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

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Cell senescence

Telomere length is a limiting factor for cell replication.

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
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.
TelomeScan is genetically engineered GFP gene-carrying adenovirus of which viral replication is under the control of hTERT promoter.
Fluorescent Detection of Cancer Cells by TelomeScan

Normal cells
(Telomerase negative)
No virus replication and GFP production

Virus infection

Cancer cells
(Telomerase positive)
Virus replication and GFP production

Fluorescence
Cancer Specific GFP Expression by TelomeScan

**SW620; Human Colon Cancer Cells**
- 2 h
- 12 h
- 24 h
- 48 h
- 72 h

**HT29; Human Colon Cancer Cells**
- 2 h
- 12 h
- 24 h
- 48 h
- 72 h

**NHLF; Normal Fibroblast**
- 24 h
- 72 h
- 120 h

Tumor Metastasis and Circulating Tumor Cells (CTC)

1. Acquisition of invasive phenotype
2. Local invasion: cells invade into surrounding stroma, then intravasate to enter hematogenous circulation
3. Physical translocation from primary tumor to distant organ
4. Epithelial-Mesenchymal Transition (EMT)
5. CTCs transit to distant organ
6. CTCs extravasate and invade into the parenchyma of foreign tissue
7. Colonization
8. Survival at secondary site
9. Adaptation and proliferation to form metastases

Differentiated cancer cell
Transitioning cancer cell
Cancer stem cell
Stromal cell
Inflammatory cell
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.

CTC is separated by anti-EpCAM

WBC is removed by anti-CD45
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)

Predictive Value: OS of Patients with <5 or ≥5 CTC at Baseline (N=177)
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%
Sensitivity of TelomeScan in NSCLC Cell Lines

- Quantitative measurement of GFP expression by FACS
- TelomeScan is feasible to detect CTCs in Lung Cancer Patients.
EpCAM and Cytokeratin Independent GFP expression in NSCLC Cell Lines

<table>
<thead>
<tr>
<th>Cytokeratin</th>
<th>EpCAM</th>
<th>TelomeScan (GFP)</th>
<th>Cytokeratin</th>
<th>EpCAM</th>
<th>TelomeScan (GFP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC827</td>
<td></td>
<td></td>
<td>A549</td>
<td></td>
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<tr>
<td>H358</td>
<td></td>
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<td>H460</td>
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<tr>
<td>H2170</td>
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<td>H1299</td>
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<td>H1975</td>
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<td>H522</td>
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<td>H322</td>
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<td>H661</td>
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<td>H1650</td>
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<td>H1703</td>
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</tbody>
</table>

- EpCAM Negative
- EpCAM/CK Negative
High Sensitivity of TelomeScan

Number of spiked cells

Bright

GFP

No. of spiked A549 cells

No. of detected GFP+/CD45- cells

Linearlity

y = 0.9273x - 5.4694

R² = 0.9583

14th Anti-Tumor Drug Development Forum
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
Phenotyping of CTC using TelomeScan and Immunostaining

**TelomeScan** (GFP)
- CTC detection

**CD45**
- Elimination of WBC

**Vimentin**
- Mesenchymal marker

**E-cadherin (E-cad)**
- Epithelial marker

**Bright Field**

**CTC detection**

**H661 (NSLCS)**

**Merge: GFP+CD45+Vim**

**Merge: GFP+CD45+E-cad**

**EMT: Epithelial to Mesenchymal Transition**
## Multiple Immunostaining Identifies Characteristics of NSCLC Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>GFP</th>
<th>CD45</th>
<th>Vimentin</th>
<th>E-Cadherin</th>
<th>Merged</th>
<th>Bright field</th>
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</thead>
<tbody>
<tr>
<td><strong>Epithelial</strong></td>
<td></td>
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<tr>
<td>H322</td>
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<tr>
<td>A549</td>
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<tr>
<td><strong>Mesenchymal (EMT)</strong></td>
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<tr>
<td>H661</td>
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<tr>
<td>H1299</td>
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</table>
# CTC Detection from NSCLC Patients

<table>
<thead>
<tr>
<th>TelomeScan</th>
<th>PBMC</th>
<th>Phenotyping marker</th>
<th>Merge</th>
<th>Bright field</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient #1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFP</td>
<td>CD45</td>
<td>Vim</td>
<td>E-Cad</td>
<td>Merge</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Patient #2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CD14</td>
<td></td>
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</tr>
</tbody>
</table>

**CTC Detection from NSCLC Patients**

**Patient #1**
- TelomeScan: GFP
- PBMC: CD45
- Phenotyping marker: Vim, E-Cad
- Merge
- Bright field

**Patient #2**
- TelomeScan: CD14
- PBMC: CD14
- Phenotyping marker: Vim
- Merge
- Bright field
# CTC Detection from Patients of Various Types of Cancer

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>TelomeScan</th>
<th>PBMC</th>
<th>Phenotyping marker</th>
<th>Merge</th>
<th>Bright field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas #1</td>
<td>GFP</td>
<td>CD45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas #2</td>
<td></td>
<td></td>
<td>Vim, E-Cad</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stomach</td>
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</tr>
<tr>
<td>Breast</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td></td>
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</tr>
</tbody>
</table>

**Phenotyping markers**:
- GFP
- CD45
- CD14
- Vim
- E-Cad
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
# Genetic Mutational Analysis Test for EGFR in NSCLC Cell Lines

Virus infection does not influence CTC mutational analysis

<table>
<thead>
<tr>
<th>Cells</th>
<th>Virus MOI (MOI)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-9</td>
<td>0</td>
<td>Exon 19del</td>
</tr>
<tr>
<td>(EGFR exon 19 deletion mutation)</td>
<td>10</td>
<td>Exon 19del</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Exon 19del</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>Exon 19del</td>
</tr>
</tbody>
</table>

EGFR mutation was detected from NSCLC cells

<table>
<thead>
<tr>
<th>Cells</th>
<th># of cell</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>H358</td>
<td>1</td>
<td>PCR failed</td>
</tr>
<tr>
<td>EGFR Wild Type</td>
<td>3</td>
<td>WT</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>WT</td>
</tr>
<tr>
<td>HCC827</td>
<td>1</td>
<td>PCR failed</td>
</tr>
<tr>
<td>EGFR E746-A750del</td>
<td>3</td>
<td>E746-A750del</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>E746-A750del</td>
</tr>
</tbody>
</table>

EGFR mutation was detected from 3 NSCLC cells with PBMC contaminant

<table>
<thead>
<tr>
<th>Cells</th>
<th># of cell</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC827</td>
<td>3 cancer cells + 12 PBMC</td>
<td>E746-A750del detected</td>
</tr>
<tr>
<td>EGFR E746-A750del</td>
<td>3 cancer cells + 17 PBMC</td>
<td>E746-A750del detected</td>
</tr>
<tr>
<td></td>
<td>3 cancer cells + 17 PBMC</td>
<td>E746-A750del detected</td>
</tr>
</tbody>
</table>
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