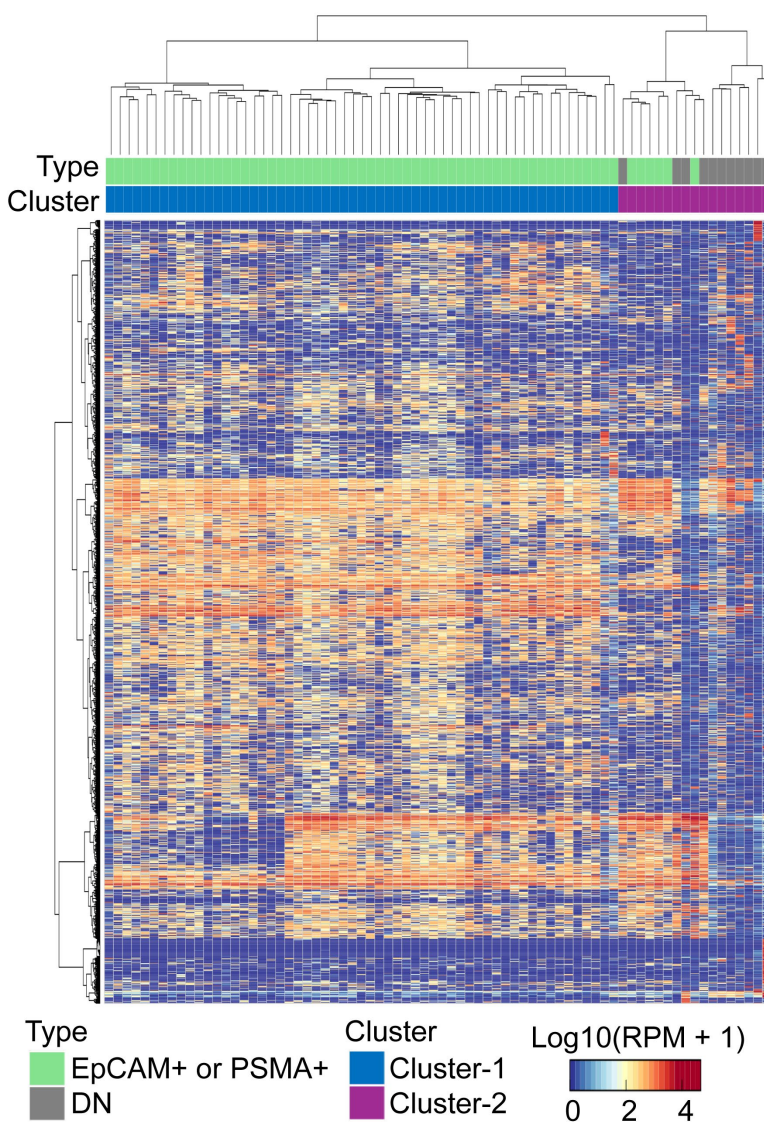
Genomic amplifications and deletions in CNV between EpCAM+ and DN CTCs indistinguishable

WES analysis for prostate CTCs displaying CNVs



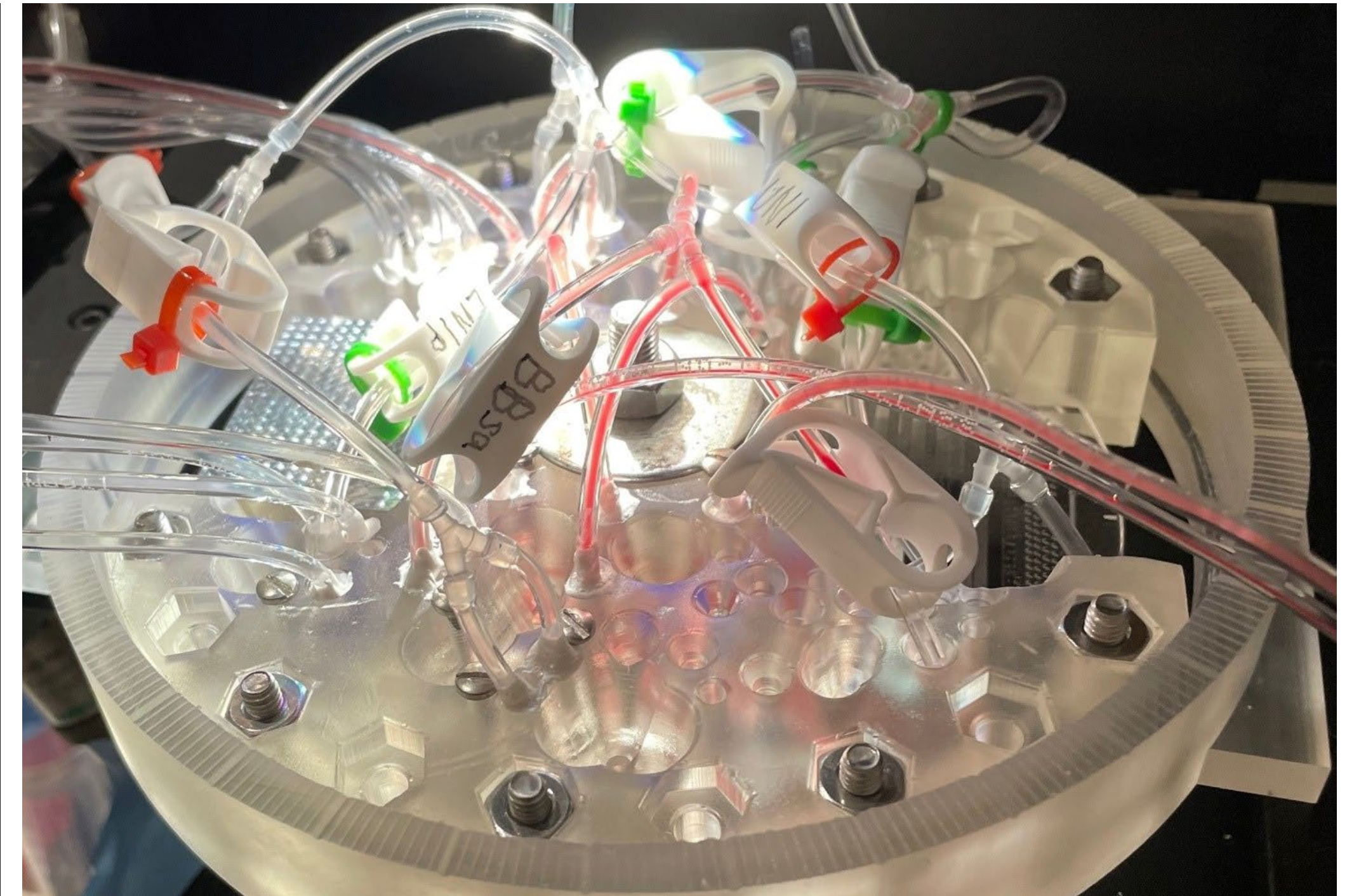
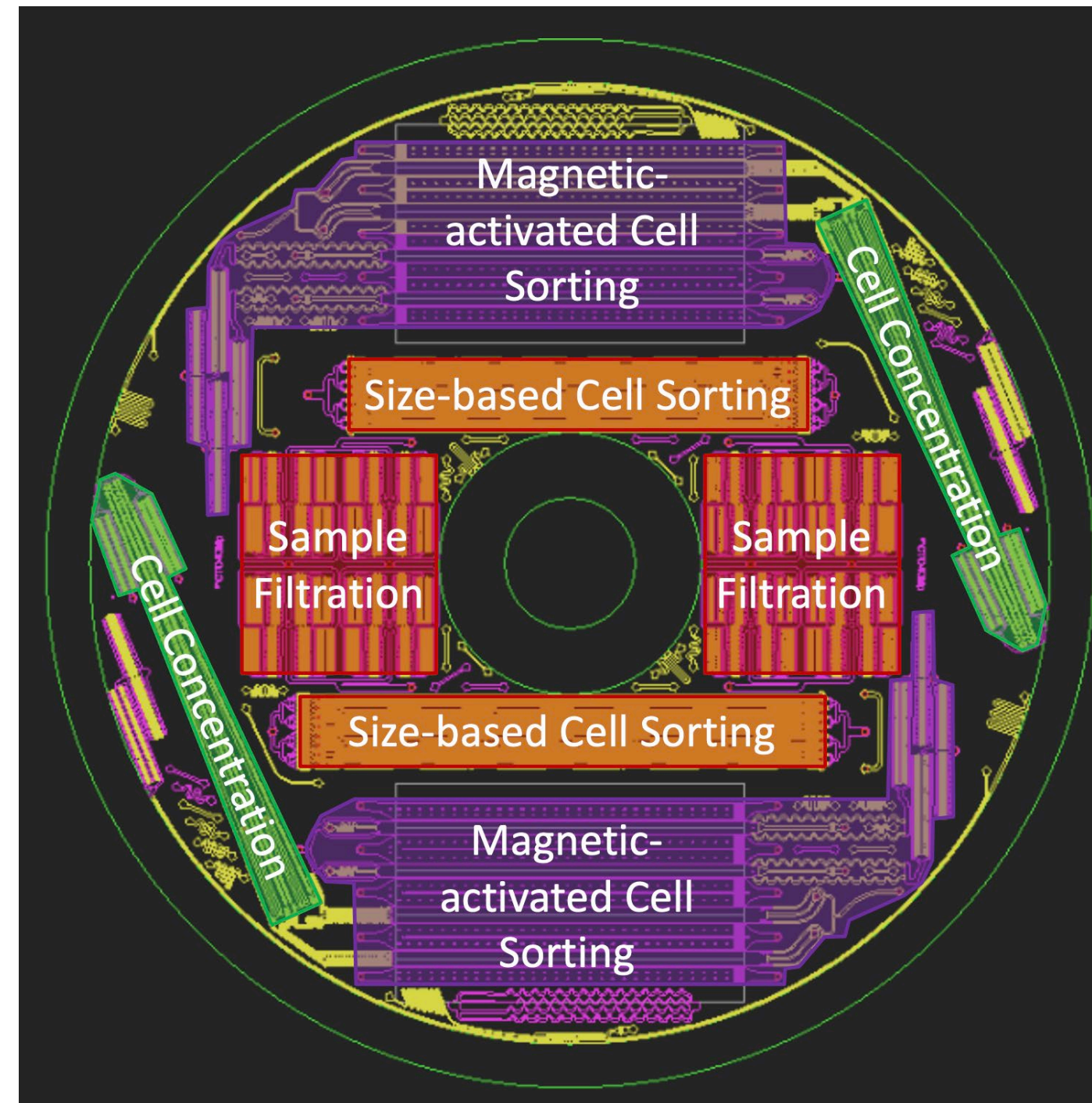
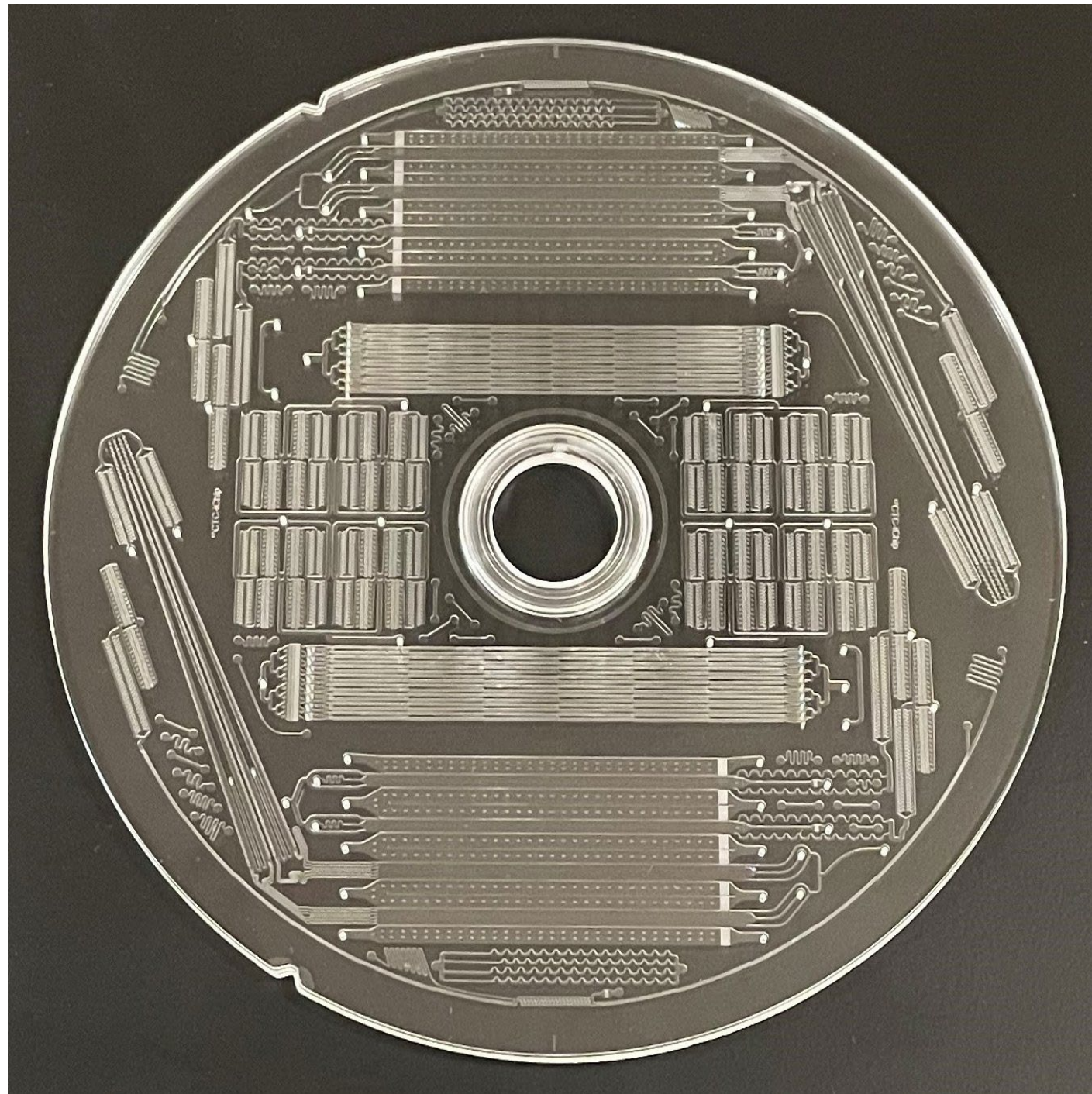
Whole Exome Sequencing (WES) of CTCs identifies functionally significant mutations within known cancer genes beyond those detectable using ctDNA or even tissue needle biopsies

Single-cell RNA-seq analysis for prostate CTCs displaying CNVs



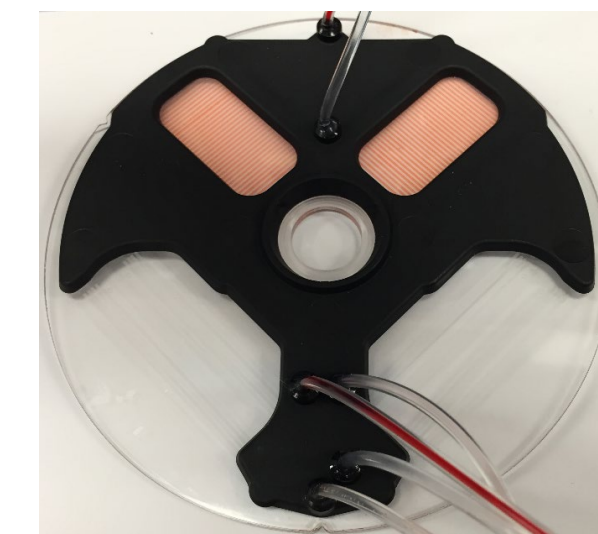
Two distinct hierarchical clusters based on RNA expression. DN CTCs are small, mesenchymal-like cells with neuroendocrine features.

Integrated monolithic CTC-Chip for early diagnosis

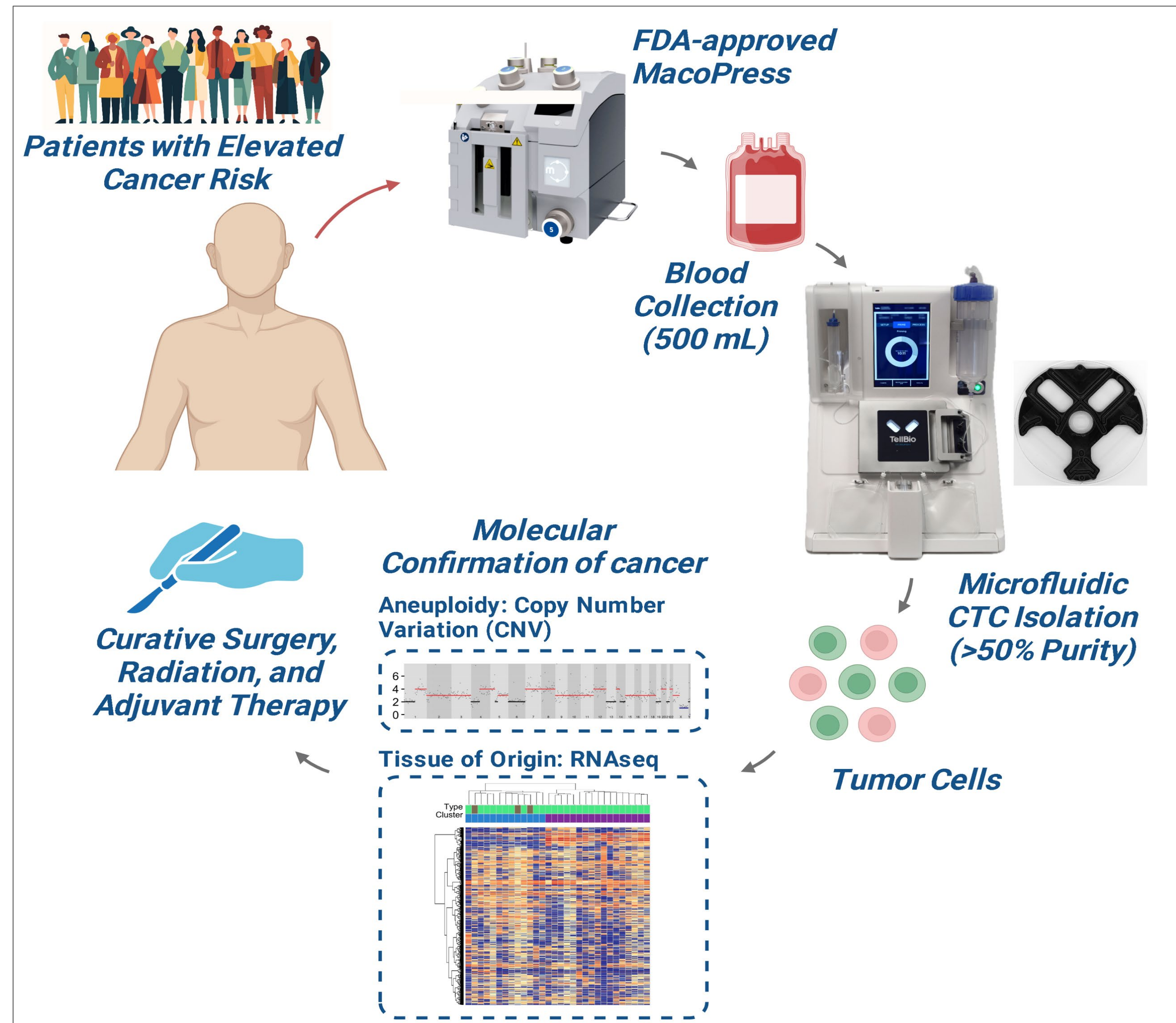


Jon Edd

300 million cells/min
1 CTC in 200 billion blood cells
<1 sec within the chip



CTCs from a rare event to a reliable biomarker



Acknowledgements

Engineering/Innovation

Avanish Mishra
Jon Edd
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Fatih Sarioglu
Ajay Shah
Joe Martell
Keith Wong
Eduardo Reategui
Dino DiCarlo
Palaniappan Sethu
Aman Russom
Chia-Hsien Hsu
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Biology/Clinical

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S. Maheswaran
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Rick Lee
Ryan Sullivan
David Miyamoto
David Ting
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Min Yu
Mark Kalinich
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Elad Horwitz
Ajinkya Revandkar
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Karsen Seeger
Laura Libb
Joanna Vuille
Doug Micalizzi
Min Yang

Computational

Mike Lawrence
Esther Rheinbay
Ben Wittner

Thank you to our Patients!



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Biomedical Imaging and Bioengineering

Johnson & Johnson

