Circulating Tumor-specific Molecules: Approaches to Ultrasensitive Isolation, Analysis, and Clinical Applications

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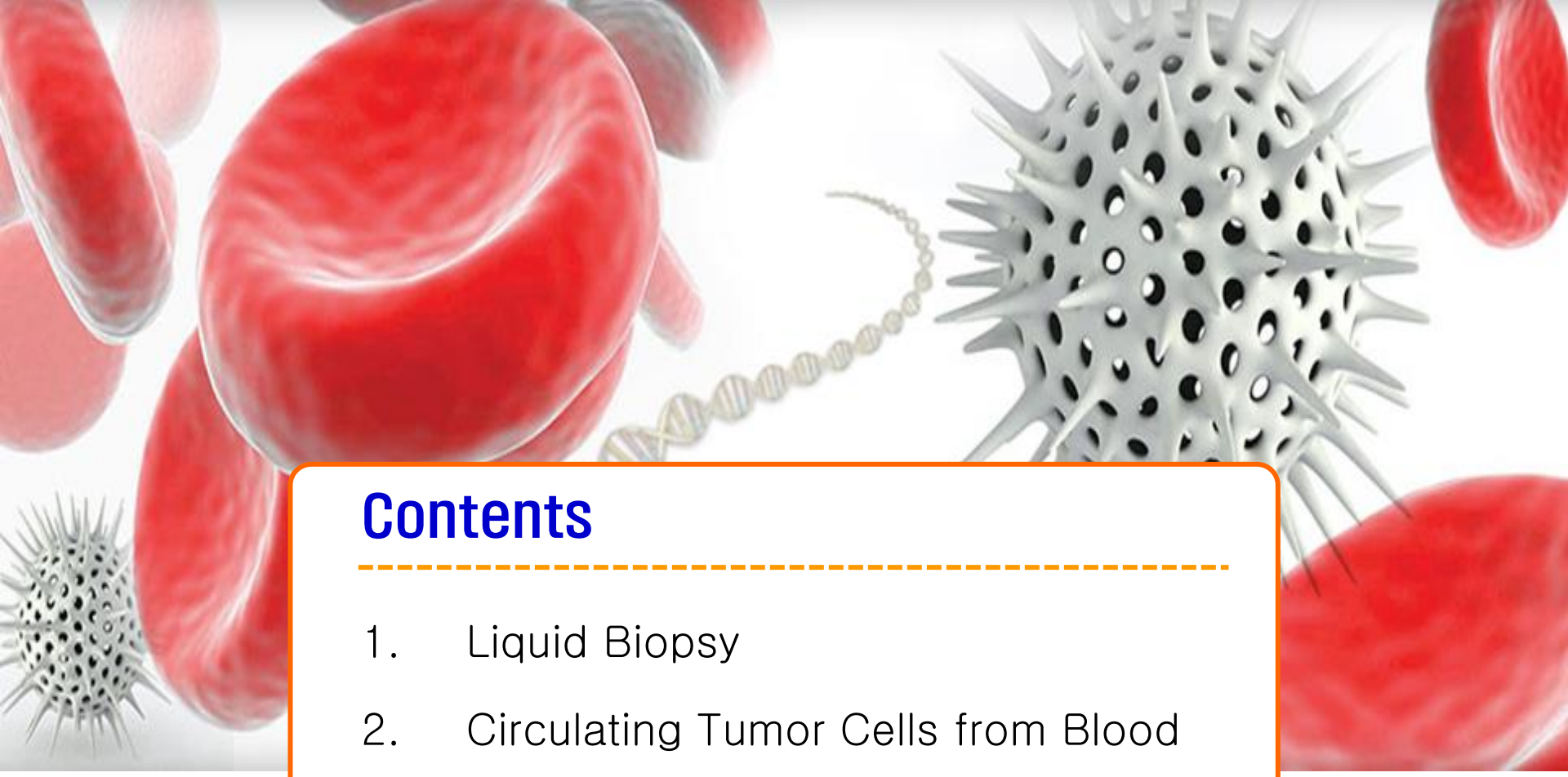
National Cancer Center

Biomarker Branch



Contents

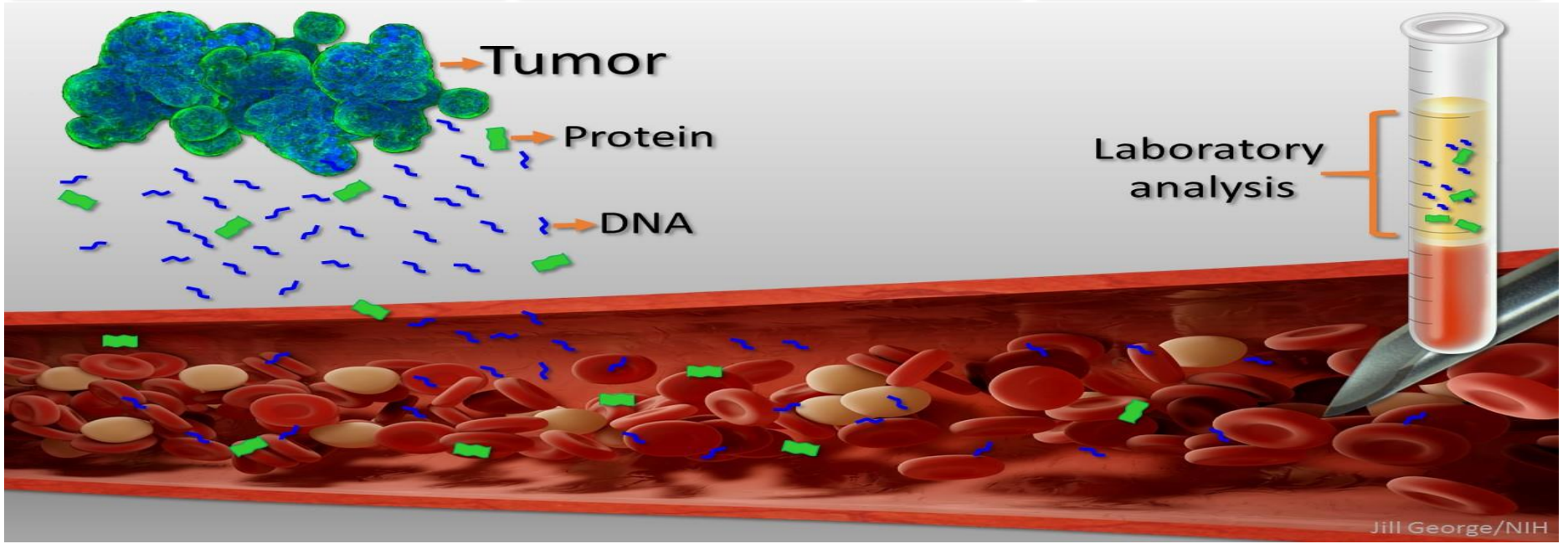
1. Liquid biopsy

Cancer Diagnosis

IMAGING

TISSUE BIOPSY

LIQUID BIOPSY



Tissue biopsy vs. Liquid biopsy

Tissue sample

Adv

- Comple
  tumor p
  express
- Best sa
  stratifica
  & non-re
  therapy
- Still the
  tumor cl

Challenges

- Invasive procedure
- Limited quantity
- Difficulties in serial repetitive collection
- Not always representative for the entire variety of malignant clones: TUMOR HETEROGENEITY

Liquid sample

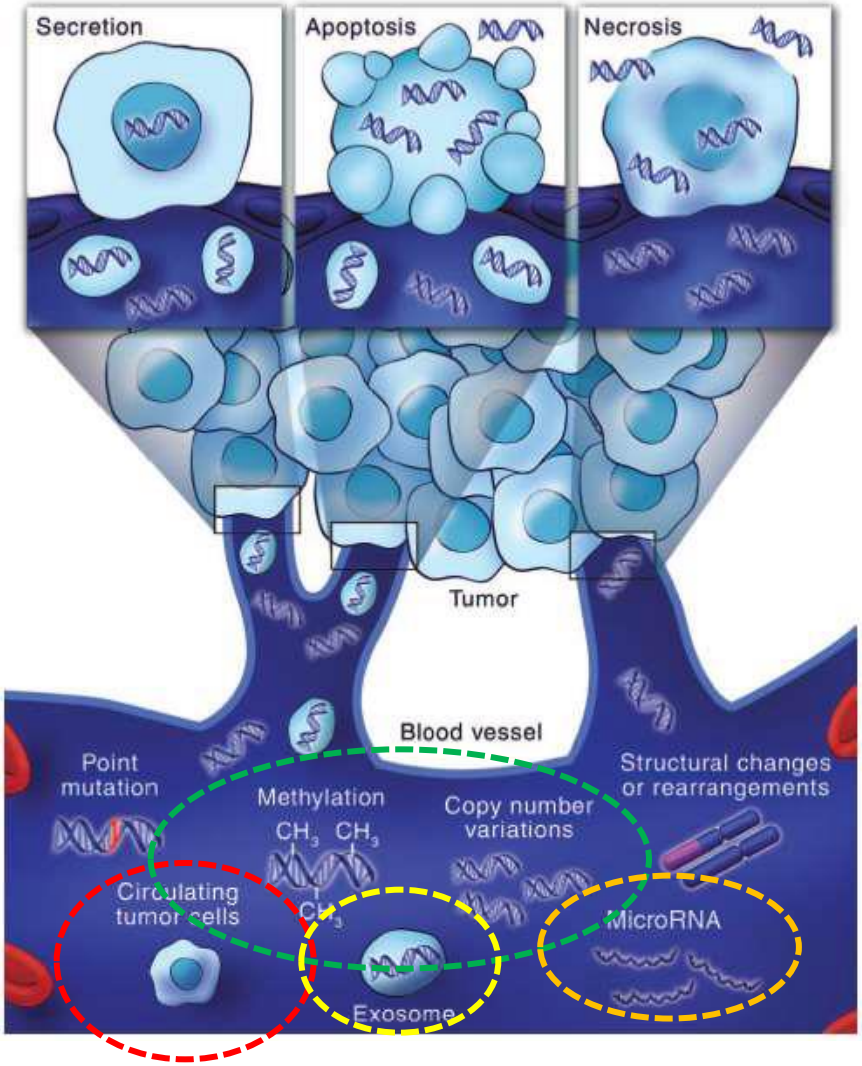
Adv

- Non-inv
- Fast & l
- Serial, r
- Informa
  heterog

Challenges

- Low biomarker concentration
- Complexity
- Lack of standardization, still used mainly in translational research

Blood as a real-time liquid biopsy



CTCs
(Circulating tumor cells)

cfDNAs
(Circulating cell-free nucleic acids)

Exosomes

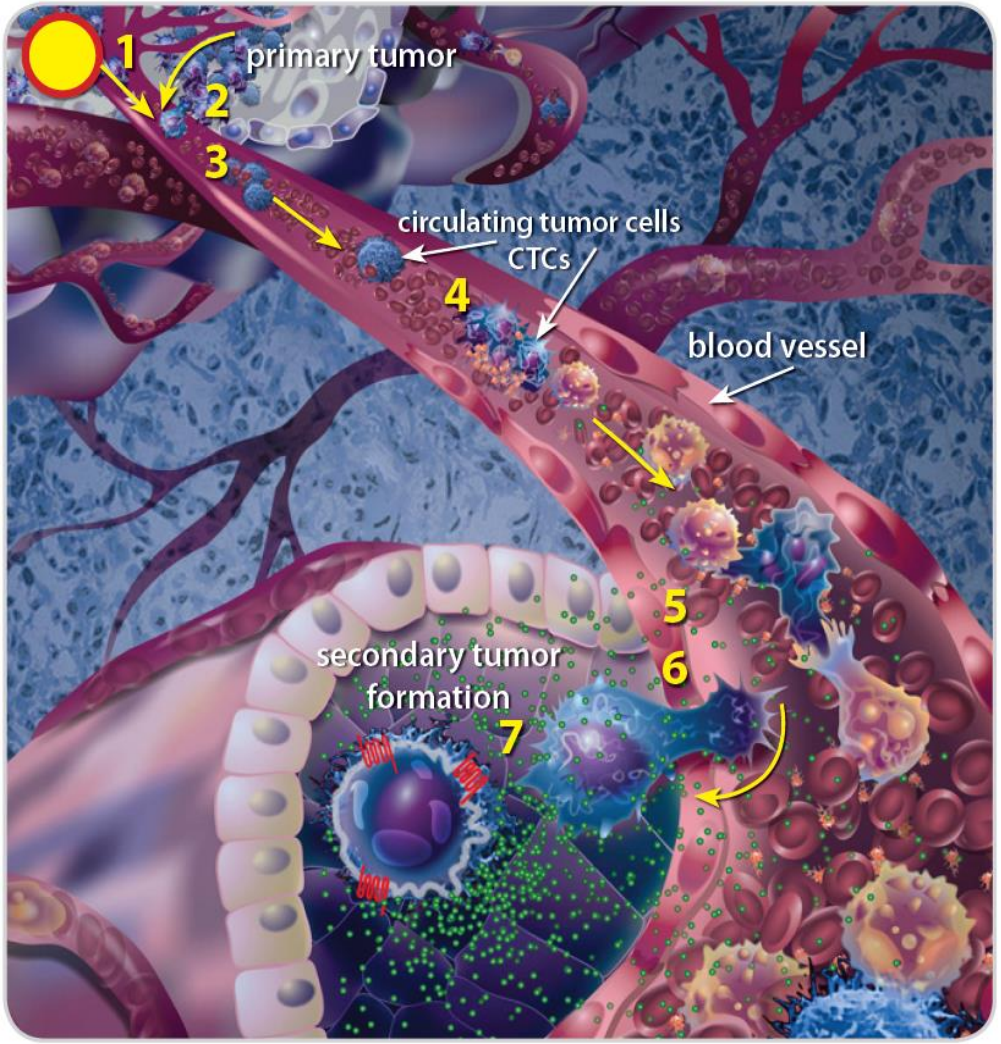
miRNA

Genome Biology, 2014, 15, 229

2. Circulating Tumor Cells from blood

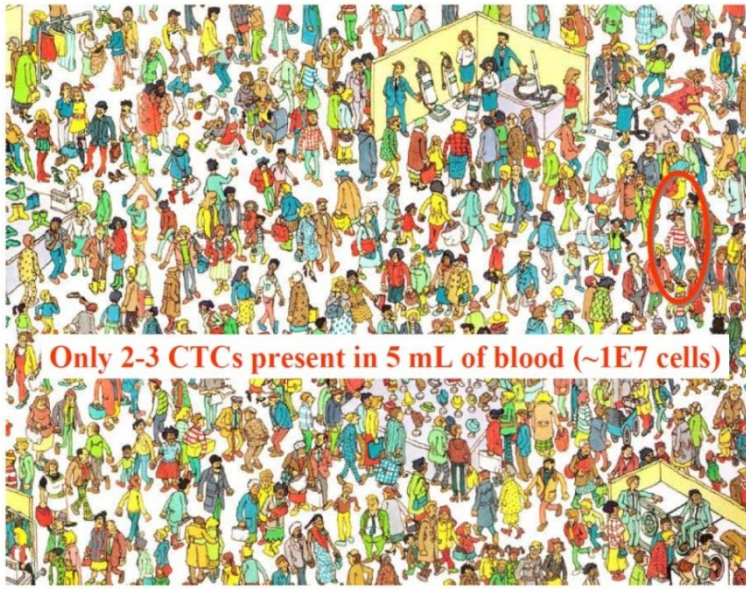
Theranostics, 2017, 8, 505-517
Gynecol. Oncol., 2017, 145, 361-365
Biomaterials, 2016, 106, 78-86
Biosen. Bioelectron.. 2016, 86, 921-926
Angew. Chem. Int. Ed, 2014, 25, 4597-602
Biomaterials, 2014, 35, 9573-9580

Circulating Tumor Cells are the Message of Metastasis



Technical Issues #1

1) **Extremely rare in the bloodstream**
   - 10^9 RBC / 1 mL Blood
   - 10^7 WBC / 1 mL Blood



10-100 cells per mL

Cell Type	CTC	Erythrocyte	Leukocyte
Size (μm)	12-25	5-7	7-15

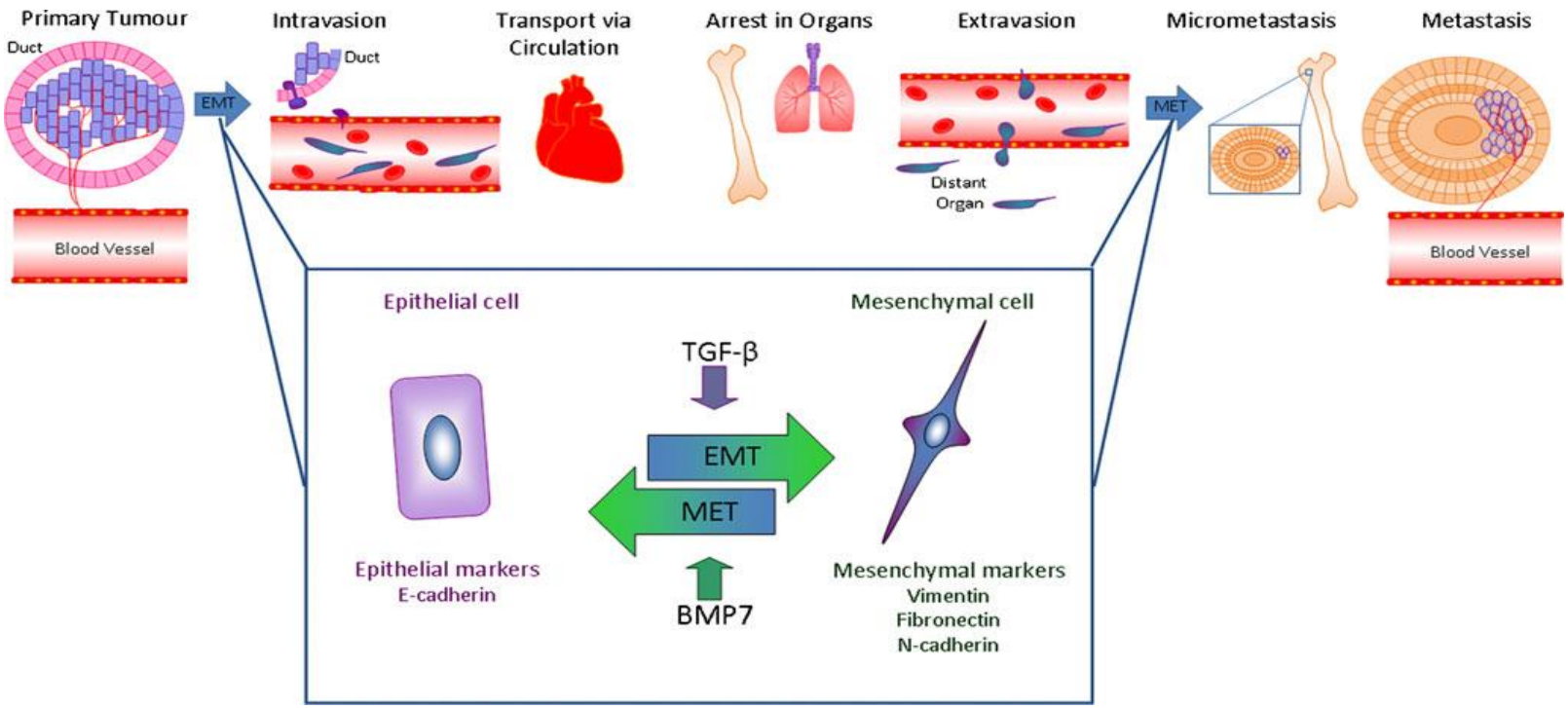
Cell Type	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Size (μm)	12-15	7-10	15-25	12-15	12-15
% of Pop	60-70	20-45	2-10	1-3	0.5-1

1,000,000 cells per mL

Technical Issues #2

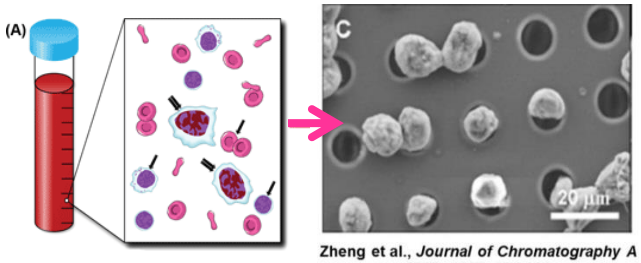
2) No known universal marker

- Epithelial cellular adhesion molecule (EpCAM) is most widely used in research but not present on 100% of CTCs
- Epithelial - mesenchymal transition (EMT)



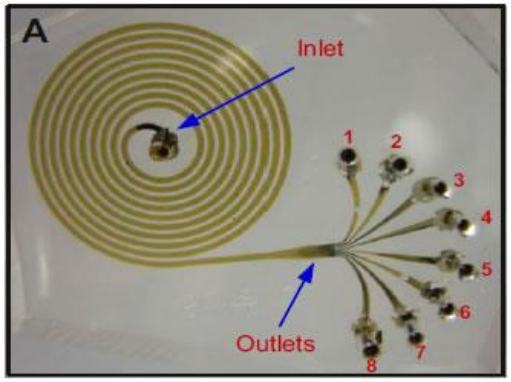
CTC Separation

By size

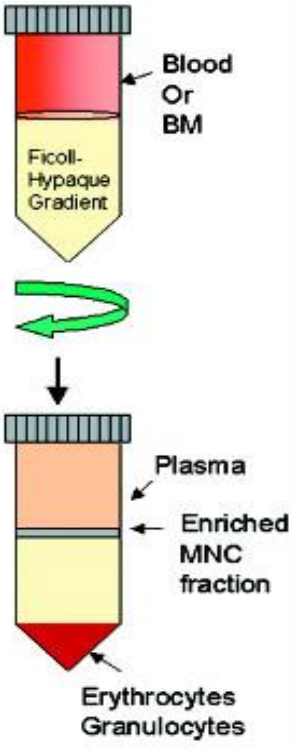


Zheng et al., *Journal of Chromatography A* 2007; 1162: 154-161

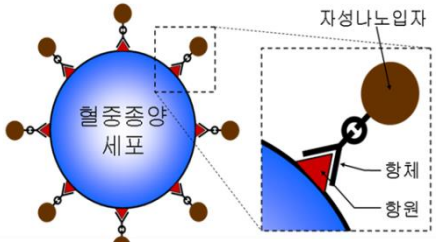
By microfluidics



By density

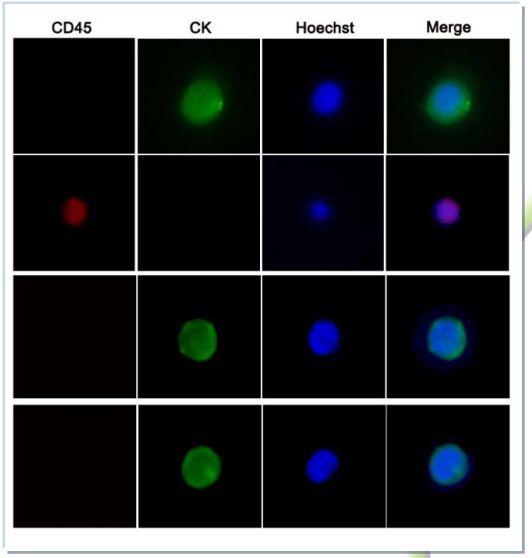


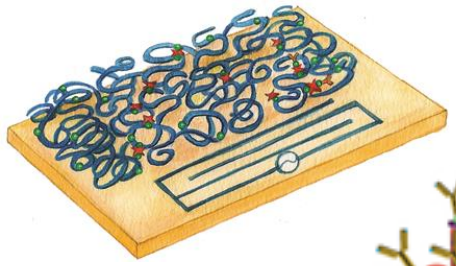
By immuno-magnetic separation (CellSearch)

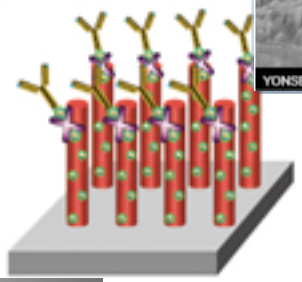


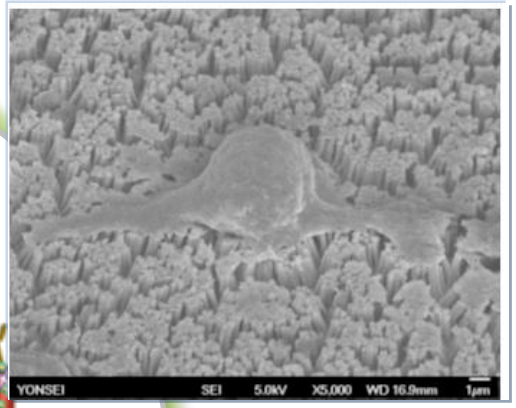
Extraction Efficiency and Purity

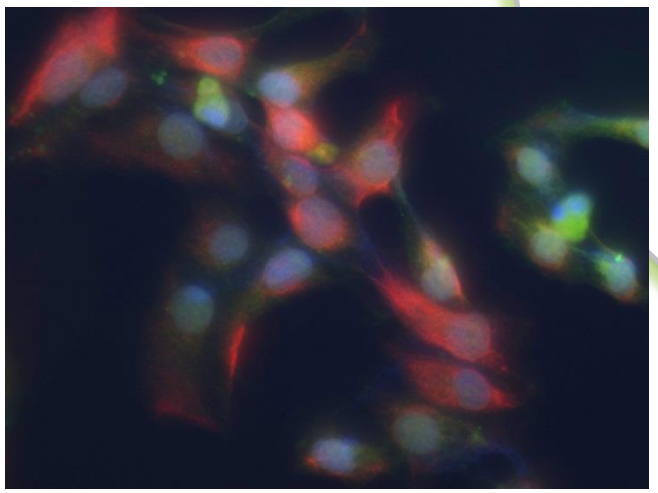
Circulating Tumor Cells: Nanochip & Microfludics

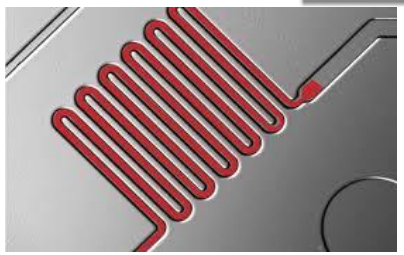


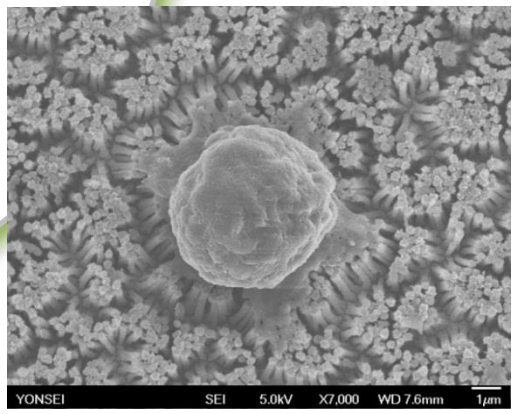




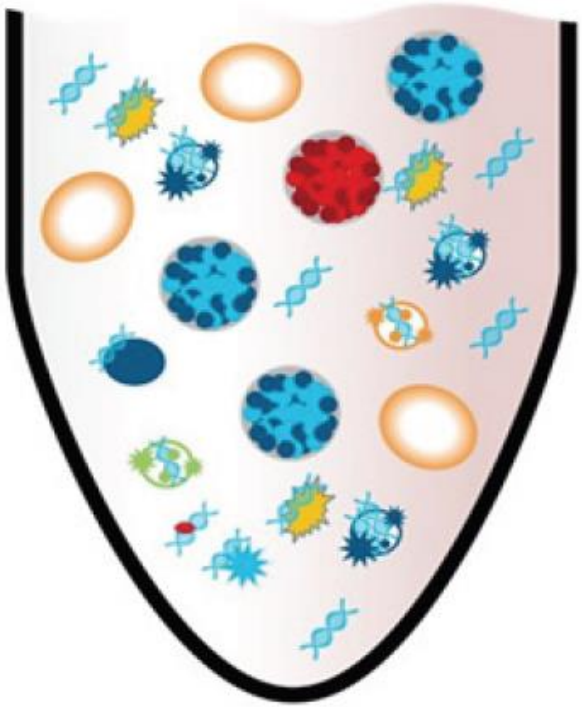






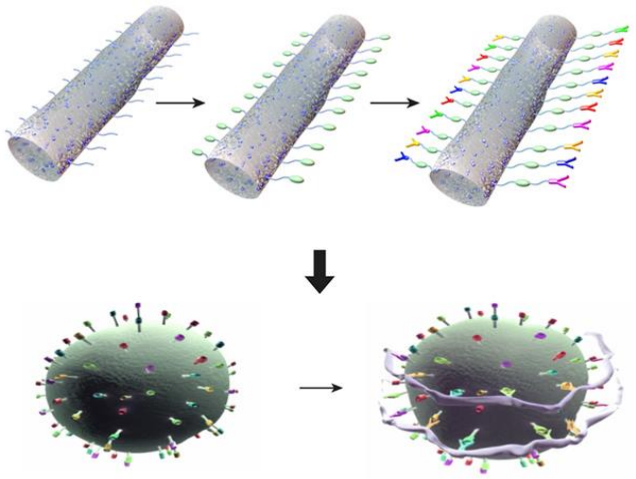


Magnetic Nanowires (MagWires)

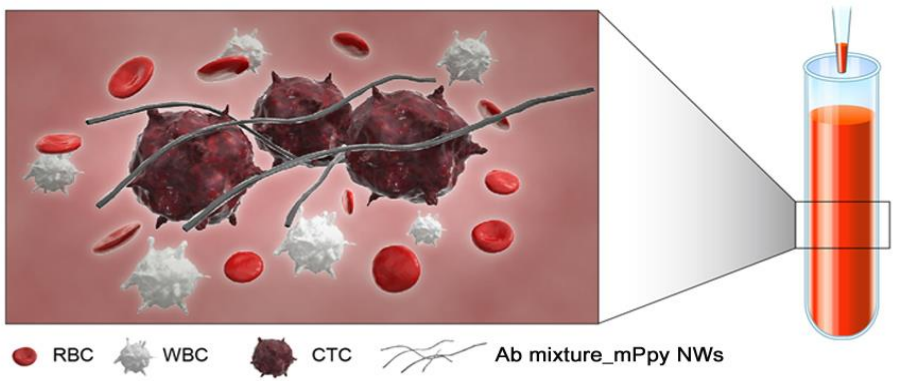


- Erythrocytes (~5×10e^{9}/mL blood)
- Leukocytes (~7×10e^{6}/mL blood)
- Circulating tumor cells (~0-10/mL blood)
- Thrombocytes (~3×10e^{8}/mL blood)
- Normal exosomes (~10e^{11}/mL blood)
- Tumor stroma exosomes (unknown)
- Tumor exosomes (~0-5×10e^{10}/mL blood*)
- Normal cfDNA (~5×10e^{9}/mL blood)
- Tumor cfDNA (~5×10e^{9}/mL blood)
- Ago2 associated miRNA (~5×10e^{9}/mL blood)
- HDL associated miRNA (~5×10e^{9}/mL blood)

Circulating Tumor Cells: Magnetic NWs

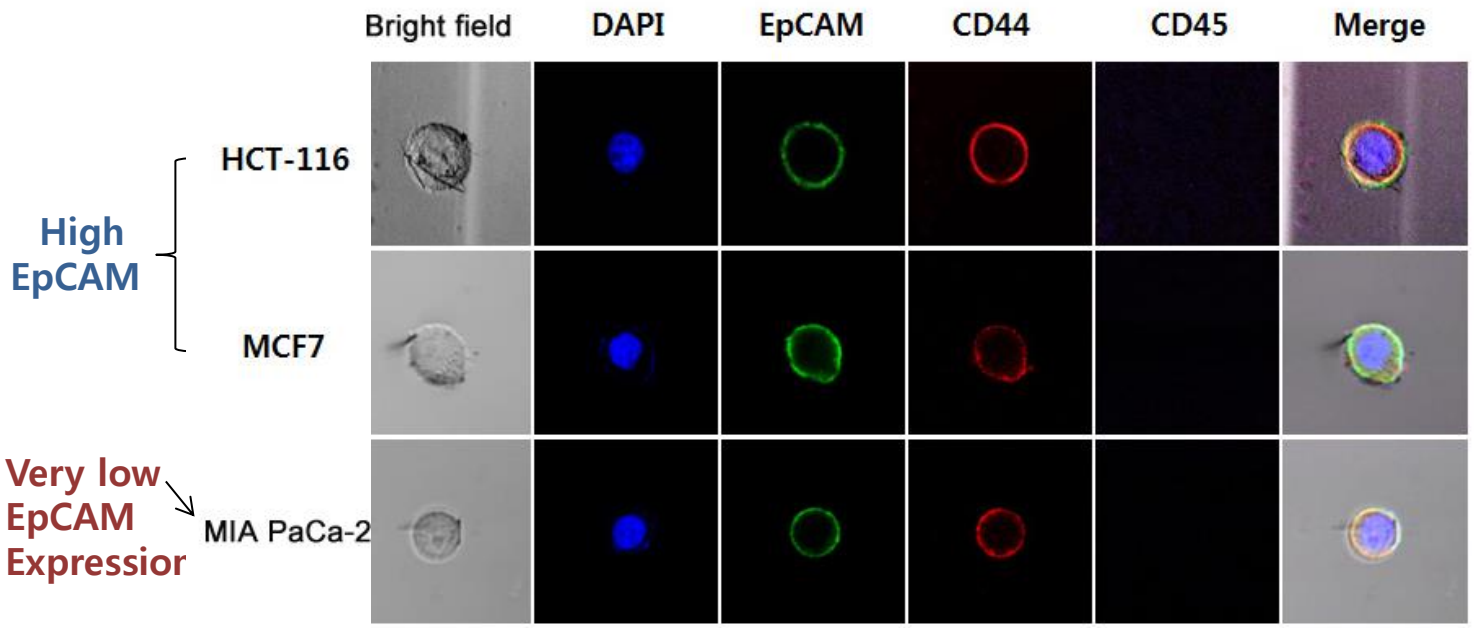


Five antibodies including epithelial / mesenchymal markers on the NW surface



Cell Recovery and Purity

: In vitro cancer cells were spiked into the blood

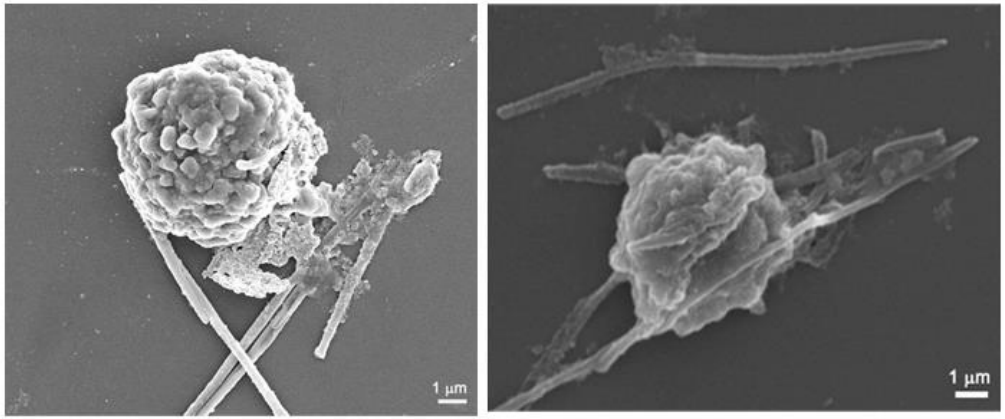


> 90% Purity Recovery

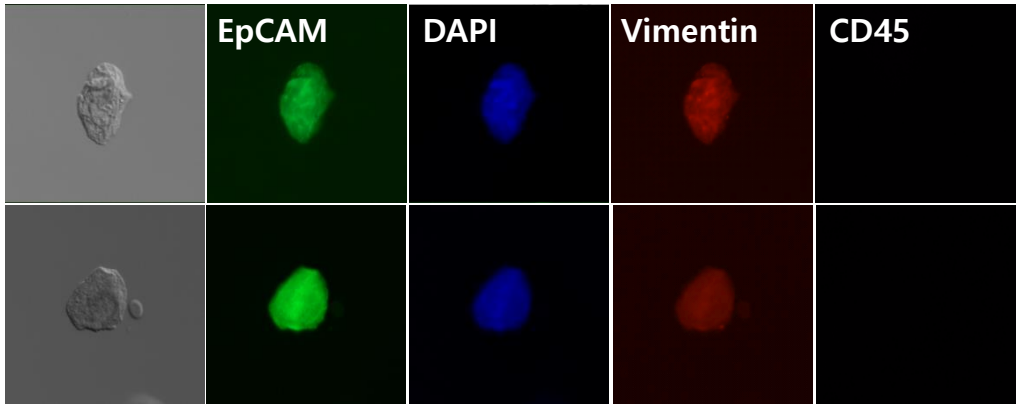
Five different types of antibodies on the nanowire ⇨ Higher isolation efficiency regardless of the EpCAM expression levels in tumor cells

Circulating Tumor Cells from Cancer Patients

SEM

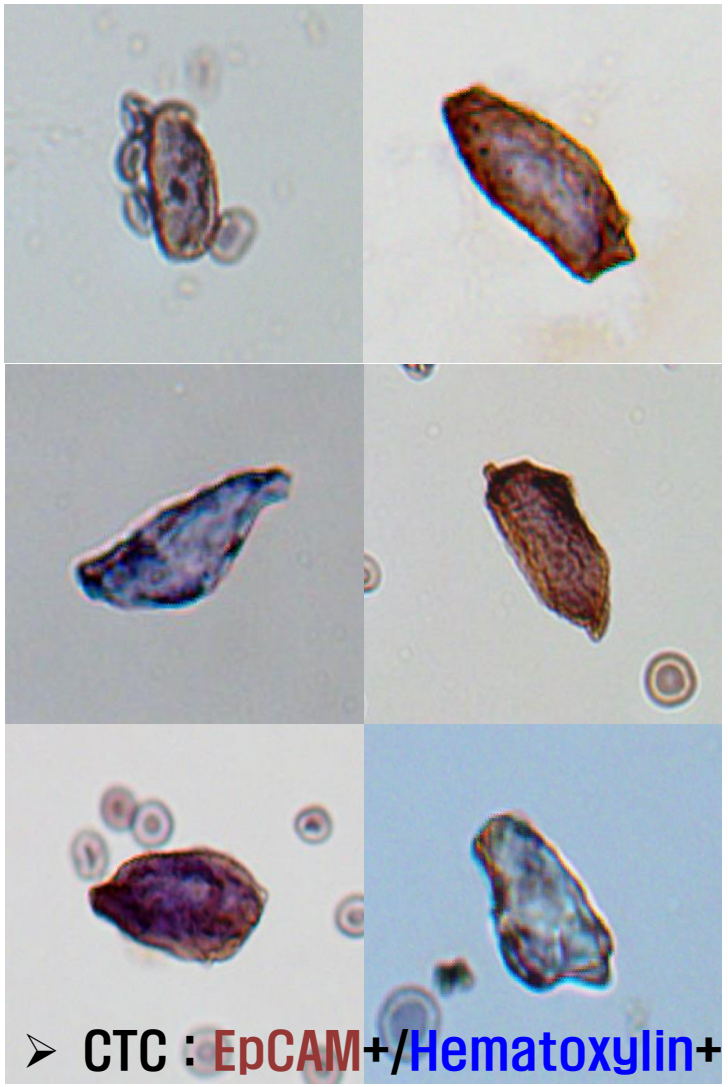


Immunofluorescence

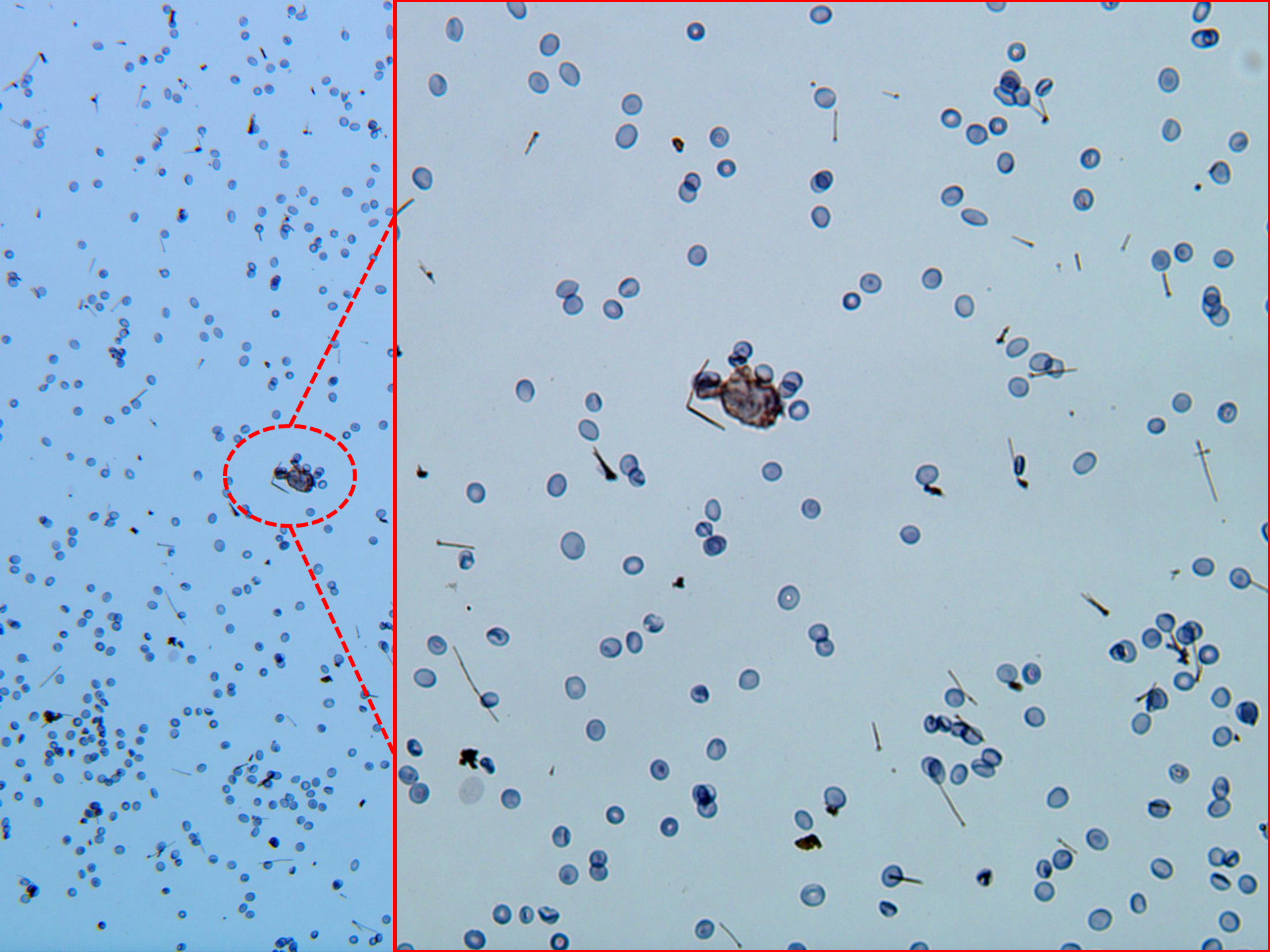


➢ CTC : EpCAM + / DAPI + / CD45 -

Immunohistochemistry

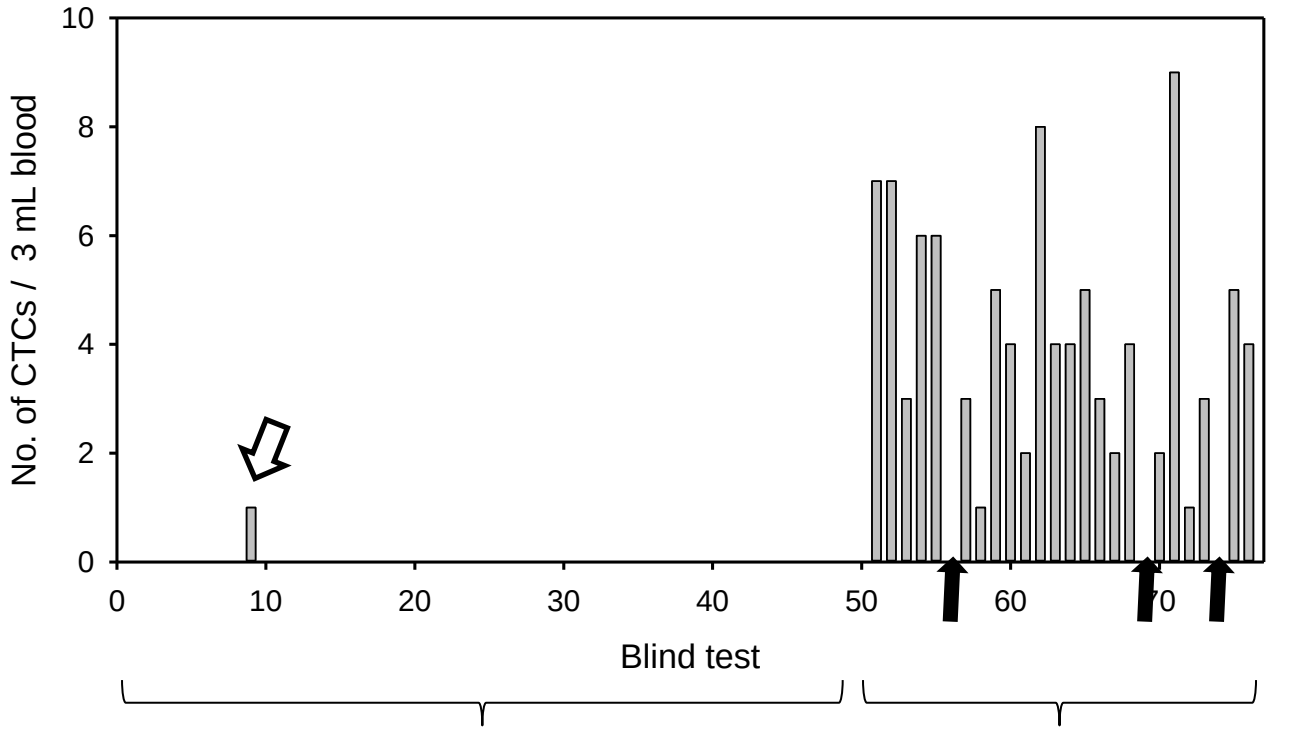


➢ CTC : EpCAM+/Hematoxylin+

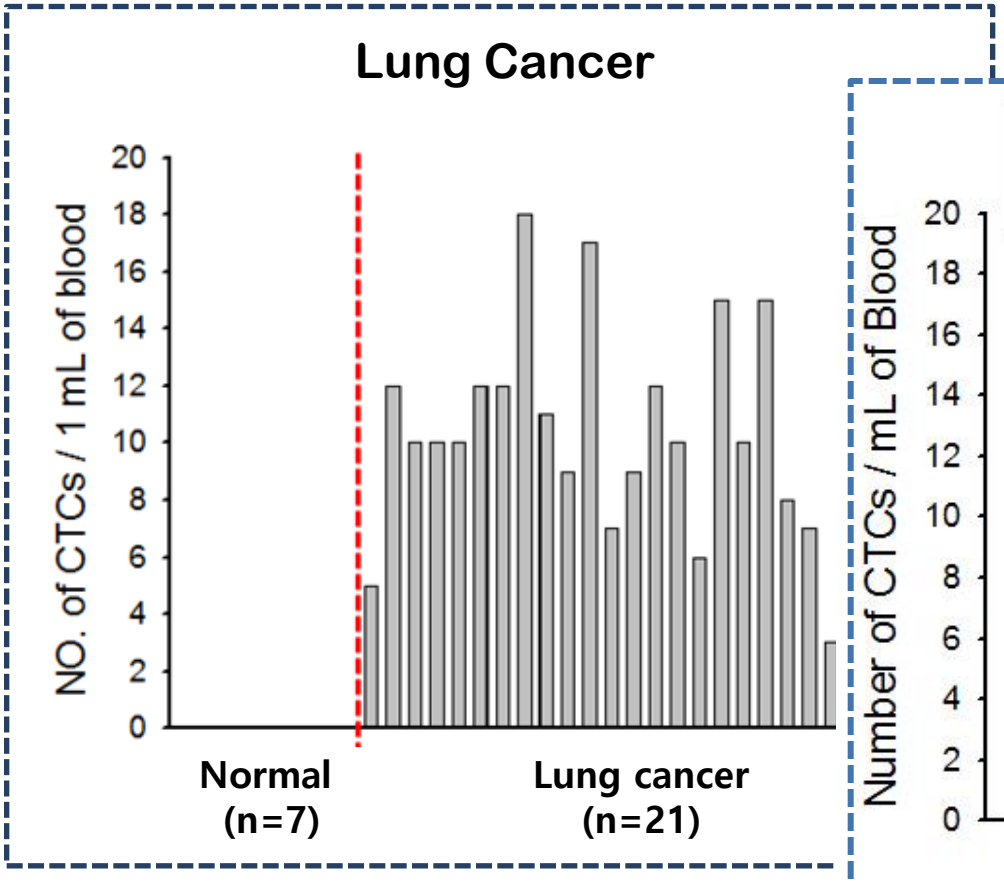


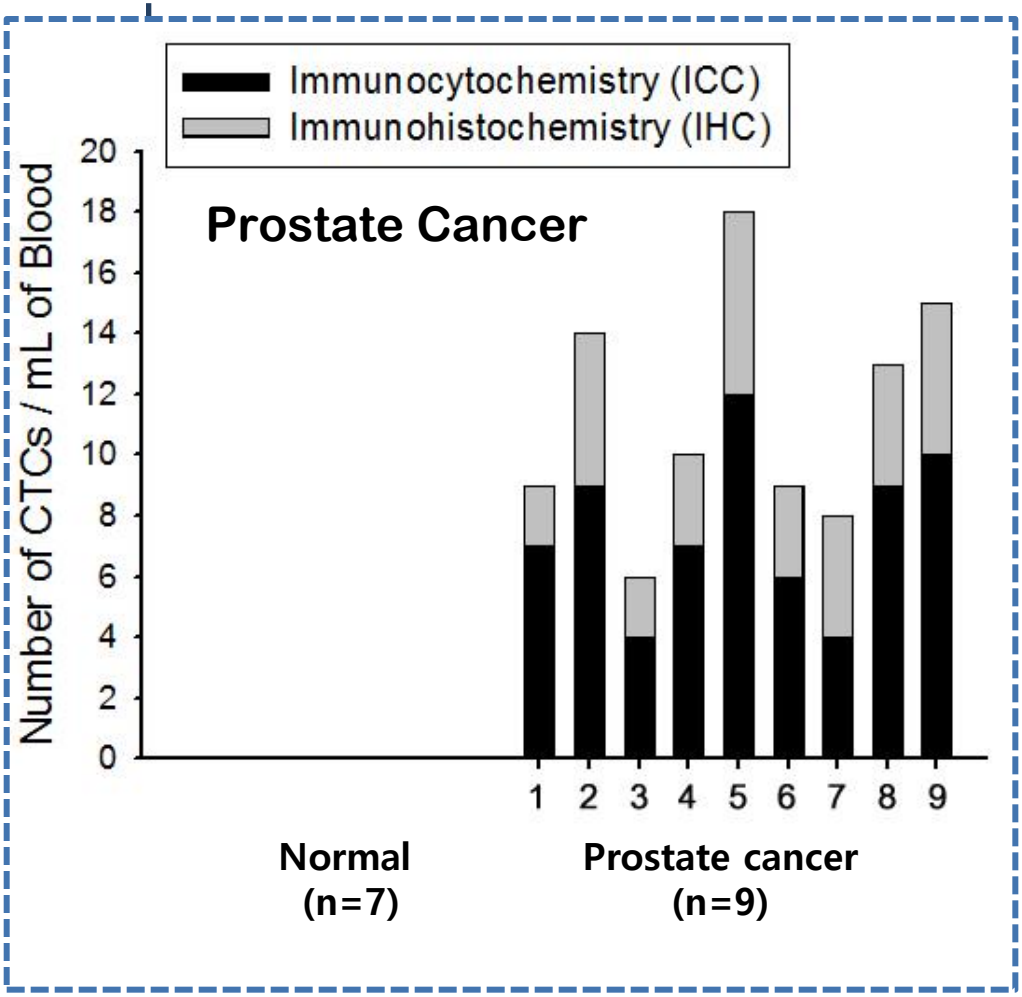
Circulating Tumor Cells_Blind Test

Early, non-metastatic Breast Cancer vs. Normal



Circulating Tumor Cells Test



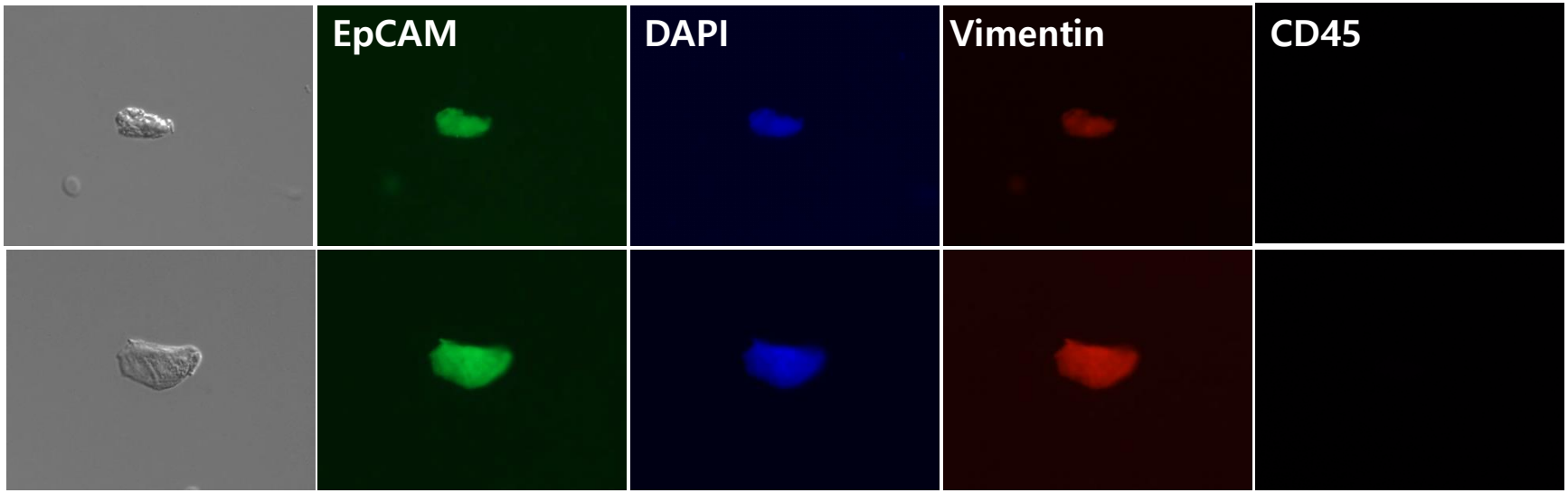


Lung Cancer Patient_Circulating Tumor Cells

Study No: 0185-001

The changes in CTC numbers before and after surgery in LC patients

Before surgery (6 CTCs / 3 mL blood)

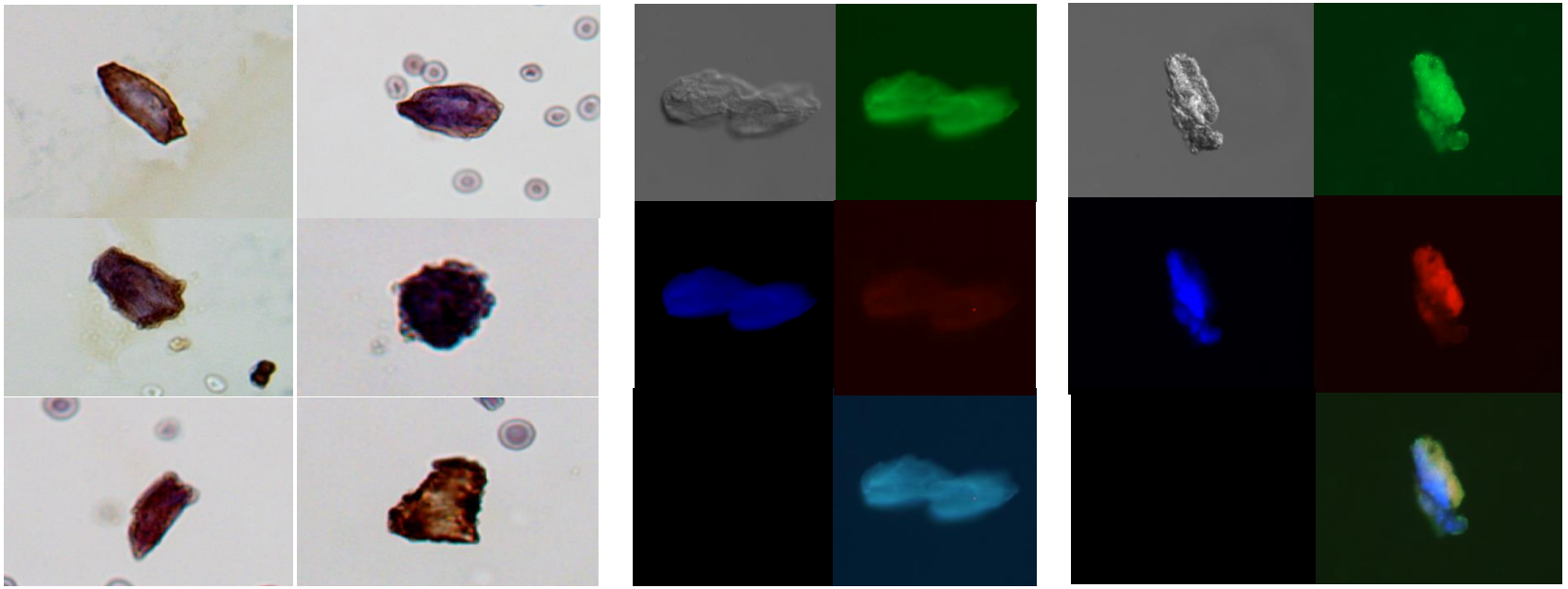


After surgery (0 CTCs / 3 mL blood)

Lung Cancer Patient_Circulating Tumor Cells

Study No: 0185-003

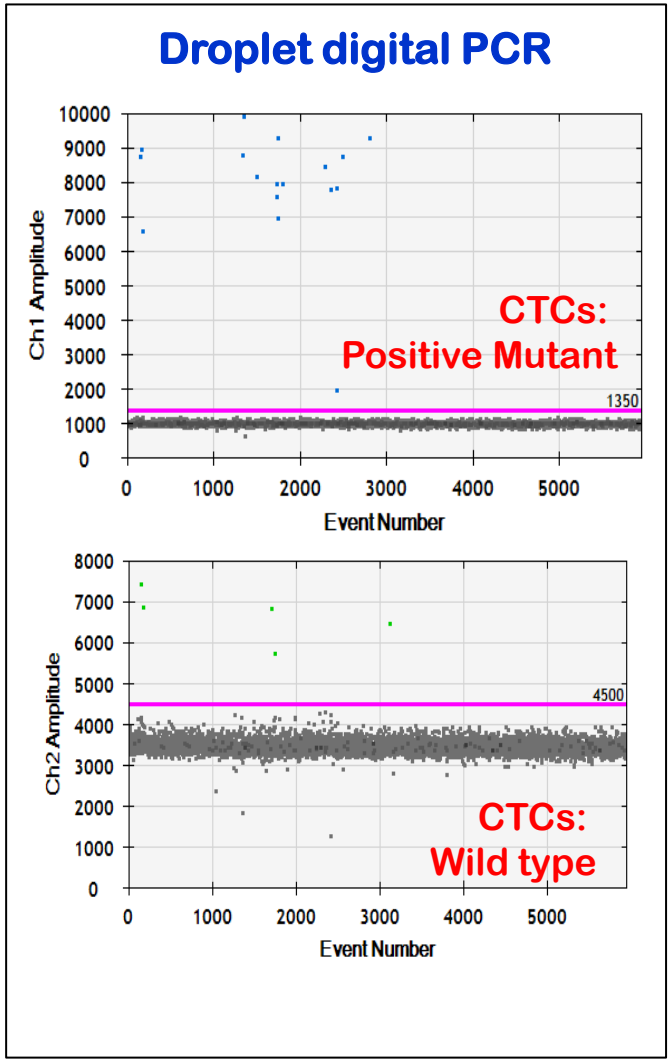
Before surgery (21 CTCs / 3 mL (3 clusters))



After surgery (9 CTCs / 3 mL (1 clusters))

The Assessment of *EGFR* Mutations by CTCs - Droplet Digital PCR

Sample ID	Stage	Primary tumor EGFR mutation	CTCs		
			No. of CTCs / mL	Mutation type	Mutant allele of CTCs, copies/Ml
NSCLC-1	IV	L858R	10	L858R	900
NSCLC-2	IV	Exon 19 Del	10	Exon 19 Del	280
NSCLC-3	IV	Exon 19 Del	12	–	0
NSCLC-4	IV	Exon 19 Del	12	–	0
NSCLC-5	IV	Exon 19 Del	18	Exon 19 Del	490
NSCLC-6	IV	L858R	11	–	0
NSCLC-7	IV	Exon 19 Del	17	–	0
NSCLC-8	IV	L858R	7	L858R	180
NSCLC-9	IV	Exon 19 Del	9	–	0
NSCLC-10	IV	L858R	12	–	0
NSCLC-11	IV	L858R	10	–	0



In four out of 11 patients, the same mutation type was confirmed in both CTCs and the primary tumors

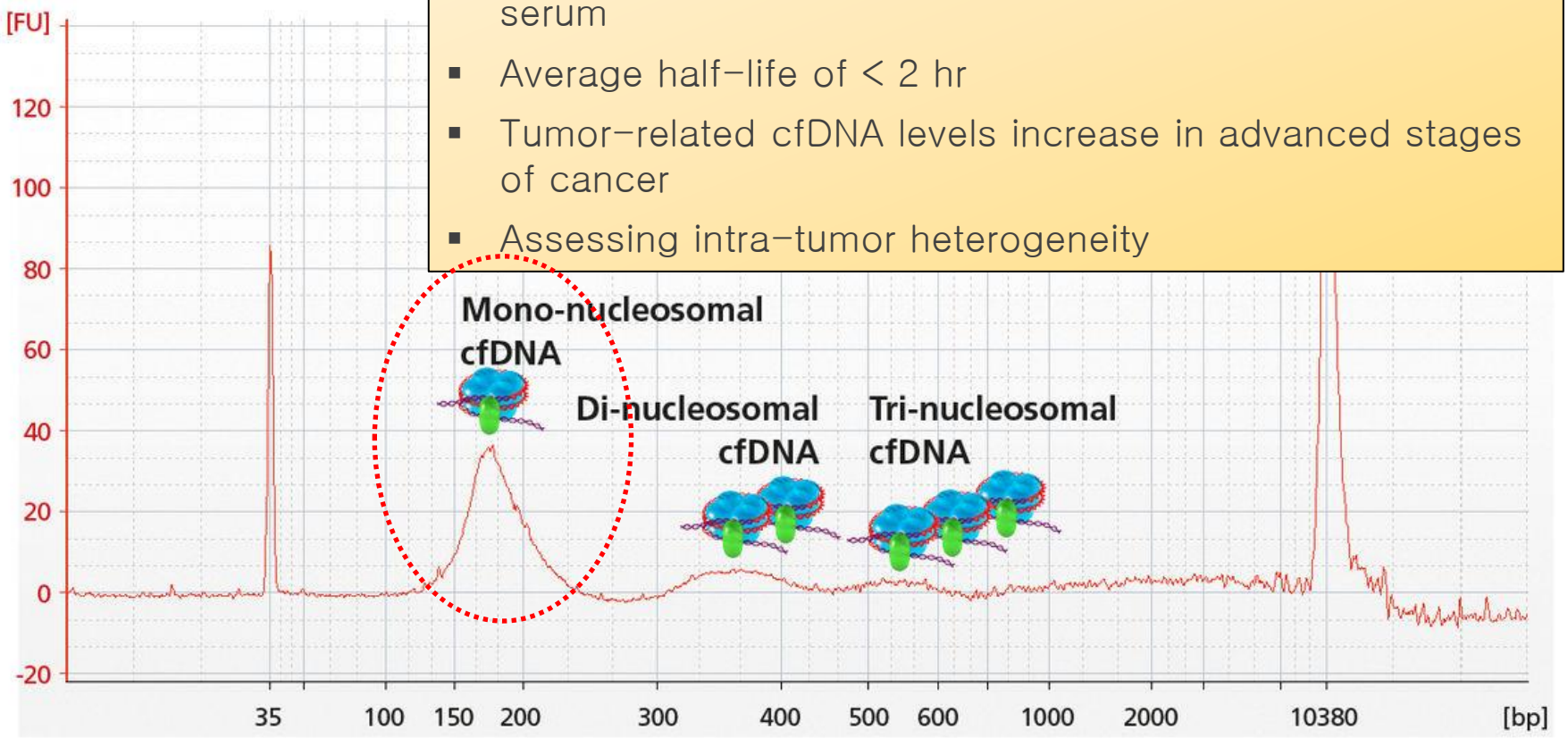
3. Circulating Tumor DNA from blood

Theranostics, 2017, 8, 399-409
Biomaterials, 2016, 101, 251-257
Theranostics, 2016, 6, 828-836
Biosen. Bioelectron., 2016, 106, 78-86
Theranostics, 2017, 8, 505-517
Biosen. Bioelectron.. 2016, 86, 864-

Circulating Cell-free DNA (cfDNA)

Tumor-related cfDNA:

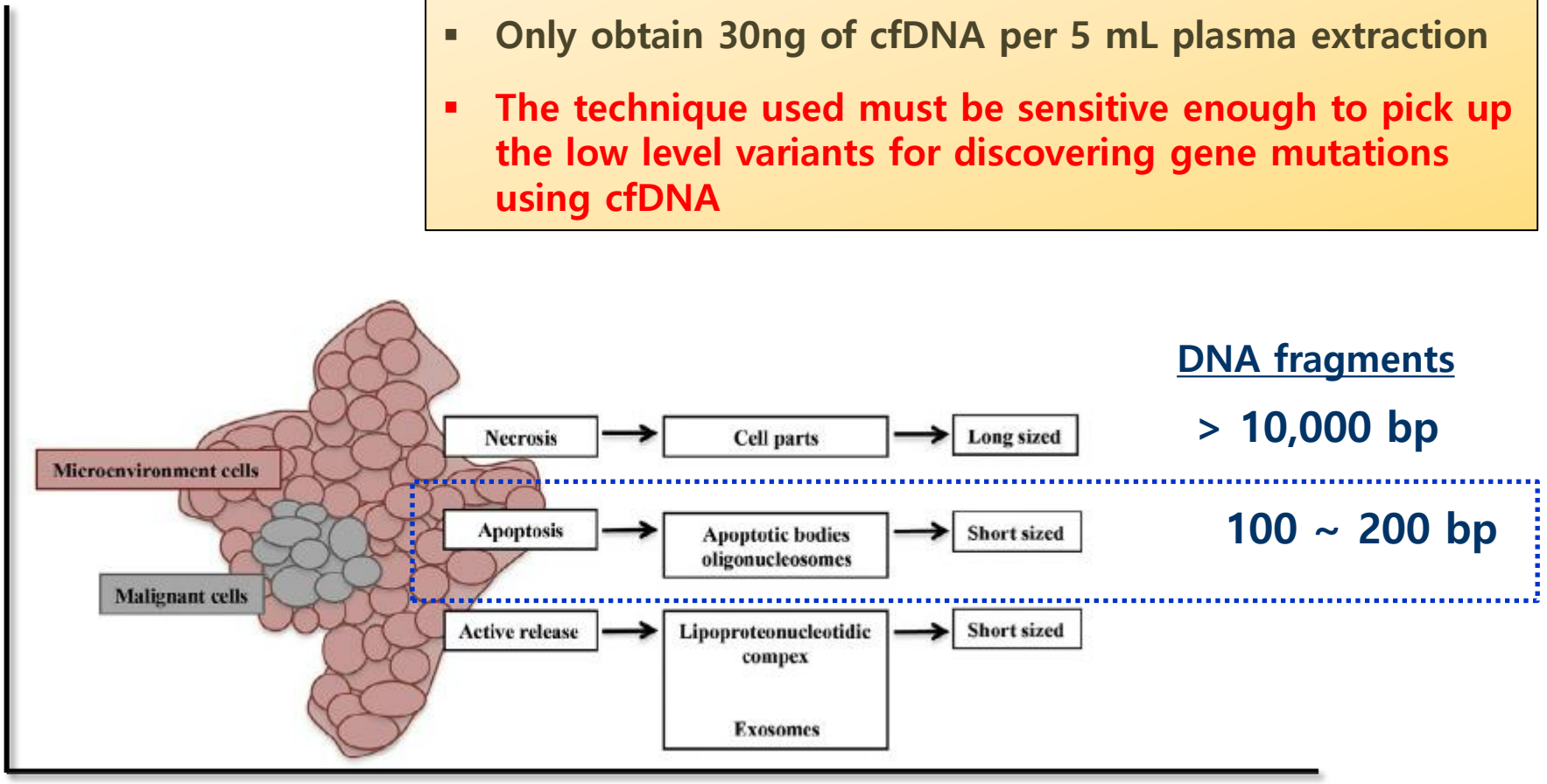
- Fragmented DNA (166 bp average length) in plasma or serum
- Average half-life of < 2 hr
- Tumor-related cfDNA levels increase in advanced stages of cancer
- Assessing intra-tumor heterogeneity



Schema of nucleosome organization. Stryer, Lubert . Biochemistry (fourth ed.). New York - Basingstoke: W. H. Freeman and Company.

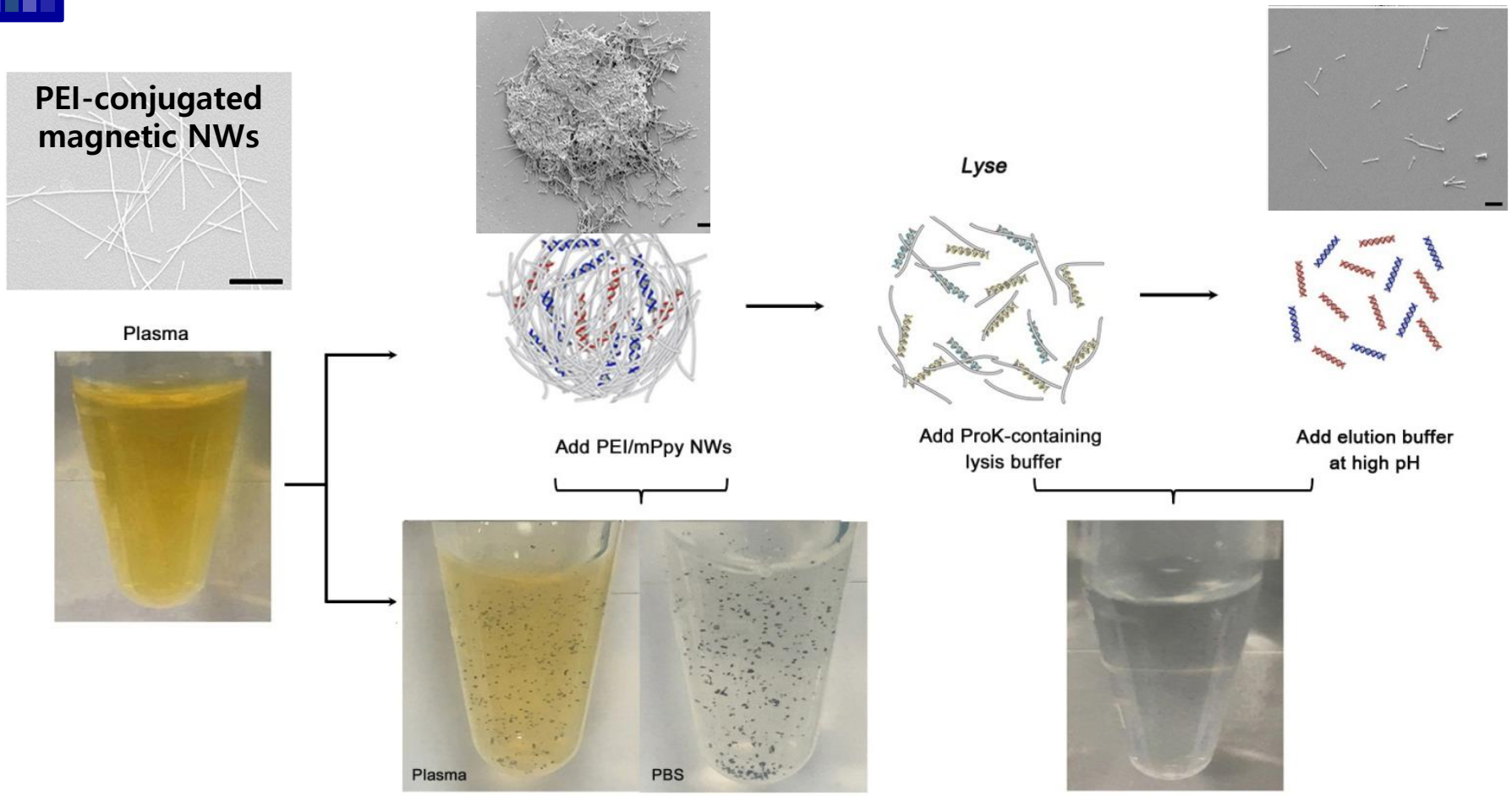
Problems with cfDNA

- **Most tumor-related cfDNA is released from the apoptotic cancer cells with small fragments**
- **Only obtain 30ng of cfDNA per 5 mL plasma extraction**
- **The technique used must be sensitive enough to pick up the low level variants for discovering gene mutations using cfDNA**



MOLECULAR ONCOLOGY 10 (2016) 481 e493

cfDNAs extraction Strategy : Magnetic nanowires



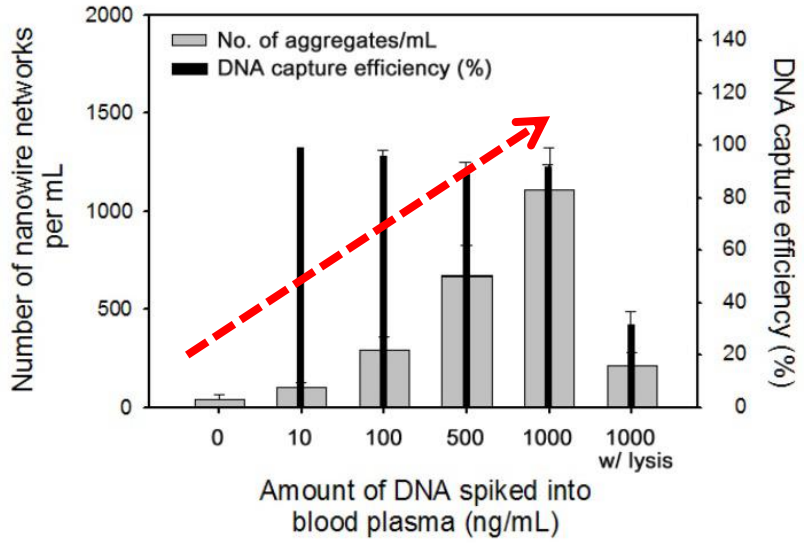
- DNA-NW aggregates increase the capture of cfDNA
- Proteinase K-containing lysis buffer enables the conversion of the aggregated structures into a homogeneous distribution of individual NWs to release cfDNA

cfDNAs extraction Strategy : Magnetic nanowires

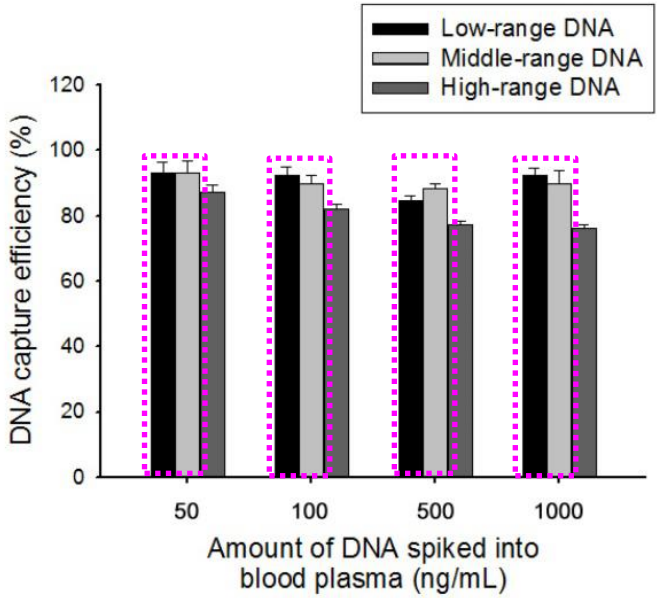
NW aggregates vs. cfDNA recovery yield

NW Capture Efficiency
(Tumor-derived cfDNAs : 100-200 bp)

A



B

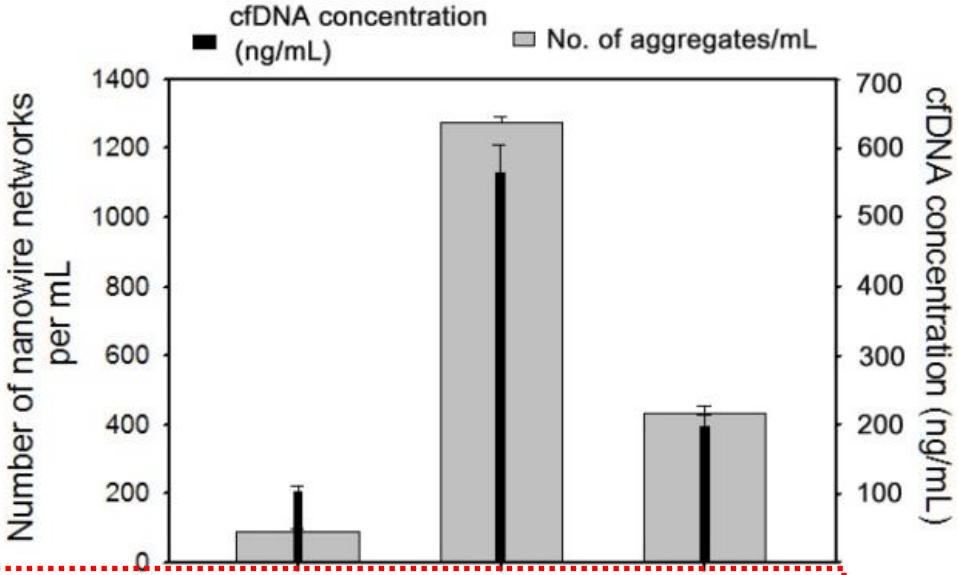


The mean number of DNA-NW aggregates is proportional to the DNA concentration

Low-range DNA : 10-100 bp
Middle-range DNA : 100-2000 bp
High-range DNA : >3.5 kb

cfDNAs extraction Strategy : Magnetic nanowires

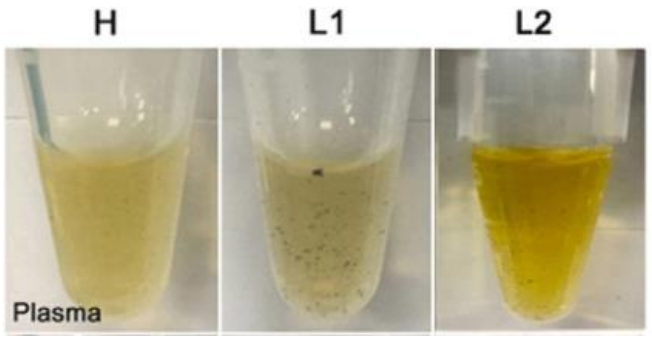
NW aggregates vs. cfDNA recovery yield vs. cfDNA in copies of EGFR mutation/μL



	Healthy	L1	L2
Mutant DNA (copies/μL)	0	4.3	2.8

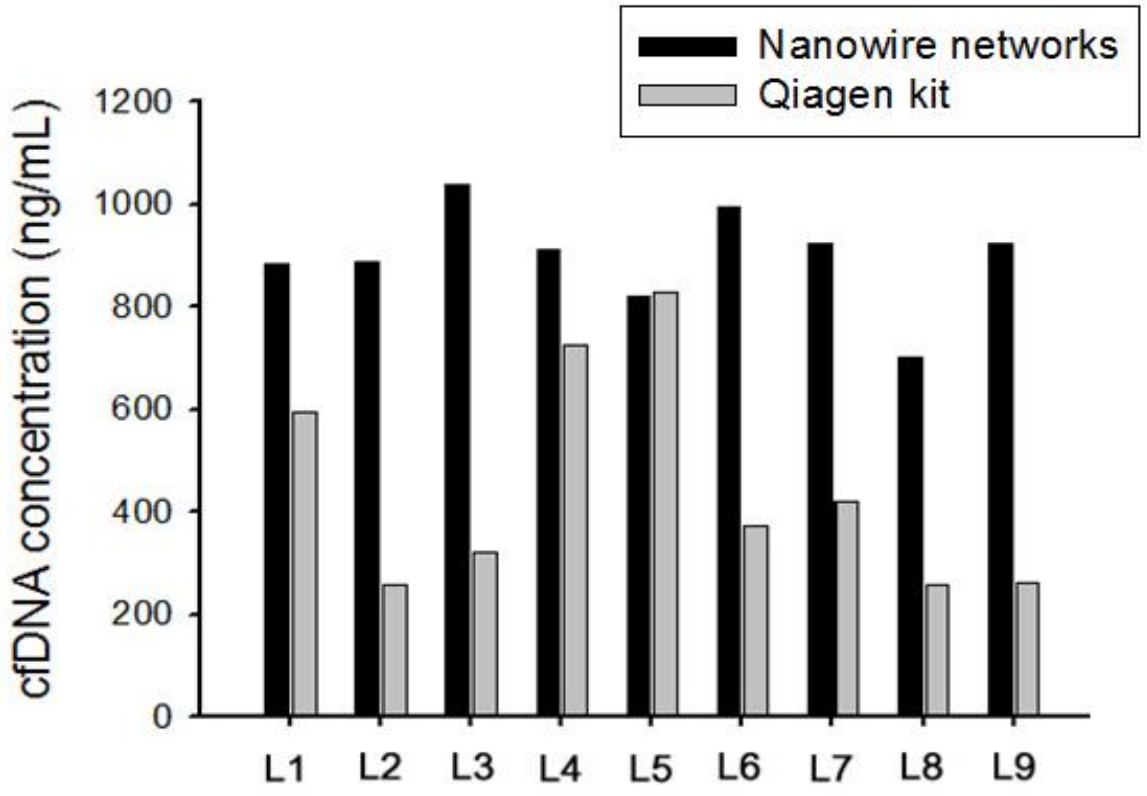
EGFR exon21 L858R (Tissue biopsy)

NW aggregates in plasma of healthy control (H) vs. patients with lung cancer (L1, L2).



NW aggregates enable highly efficient isolation of small-fragmented, tumor-related cfDNA

cfDNAs extraction Strategy : Magnetic nanowires

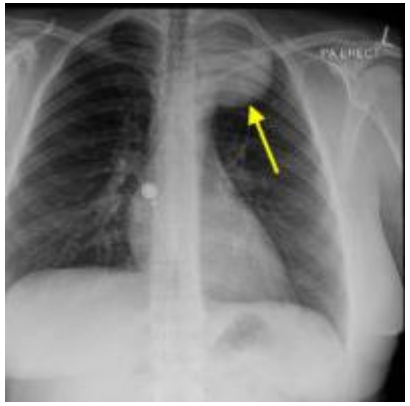


NWs efficiently bind extremely low levels of cfDNA in the plasma of lung cancer patients

Liquid biopsy

Answering questions about unmet medical need

- **By rapid biomarker assessment in cancer patients for whom solid biopsies are impossible due to restricted or extremely risky access possibilities**
- **By repeated monitoring during cancer patient follow-up to control treatment efficiency**
- **By detecting genomic alterations occurring as result of resistance to therapy**



Obtaining cancer-related biomarkers as much as possible

Acknowledgements

Present Group Members

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- Eunsook Jung
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  - Dr. Eunkyung Lee
- Bladder cancer team
  - Dr. Hokyung Seo

Thanks !