A New Molecular Probe for Rapid Detection of Circulating Tumor Cells in Whole Blood

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Circulating tumor cells (CTCs) detection

- Clinically, the number of CTCs is directly associated with cancer metastasis and patient prognosis
- Current CTC assays require multiple steps
  1) Isolation of CTCs from blood cells (physical or biological properties, biomarkers, cell functions...)
  2) Labeling CTCs by antibody-mediated methods
  3) CTC detection
- Multiple steps may cause loss and/or damage of CTCs, reducing assay sensitivity

One-step assay of circulating tumor cells (CTCs)

Need a new reporting system

Oligonucleotide Aptamer

- A class of small molecule ligands composed of short, single-stranded nucleotides
- RNA or ssDNA, usually 30-70 bases in length
- Can be selected from the random sequence library through the Systematic Evolution of Ligands by EXponential enrichment technique (SELEX)
- Can be produced by chemical synthesis

Oligonucleotide aptamer: a “chemical antibody”

- Form 3-dimensional structure
- Specifically bind to the targets through molecular recognition (similar to antigen and antibody reaction)
- Not through sequence hybridization in molecular tests

Disclosure of Relevant Financial Relationships

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**Nucleotide aptamer vs. Protein antibody**

**As a “Chemical antibody”**

- Chemical modification
  - For different purposes

- Small in size
  - Faster cell binding
  - Deeper tissue penetration

- Less/no immunogenicity
  - Suitable for in vivo use

**Nucleotide aptamer: a chemical antibody**

A synthetic RNA aptamer specific for CD30

*CD30 is a diagnostic biomarker highly expressed on tumor cell surface:*
- Hodgkin lymphoma
- Anaplastic large T cell lymphoma
- Some B- and T-cell lymphomas
- Some solid tumors

**Specific cell binding of aptamer probe in mixed marrow cells**

**Immunohistochemistry**

**Immunostaining of lymphoma tissues by aptamer probe**

**One-step assay for CTCs detection**

One-step assay for CTCs detection

1. Add blood sample
2. In minutes
3. Detection of CTCs

Aptamer-reporter

12.52% 6.46% 3.32% 1.54% 0.48%

CD45 antibody

Antibody

Tissue immunostain (color development)

Antibody

Tissue immunostain (color development)

Aptamer

Immunohistochemistry

12.78% 6.58% 3.37% 1.58% 0.48%

Nucleated red blood cells

Granulocytes

Karpas 299 cells

Lymphocytes

Blasts

Monocytes

**ALCL lymphoma**

**Hodgkin lymphoma**

**H&E stain**

**CD30 aptamer**

**CD30 antibody**
A tumor cell-activatable aptamer-reporter (TCAR)

Inactive TCAR

Tumor cell-activatable aptamer-reporter (TCAR) for one-step detection of CTCs

Optically inactive TCAR

Activation of TCAR by cell lysates

Tumor cell-activatable aptamer-reporter (TCAR) for one-step detection of CTCs

Intracellular activation of TCAR
**Cell specificity of TCAR**

**A** CD30-expressing tumor cells

**B** CD30 (−) control tumor cells

- Light microscopy
- Fluorescent microscopy

**Specific and selective detection of tumor cells by TCAR in one step assay**

- CD30+ (red) cells
- Pre-stained CD30(−) cells (green)
- Merged

**One-step assay of CTCs in lymphoma patient whole blood and marrow specimen**

- Blood
- Bone marrow

- Light microscopy
- Fluorescent microscopy

**Double-stains of CTCs by TCAR and antibody in patient whole blood**

- Aptamer
- Antibody
- Merged

**One-step assay of CTCs in patient whole blood**

- High blood concentration
- Normal blood

**One-step assay of CTCs in whole bloods via a high throughput and multi-sample platform**

- Multi-sample platform
- One-step reaction
- High throughput detection
- Tumor cell
One-step CTC assay vs. Current method

- **One-step CTC assay**
  - Require minimal amount of blood
  - Loading multiple blood samples from different patients
  - Complete in minutes

- **Current method**
  - Require 7.5 ml blood per assay
  - Loading one blood sample from single patient
  - Blood sample buffering
  - Centrifugation
  - Removal of plasma
  - Mixed with cell isolation reagents
  - Magnetic cell separation
  - Tumor cell staining
  - Repeated washing to remove free antibodies
  - Tumor cell detection
  - Take hours to complete

TCAR is a universal platform to detect cancer cells

- Different biomarker-specific **aptamer sequences** (RNA or ssDNA) to detect cancer cells of interest

EpCAM-specific TCAR for one-step assay of CTCs

- EpCAM-specific TCAR
- Merged

One-step assay of CTCs in whole blood of breast cancer patient by EpCAM-specific TCAR

- Whole blood
- DAPI
- EpCAM-TCAR
- Merged

TCAR is a universal platform to detect cancer cells

- Different biomarker-specific aptamer sequences (RNA or ssDNA) to detect cancer cells of interest
- Different **fluorochromes** to highlight cancer cells in different colors
Detection of breast tumor cells by EpCAM-specific TCAR

One-step assay of CTCs in whole blood of breast cancer patient by EpCAM-specific TCAR

Detection of different tumor cells by TCARs

Detection of different tumor cells by TCARs

Detection of different tumor cells by TCARs

Developing 2nd generation TCAR for CTC detection
Developing 2nd generation TCAR for CTC detection

Aptamer-CypH

Light view Cy3 dye Lysosomes Nuclei Merged view

Aptamer-CypH

Light view Cy7 dye Lysosomes Nuclei Merged view

Patient whole blood 2nd-TCAR DAPI Merged

One-step CTC detection by 2nd generation TCAR
<table>
<thead>
<tr>
<th><strong>Oligonucleotide aptamer: a new molecular probe for clinical applications</strong></th>
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Questions and comments?