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Powering Future Oncotherapy

14th Anti-Tumor Drug Development Forum

A Novel CTC-Detecting Technique Using TelomeScan and Its Clinical Applications

Yasuo Urata
CEO and President
Oncolys BioPharma Inc.

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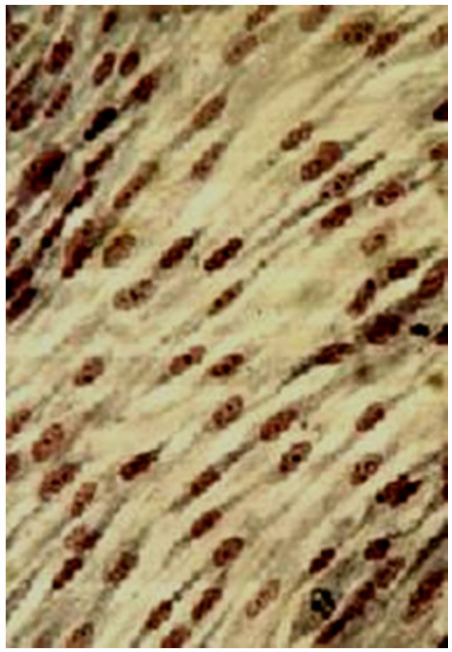
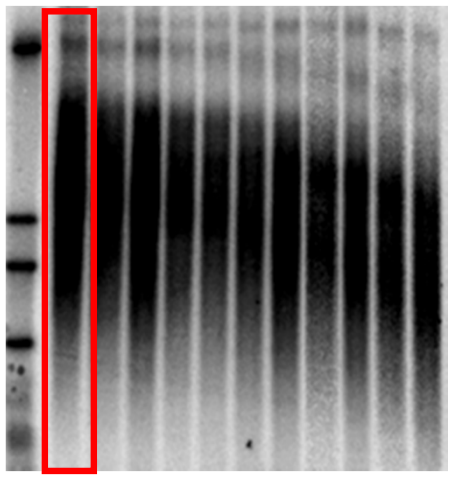
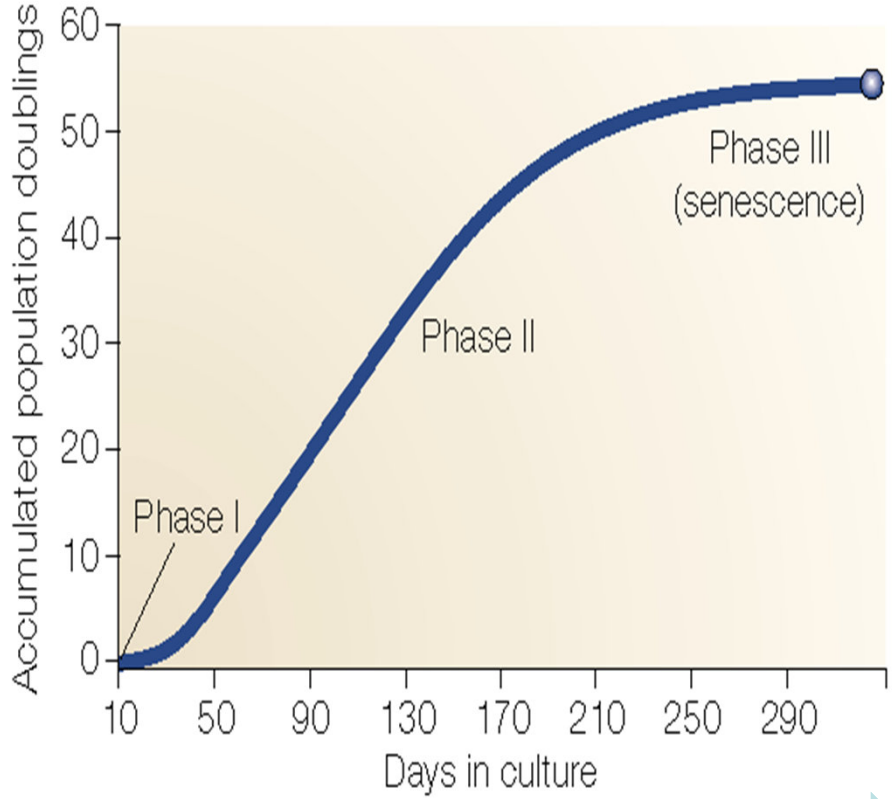
Telomere Length is a Limiting Factor for Cell Replication

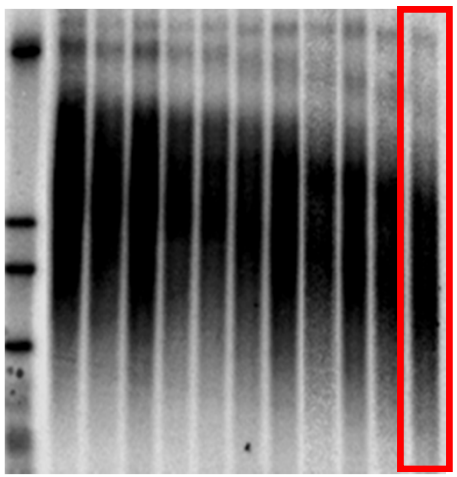




Cell senescence



Telomere shortening



Shay JW and Wright WE, *Nat Rev Mol Cell Biol.* **1**:72-6.

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Telomerase Activity in Cancer Cells and Biopsies

Cell lines

- Telomerase (+) in 98/100 immortal cell lines
- Telomerase (-) in 22/22 mortal cell lines

Biopsies

- Telomerase (+) in 90/101 human tumors
- Telomerase (-) in 50/50 human normal tissues

Telomerase is an universal marker in all types of human cancer

Tissue of origin	Cell type	Telomerase activity (no. positive/ no. tested)
Skin	Tumor	8/8
Skin	Normal	0/5
Connective	Tumor	1/1
Joint	Normal	0/1
Adipose	Tumor	1/1
Breast	Tumor	22/22
Breast	Normal	0/8
Lung	Tumor	18/18
Lung	Transformed	2/3
Lung	Normal	0/3
Stomach	Tumor	1/1
Pancreas	Tumor	3/3
Ovary	Tumor	5/5
Cervix	Tumor	3/3
Cervix	Normal	0/1
Uterus	Normal	0/1
Kidney	Tumor	8/8
Kidney	Transformed	1/1
Bladder	Tumor	3/3
Bladder	Normal	0/1
Colon	Tumor	7/7
Prostate	Tumor	2/2
Prostate	Transformed	0/1
Prostate	Normal	0/2
CNS	Tumor	3/3
Retina	Transformed	1/1
Blood	Tumor	9/9

Kim NW, et al. Science. (1994)

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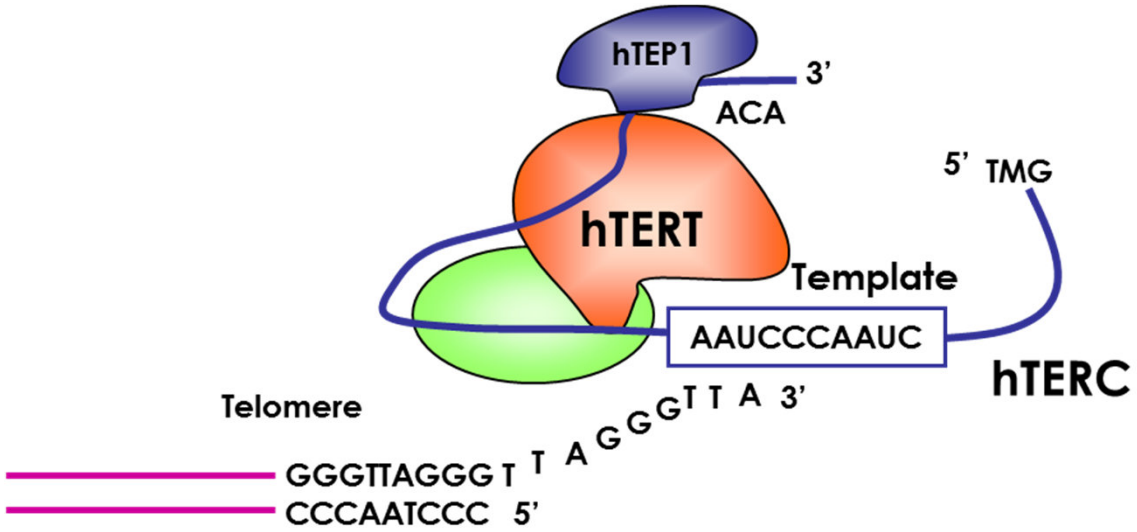
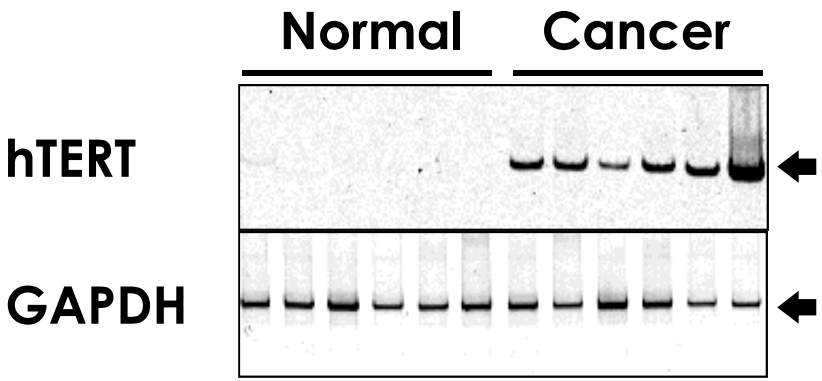
Telomerase and hTERT





Structure of Telomerase Complex

Human telomerase is a complex consisting of template RNA and enzyme subunits including hTERT, human telomerase reverse transcriptase.

Distribution of hTERT

hTERT is expressing in cancer and some normal stem cells but not in normal somatic cells.

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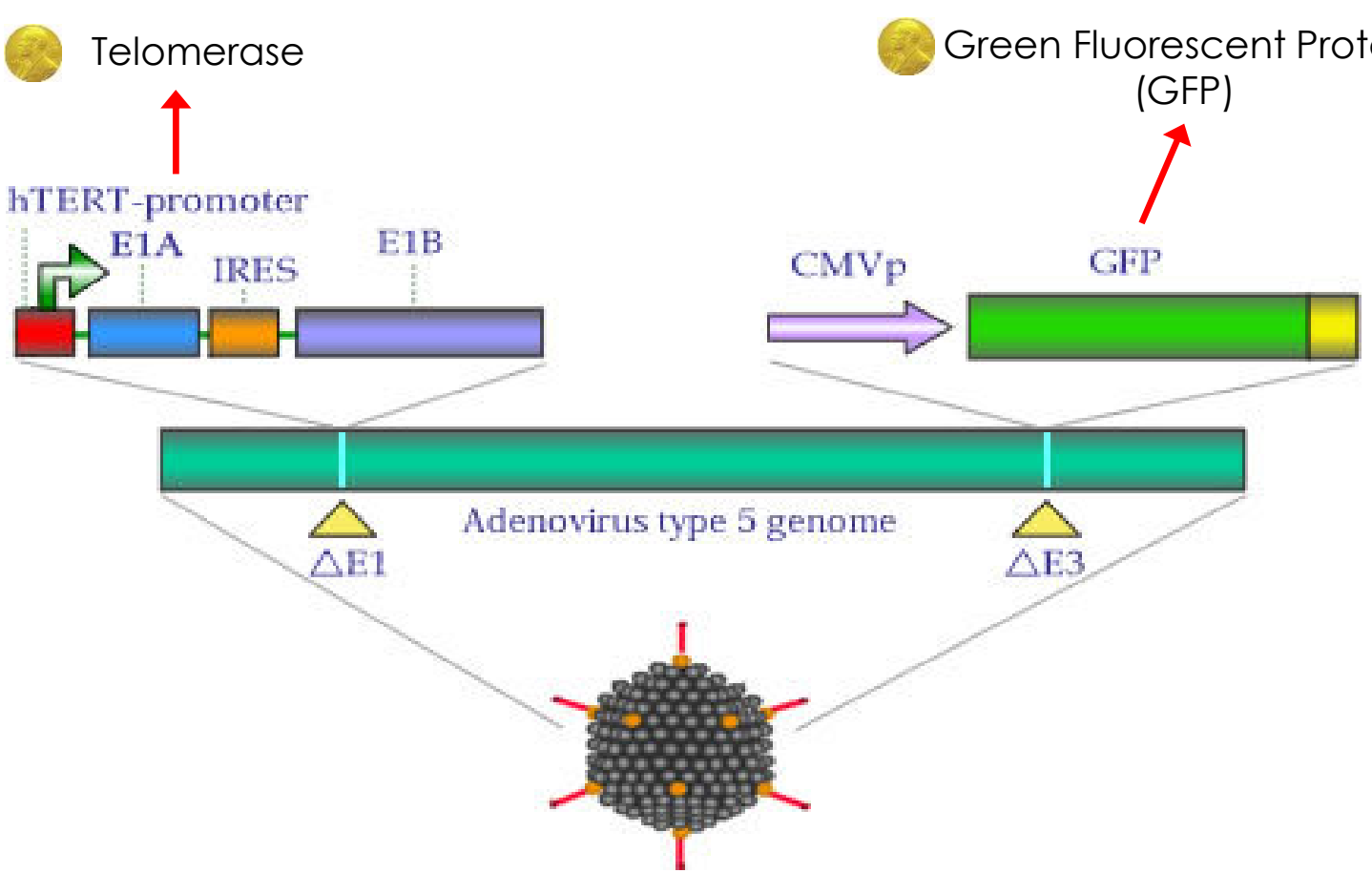
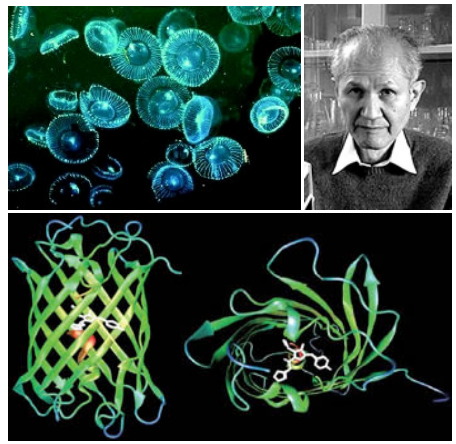
Structure of TelomeScan





TelomeScan is genetically engineered GFP gene-carrying adenovirus of which viral replication is under the control of hTERT promoter.

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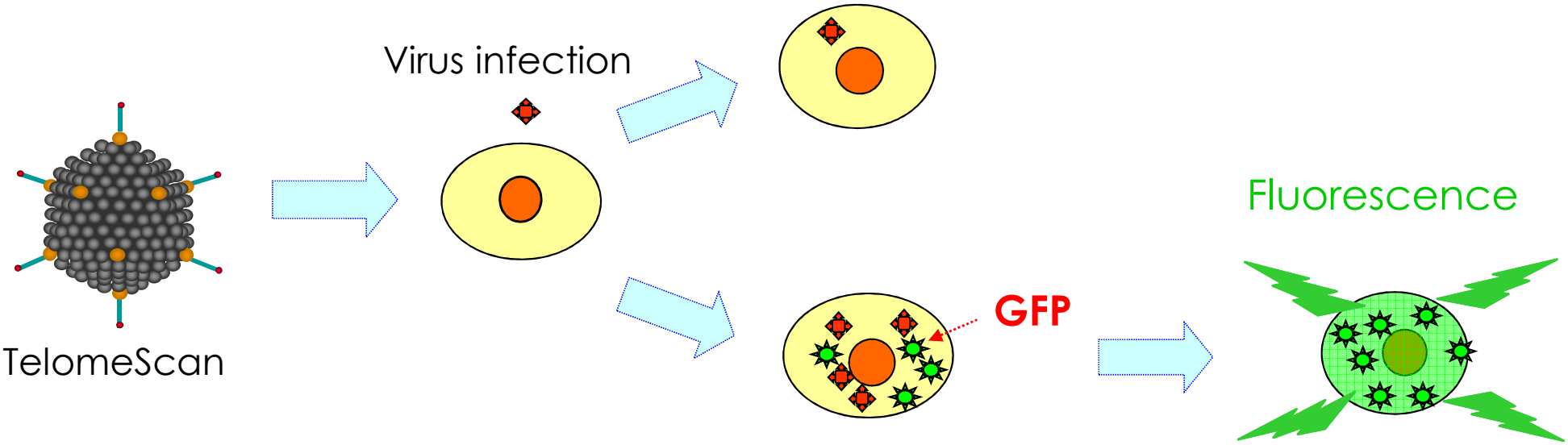
Fluorescent Detection of Cancer Cells by TelomeScan

Normal cells

(Telomerase negative)

No virus replication and GFP production



Cancer cells

(Telomerase positive)

Virus replication and GFP production

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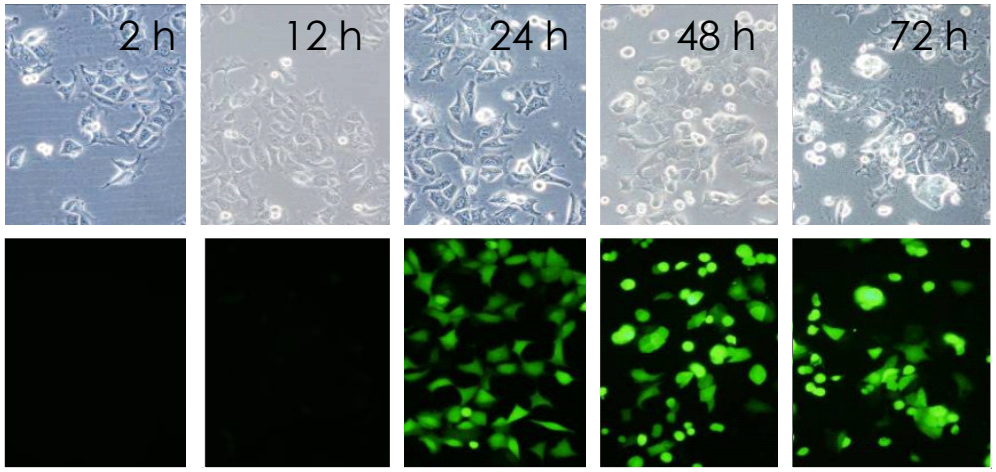
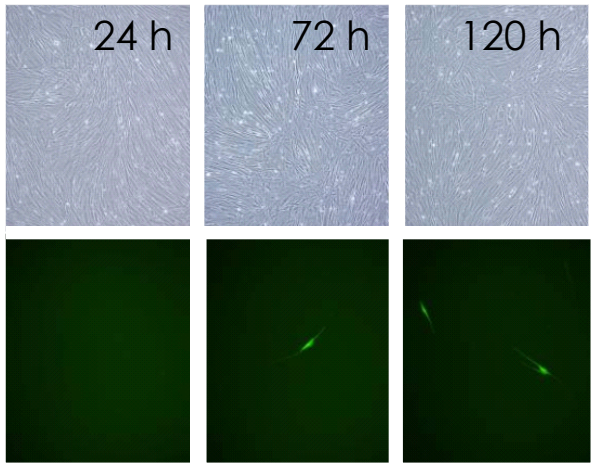
Cancer Specific GFP Expression by TelomeScan

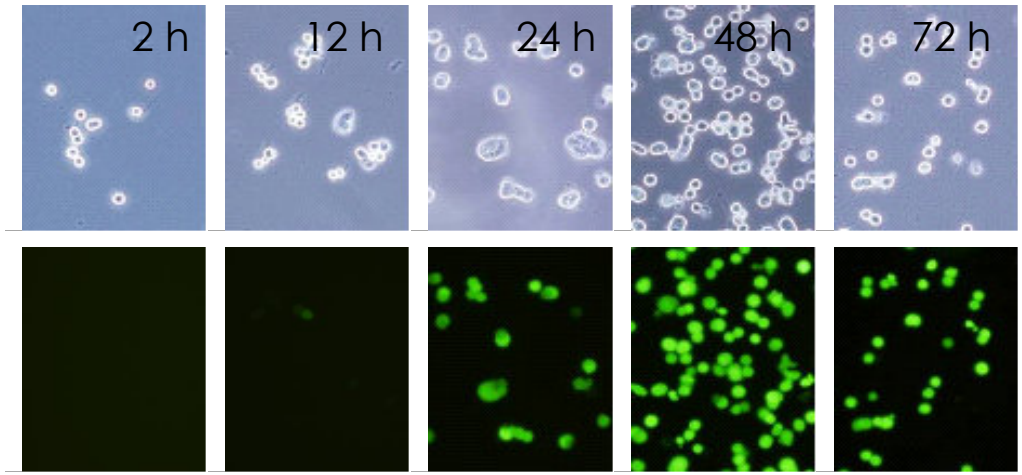
SW620; Human Colon Cancer Cells



NHLF; Normal Fibroblast



HT29; Human Colon Cancer Cells



Kishimoto et al., Nat Med. 12:1213-1219. (2006)

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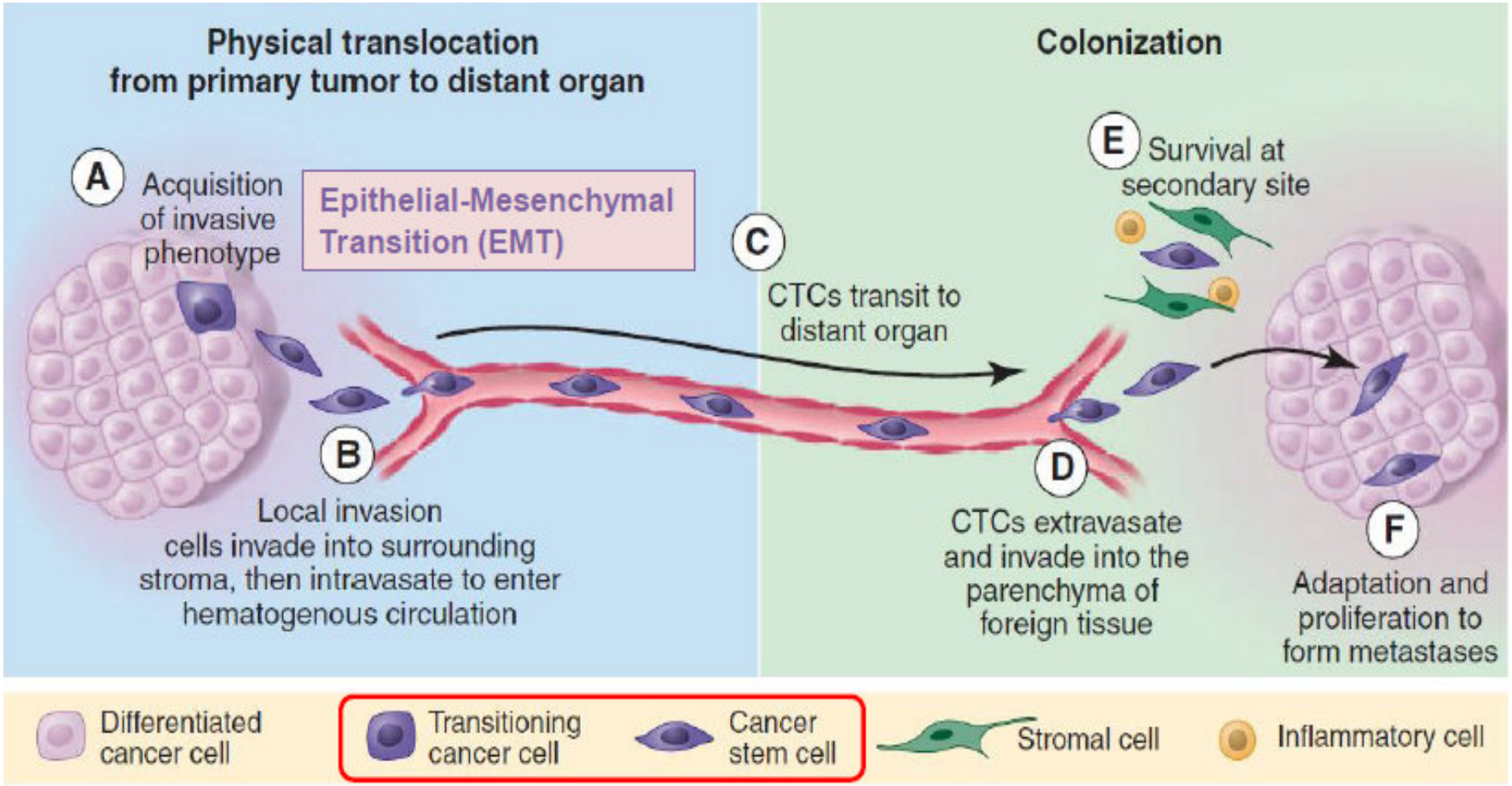
Tumor Metastasis and Circulating Tumor Cells (CTC)



Chaffer CL and Weinberg RA. Science, 331: 1559-1564, 2011.

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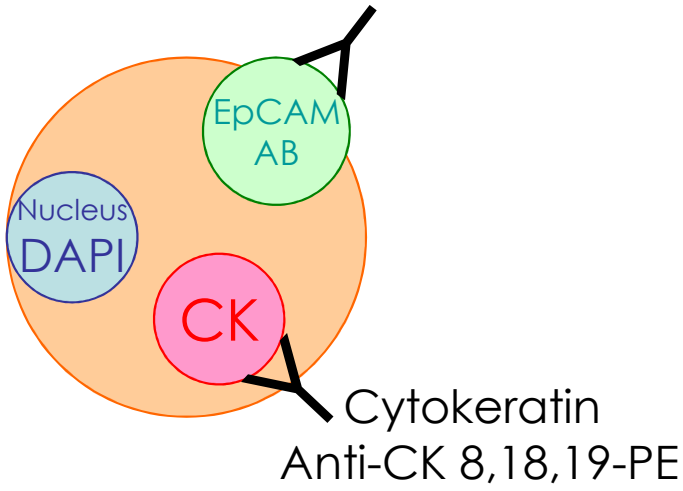
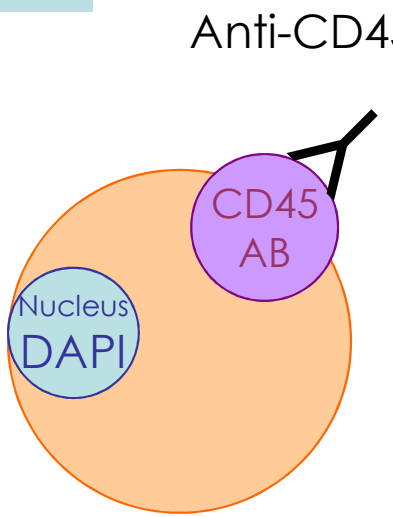
Veridex CellSearch System for CTC detection

CTC



WBC



CTC is separated by anti-EpCAM

WBC is removed by anti-CD45

The FDA-approved Veridex LLC CellSearch Circulating Tumor Cell Kit is intended for the enumeration of circulating tumor cells (CTC) of epithelia origin (CD45-, EpCAM+, and cytokeratins8, 18+, and/or 19+) in peripheral blood samples.

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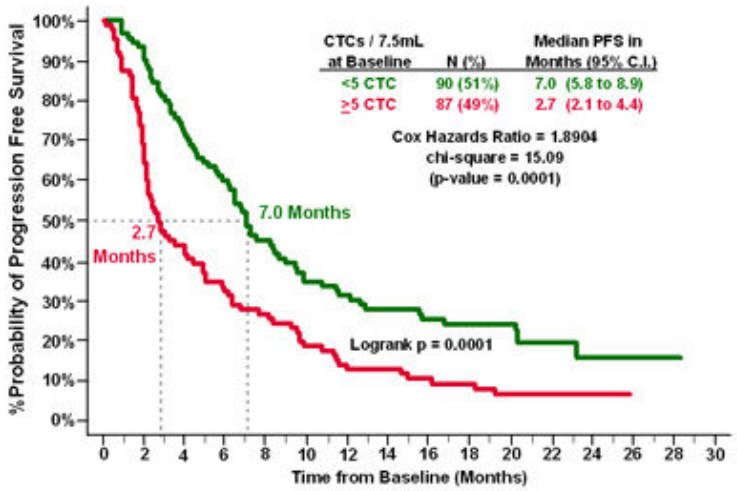
Correlation of Prognosis and CTC number detected by CellSearch

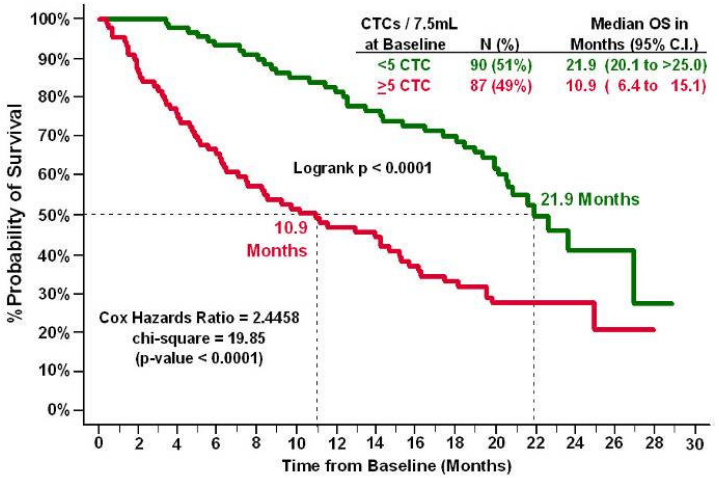
- The assay predicts progression-free survival (PFS) and overall survival (OS) in patients treated for metastatic breast, colon and prostate cancer.
- A CTC count of 5 cells / 7.5mL blood or greater as determined by the assay is predictive of shorter PFS and OS for patients with metastatic breast and/or prostate cancer.
- A CTC count of 5 cells / 7.5mL blood or greater is predictive of shorter PFS and OS for patients with metastatic colon cancer.

Predictive Value: PFS of Patients with <5 or ≥5 CTC at Baseline (N=177)



N Engl J Med 351:781, 2004

Predictive Value: OS of Patients with < 5 or ≥ 5 CTC at Baseline (N=177)



N Engl J Med 351:781, 2004

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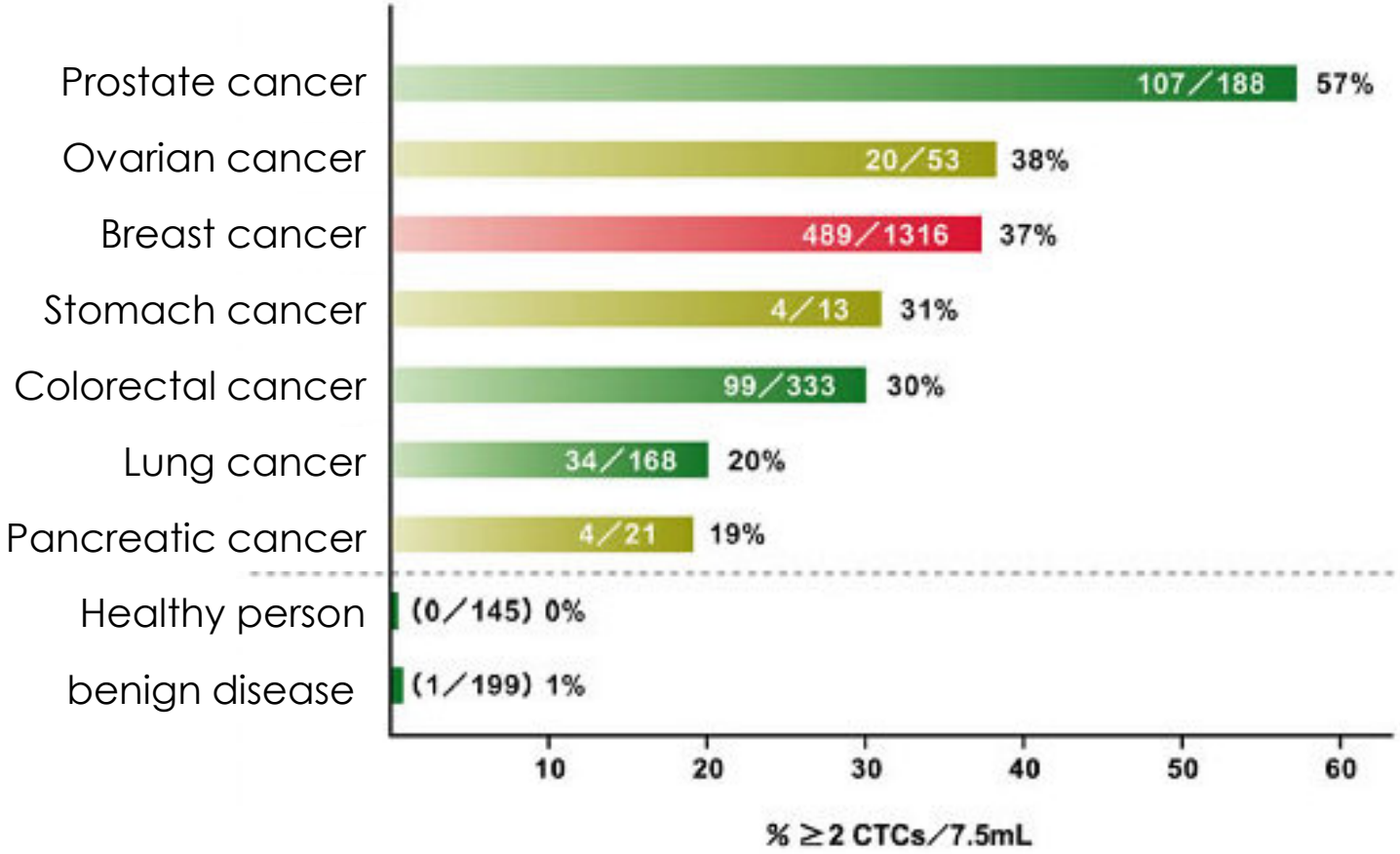
Restricted Sensitivity of CellSearch

CTC detection rate of CellSearch™ system in various metastatic cancer patients



- Measured range of CTC number (mean ± S.D.); 0~23,618 cells/7.5ml (60 ± 693 cells)
- Sample ratio that had more than 2 cells/7.5ml (Ratio); Mean 36%
- Sample ratio that had more than 5 cells/7.5ml (Ratio); Mean 24%

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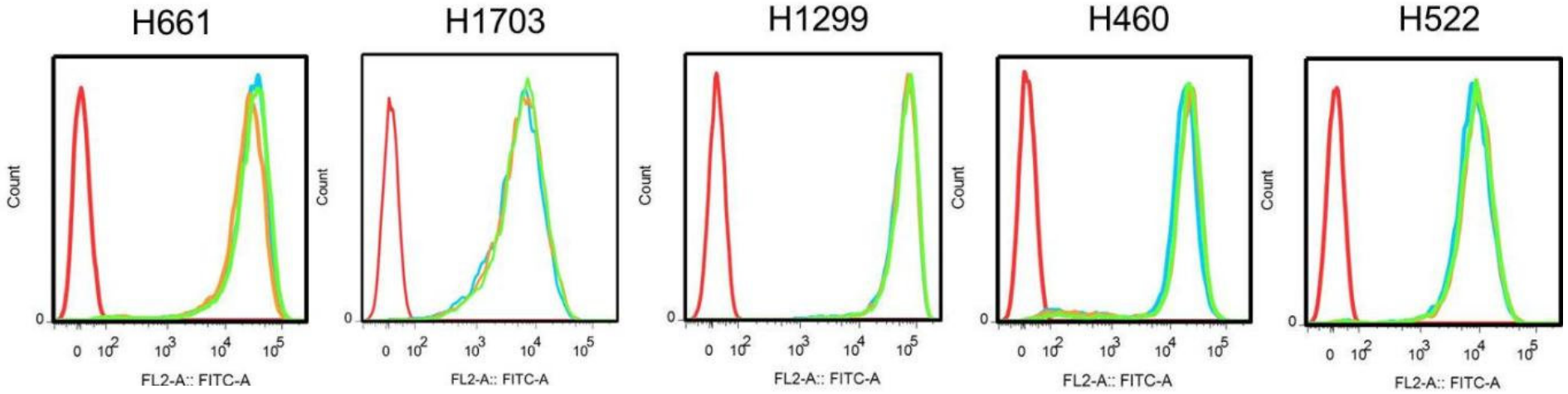
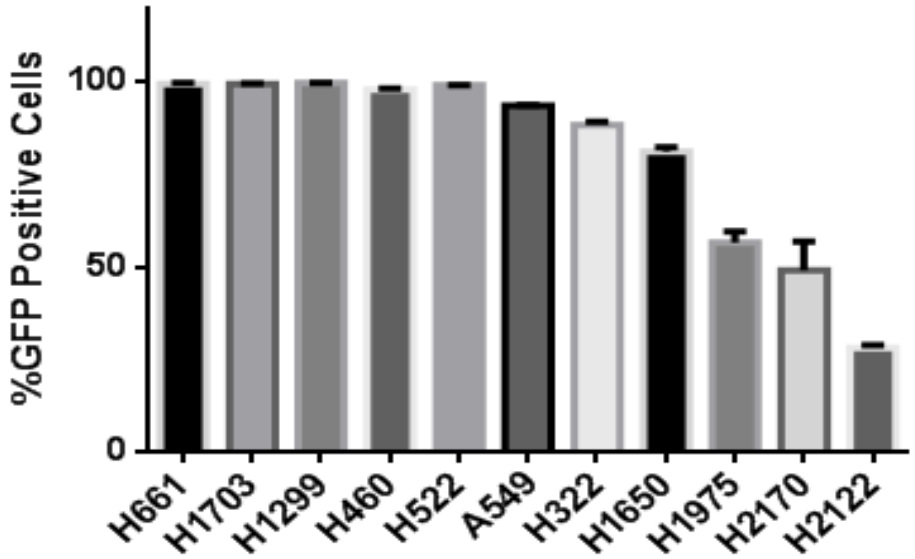
Sensitivity of TelomeScan in NSCLC Cell Lines





- Quantitative measurement of GFP expression by FACS
- TelomeScan is feasible to detect CTCs in Lung Cancer Patients.

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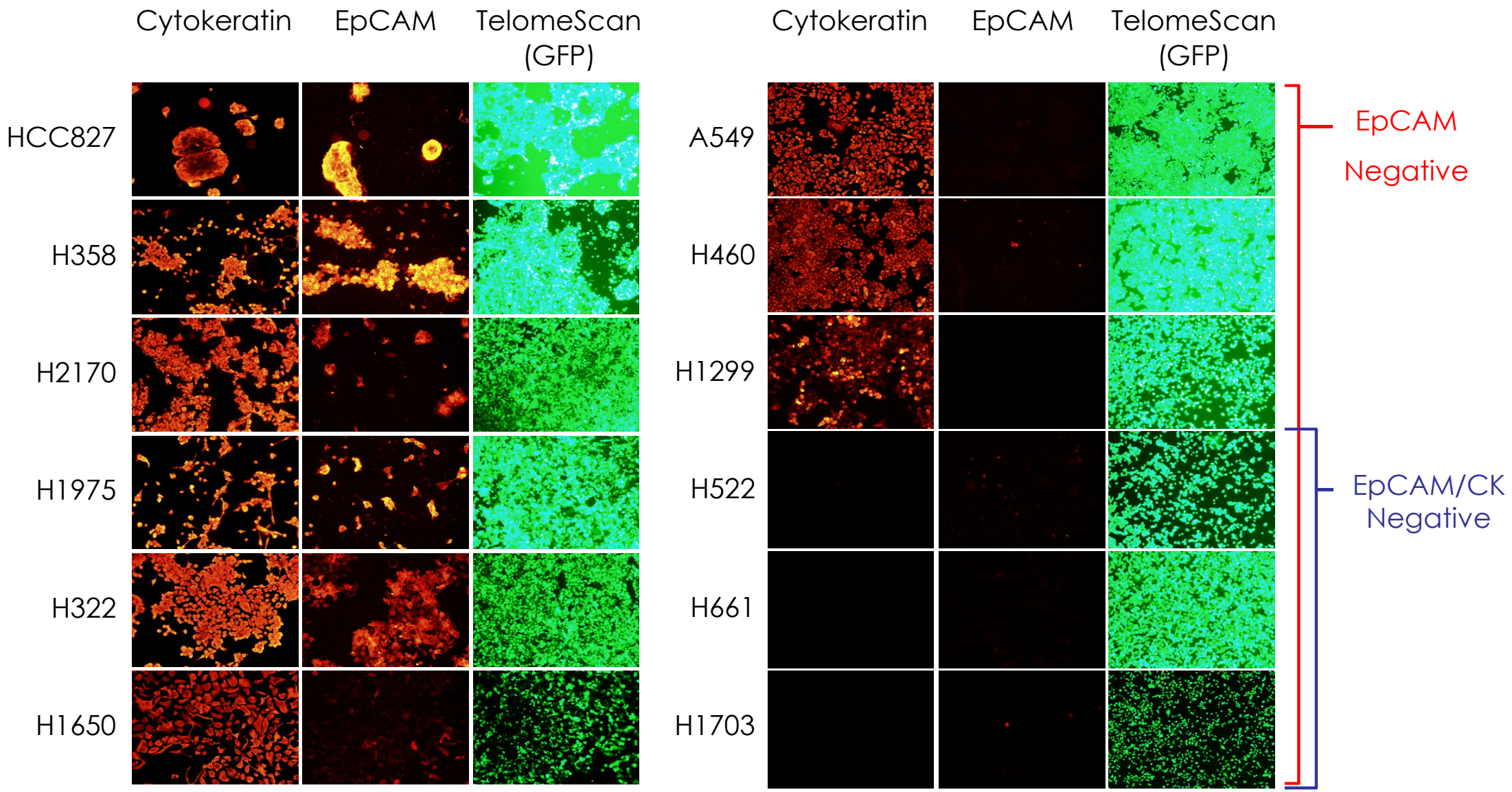
EpCAM and Cytokeratin Independent GFP expression in NSCLC Cell Lines



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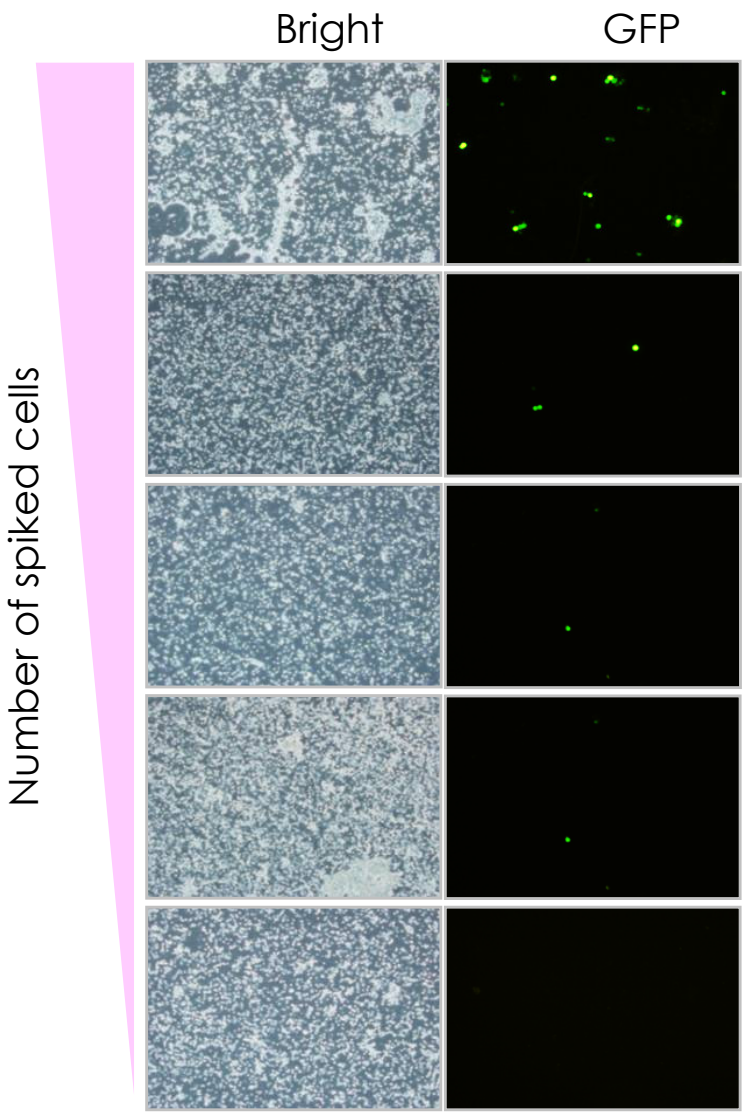
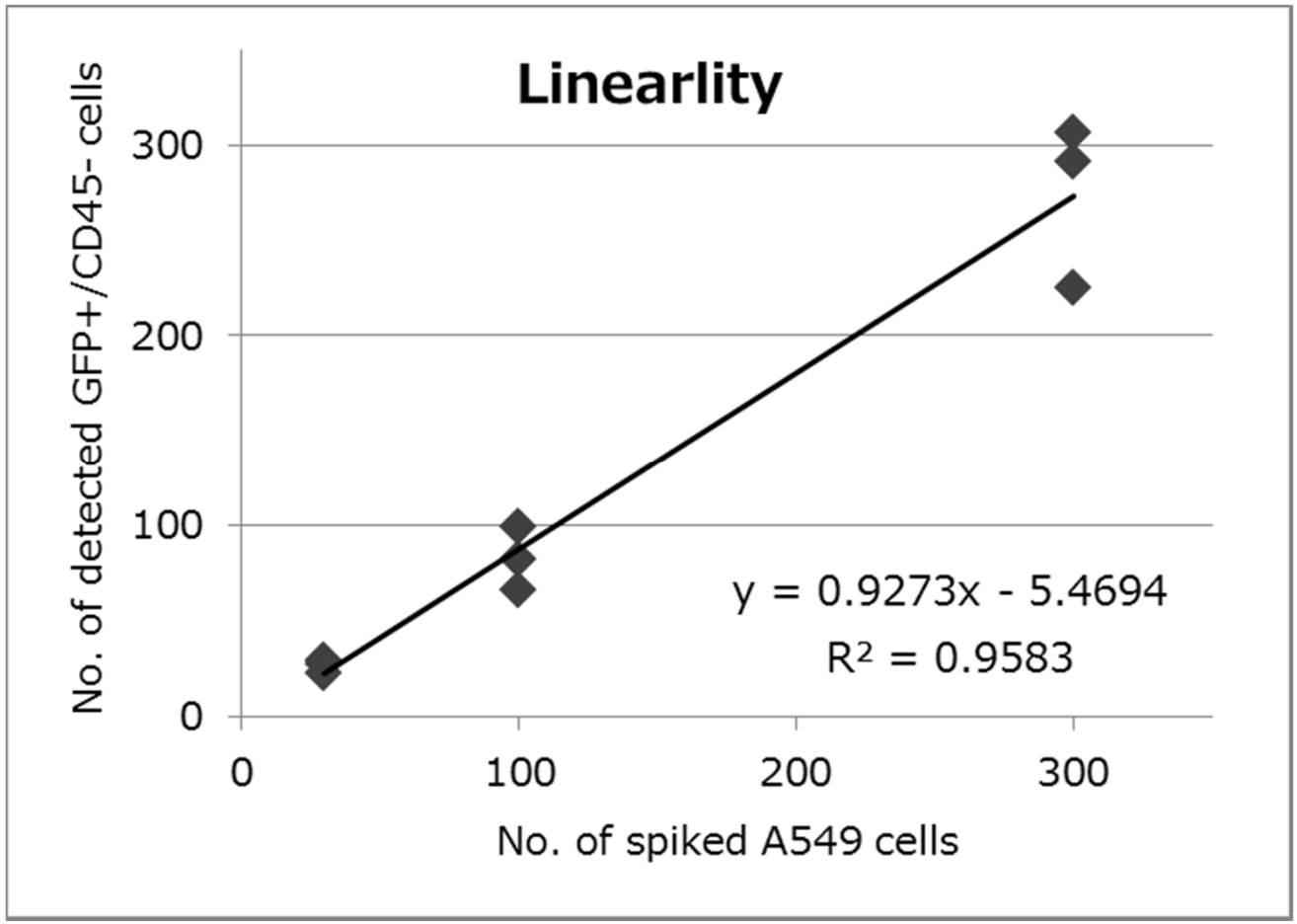
High Sensitivity of TelomeScan





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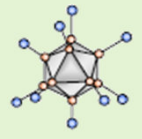
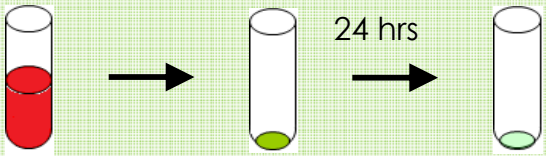
Clinical Application of TelomeScan for CTC detection and Analyses

Sample Preparation

TelomeScan kit

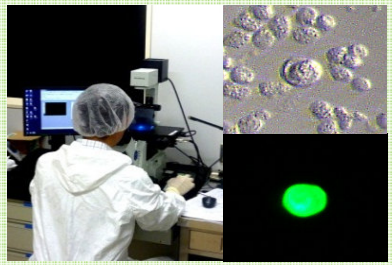


Lysis Infection Immunostaining



Whole blood PBMC

CTC Enumeration

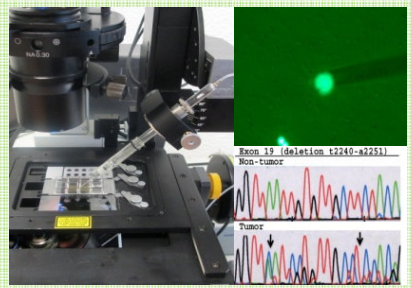


T-CAS

TelomeScan CTC Analysis System

- Super early detection of cancer
- Prognosis definition
- Evaluation of malignancy

CTC isolation and its gene analysis



T-GEN

TelomeScan Genotyping System

- Gene analysis

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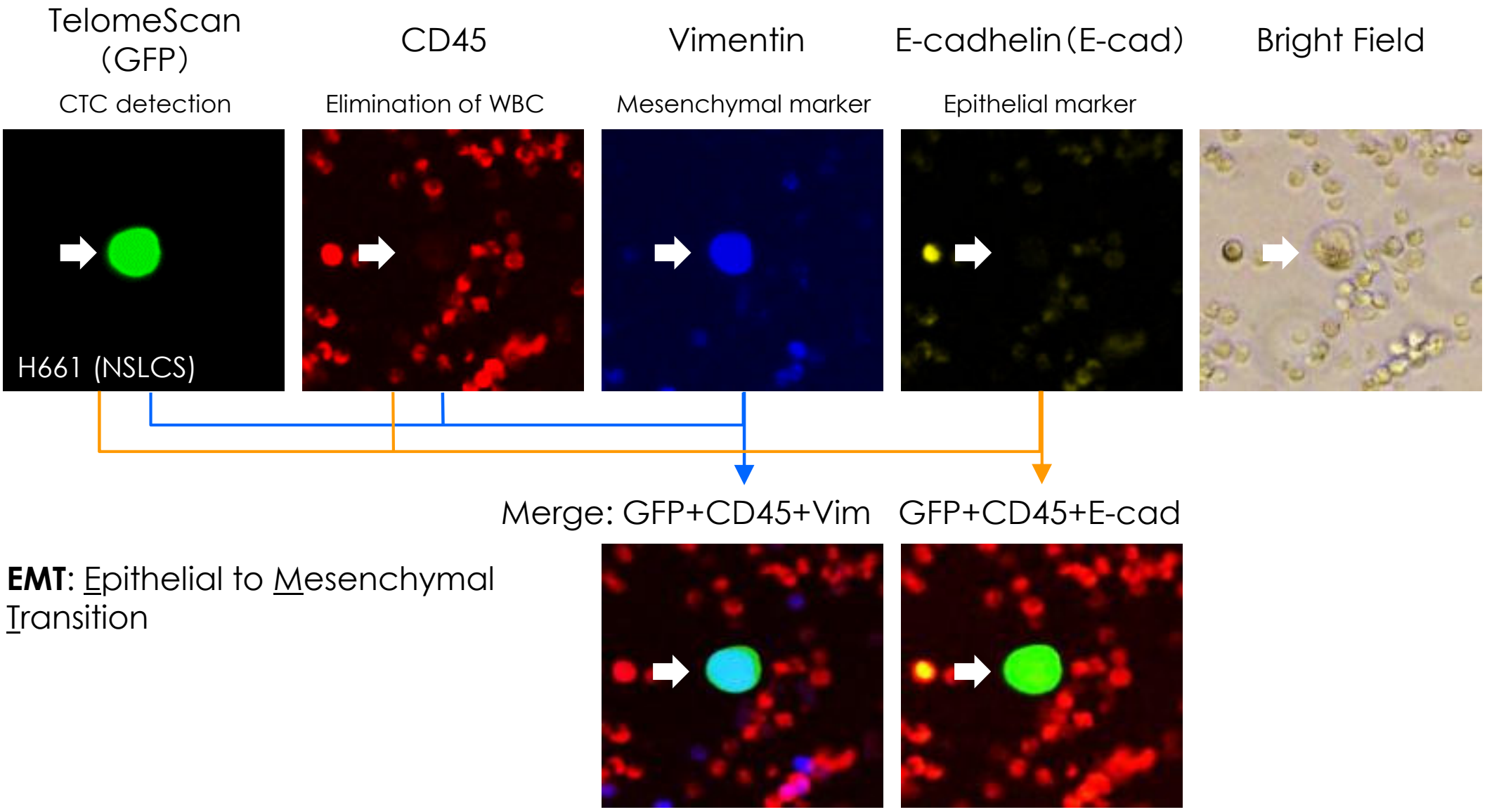
Phenotyping of CTC using TelomeScan amd Immunostaining



EMT: Epithelial to Mesenchymal Transition

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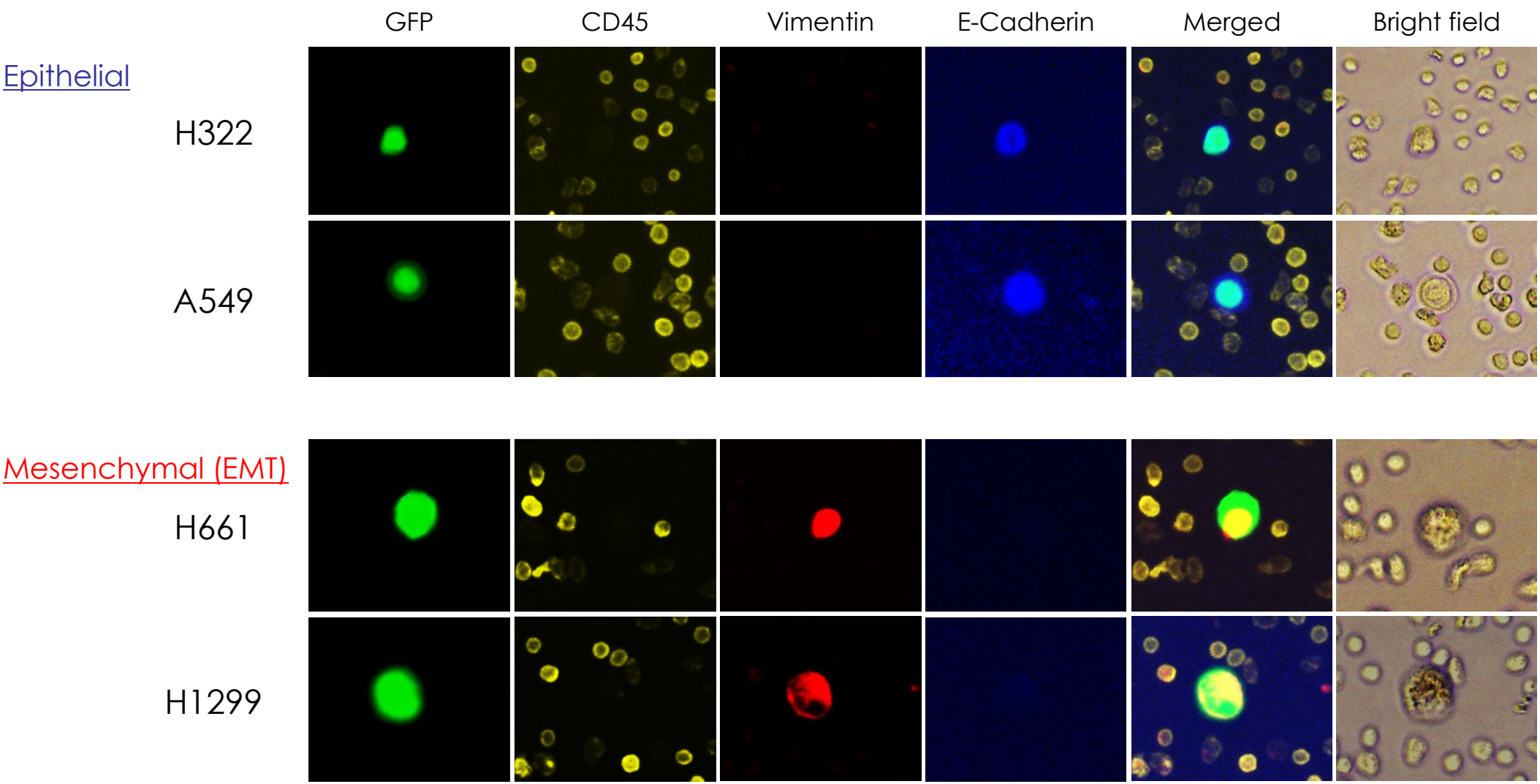
Multiple Immunostaining Identifies Characteristics of NSCLC Cell Lines



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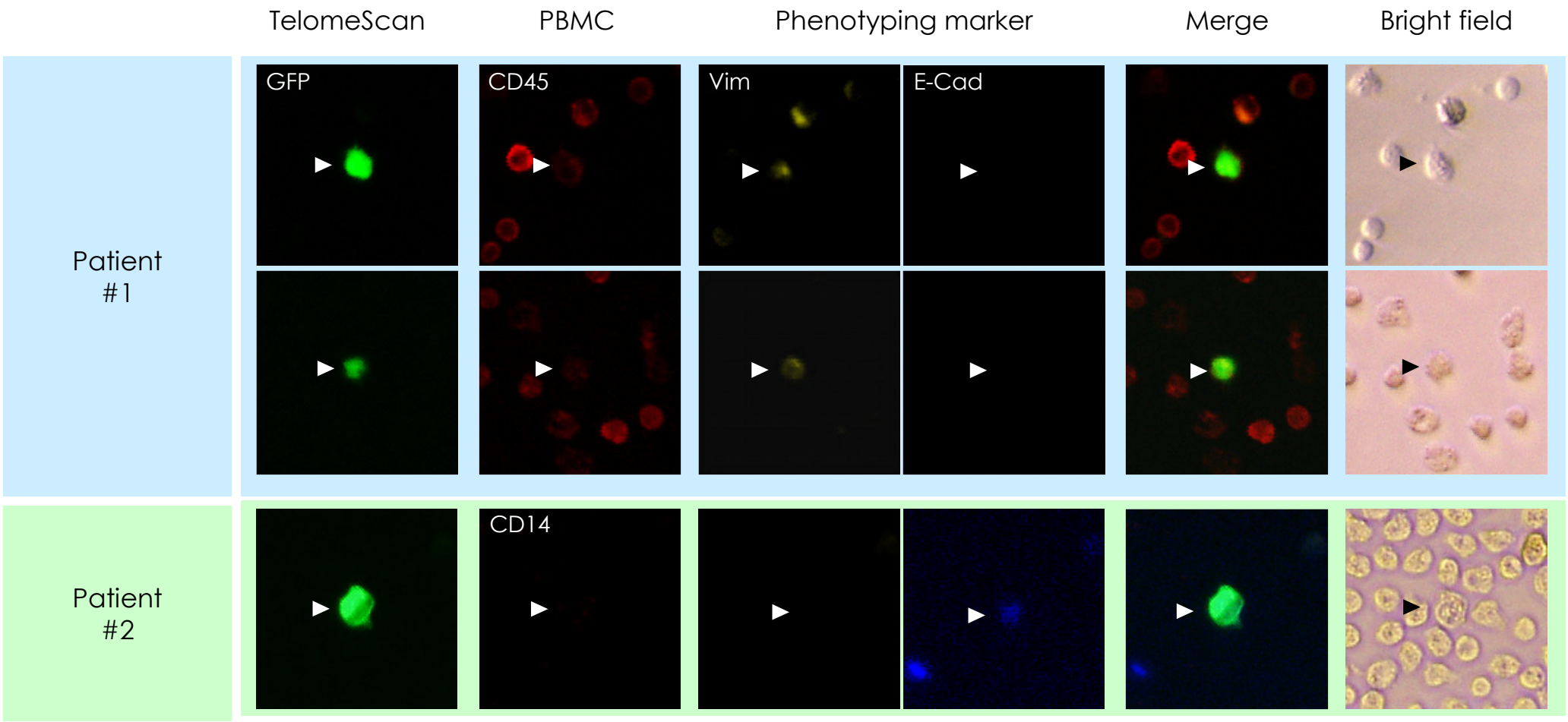
CTC Detection from NSCLC Patients



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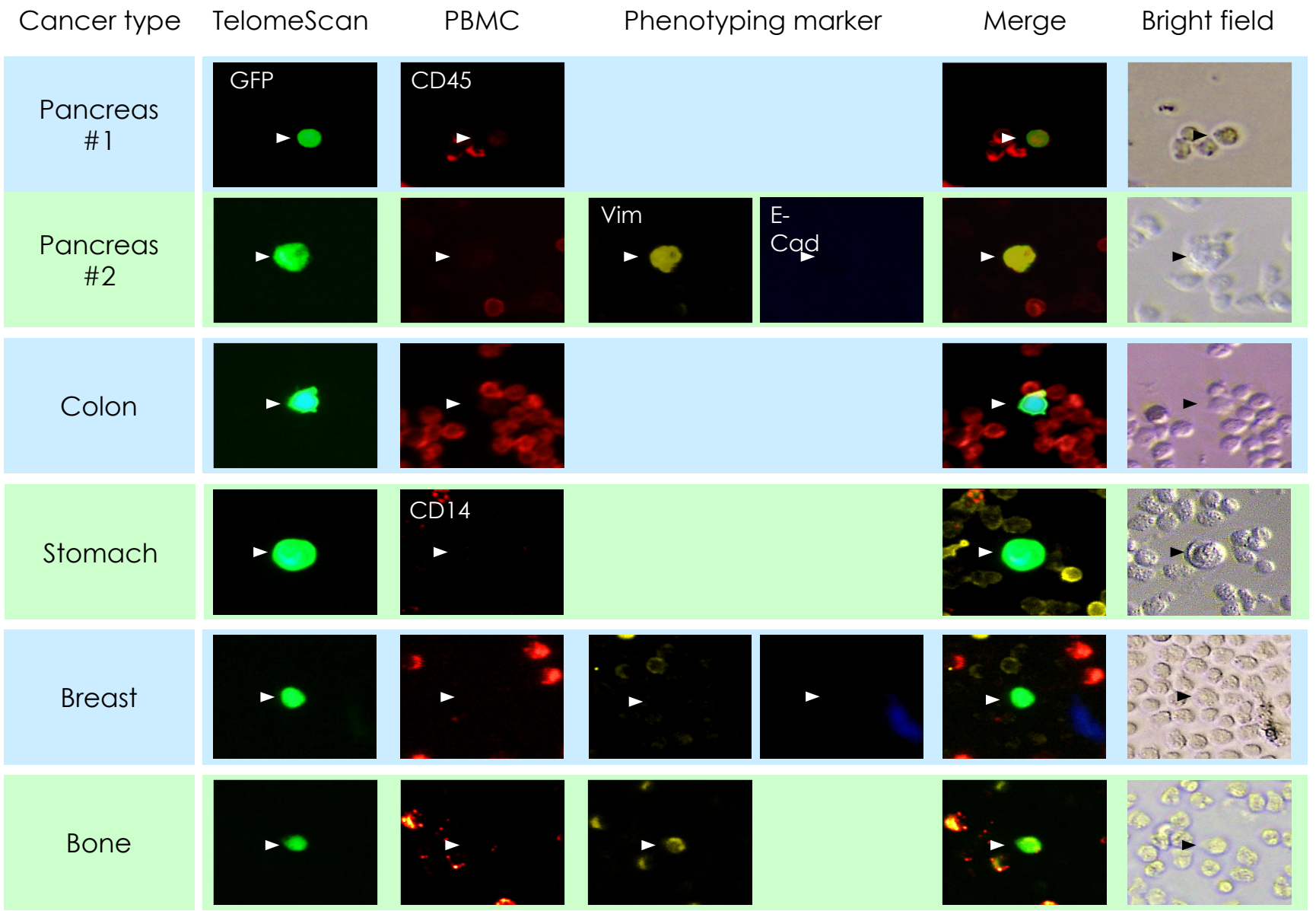
CTC Detection from Patients of Various Types of Cancer



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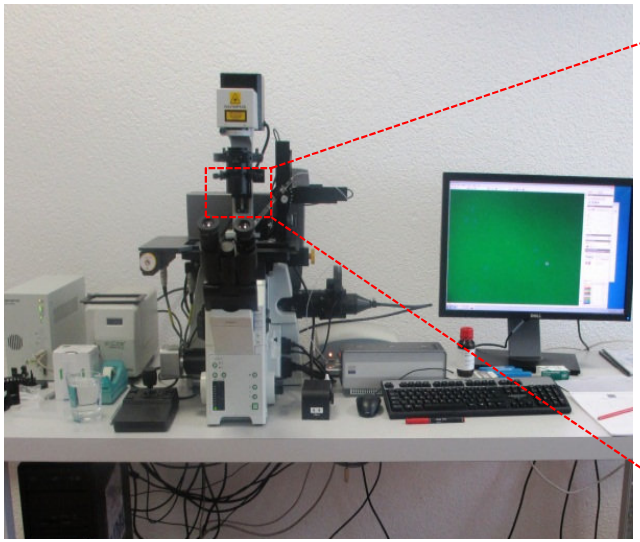
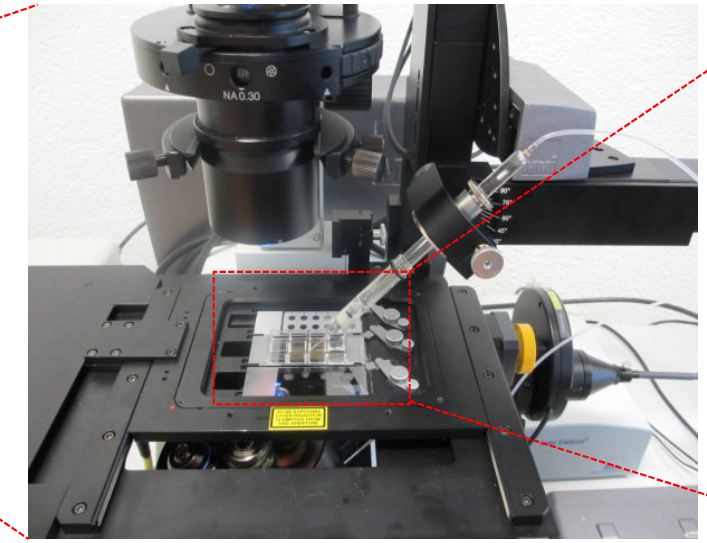
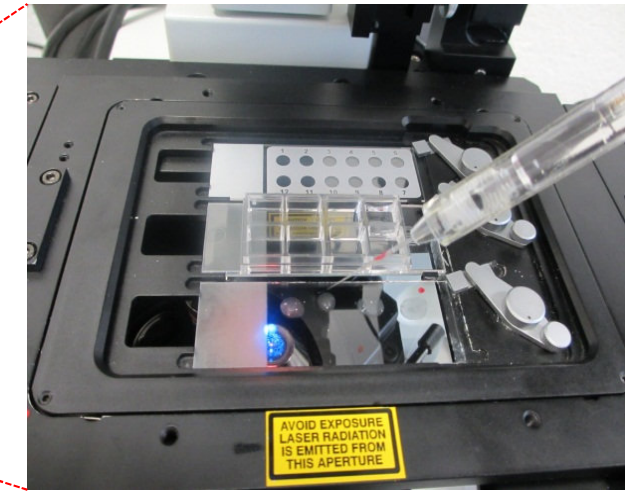
CTC Isolation Using Single-Cell Isolator



Nano-litter scale manipulator equipped with Microscope

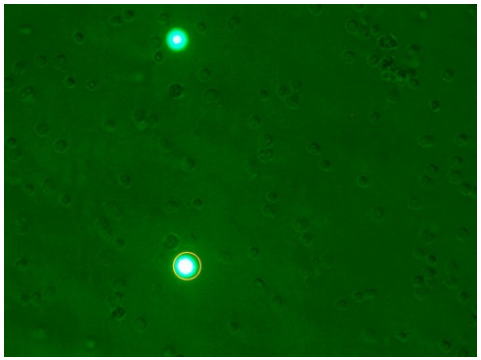


X-Y-Z coordinates were calculated and cell collection is achieved automatically

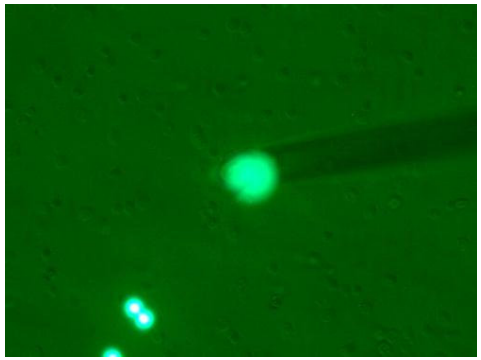


A cell is collected and drained out on PCR tube or glass slide

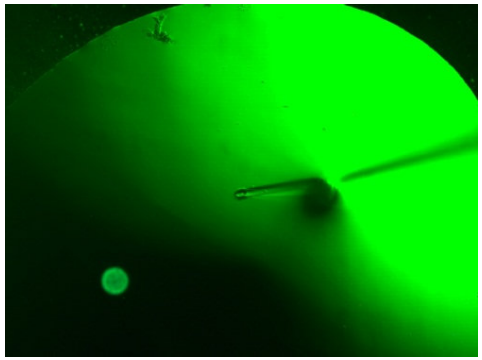
Define a cell



Capture



Deposit



Cells are collected on cap of PCR tube and directly processed for genetic mutational analysis

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Genetic Mutational Analysis Test for EGFR in NSCLC Cell Lines

Virus infection does not influence CTC mutational analysis

Cells	Virus MOI (MOI)	Result
PC-9 (EGFR exon 19 deletion mutation)	0	Exon 19del
	10	Exon 19del
	100	Exon 19del
	1000	Exon 19del

EGFR mutation was detected from NSCLC cells

Cells	# of cell	Result
H358 EGFR Wild Type	1	PCR failed
	3	WT
	10	WT
HCC827 EGFR E746-A750del	1	PCR failed
	3	E746-A750del
	10	E746-A750del

EGFR mutation was detected from 3 NSCLC cells with PBMC contaminant

Cells	# of cell	Result
HCC827 EGFR E746-A750del	3 cancer cells + 12 PBMC	E746-A750del detected
	3 cancer cells + 17 PBMC	E746-A750del detected
	3 cancer cells + 17 PBMC	E746-A750del detected

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Specification of TelomeScan Systems

- T-CAS (TelomeScan CTC Analysis System) is;
  1. To detect telomerase-positive tumor cells
  2. Not depends on EpCAM enrichment
  3. Biological tool to detect only living CTC (L-CTC)

- T-GEN (TelomeScan Genotyping System) is;
  1. To isolate a single L-CTC
  2. To identify allele specific gene mutation of EGFR
  3. Gene analysis system without biopsy