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(54) **Title**: CEACAM1 BASED POINT-OF-CARE CANCER DIAGNOSTIC

(57) **Abstract**: The present technology regards the utilization of CEACAM1 as a biomarker for cancer. In particular the present invention regards the detection and measurement of soluble CEACAM1 levels for the detection and diagnosis of melanoma.

# CEACAM1 BASED POINT-OF-CARE CANCER DIAGNOSTIC

## CROSS-REFERENCE TO RELATED APPLICATIONS/INCORPORATION BY REFERENCE

**[0001]** The present application is related to and claims priority from U.S. Provisional Patent Application serial number 60/985,484, filed November 5, 2007, and titled "CEACAM1 Based Point-of-care cancer diagnostic," the contents of which are herein incorporated by reference in their entirety.

## FIELD OF THE INVENTION

**[0002]** This invention relates generally to the field of cancer detection, and more specifically to the utilization of CEACAM1 as a biomarker for the point-of-care diagnosis of cancer.

## BACKGROUND OF THE INVENTION

**[0003]** Cancer is a term for diseases in which abnormal cells divide without control. Cancer cells can invade nearby tissues and can spread to other parts of the body through the blood and lymph systems. There are several types of cancer, including without limitation, carcinoma which is cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is cancer that starts in blood-forming tissue such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the blood. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord.

**[0004]** The human carcinoembryonic Ag (CEA)3 protein family encompasses several proteins with different biochemical features. These proteins are encoded by 29 genes tandemly arranged on chromosome 19q13.2. CEA family genes have been classified into two major subfamilies, the CEA cell adhesion molecule (CEACAM) and the pregnancy-specific glycoprotein subgroups. The CEACAM proteins, which are part of the larger Ig superfamily, include CEACAM1, -3, -4, -5, -6, and -8. They share a common basic structure of sequentially ordered different Ig-like domain(s) and are able to interact with each other. For example, it has been reported that various CEACAM proteins, such as CEACAM1 or CEACAM5, exhibit both homophilic and heterophilic interactions.

**[0005]** CEACAM1 (CD66a), a transmembrane protein and member of the carcinoembryonic Ags family (belongs to IgSF), contains two ITIM sequences located within its cytosolic tail. CEACAM1 interacts with other known CD66 proteins (both homophilic and heterophilic interactions), including CD66a, CD66c, and CD66e proteins. It is expressed on a wide spectrum of cells, ranging from epithelial to hemopoietic origin. Among CD66 proteins tested, only the CD66a protein is expressed on the surface of activated CD16-negative NK cells.

**[0006]** Point-of-care testing refers to a medical test which is carried out at sites other than a central laboratory, and comprises testing at or near the site of patient care. The value added by Point-of-Care Diagnostics is significant, and includes for example: improved patient outcomes attributable to immediate on-site actionable healthcare resulting from immediate test results (i.e. - reduced time for start of treatment); access to areas lacking clinical laboratory infrastructures (i.e. - rural areas, disaster areas, and developing countries); and avoidance of sample identification and sample transport problems.

## BRIEF SUMMARY OF THE INVENTION

**[0007]** The present technology generally regards methods for diagnosing cancer comprising the detection of soluble CEACAM1 in a biological sample. In one particular aspect, this technology relates to an immunochromatographic strip for the point-of-care detection of soluble CEACAM1 in a biological sample for the detection and prognosis of melanoma.

**[0008]** The present technology further regards a diagnostic device for point-of-care detection of cancer comprising a matrix material having an application zone wherein a test fluid is applied, the test fluid moving by capillary action into a reaction zone comprising a mobile phase having a first CEACAM1 binding element (i.e. – a first CEACAM1 antibody or fragment thereof) conjugated to a detectable moiety, the test fluid then moving by capillary action through the reaction zone into one or more capture zones having a stationary phase comprising a second CEACAM1 binding element (i.e. – a second CEACAM1 antibody or fragment thereof), the detection of CEACAM1 in the test sample being dependent upon the presence of said detectable moiety within the CEACAM1 capture zone.

## BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

**[0009] FIGURE 1.** Presentation of data showing that soluble CEACAM1 is increased in melanoma patients.

**[0010] FIGURE 2.** Schematic representation of an immunochromatographic strip for the point-of-care detection of CEACAM1 in a biological sample.

**[0011] FIGURE 3.** Schematic representation of one embodiment of a lateral-flow point-of-care rapid detection system, for the detection and measurement of CEACAM1 in a test sample.

## DETAILED DESCRIPTION OF THE INVENTION

**[0012]** The present technology generally regards the use of soluble CEACAM1 for the diagnosis and for predicting the prognosis of cancer, including for example melanoma. The present technology also comprises in part a immunochromatographic strip for the point-of-care detection of CEACAM1 in a biological sample.

**[0013]** CEACAM1 appears as a soluble molecule in the peripheral blood. The present technology regards the diagnosis of cancer comprising the detection of soluble CEACAM1 in a biological sample. The data shown in Table 1 and presented in Figure 1 demonstrates that soluble CEACAM1 levels function as a biomarker for at least one type of cancer, namely melanoma (a form of skin cancer that begins in melanocytes (the cells that make the pigment melanin). As shown in Figure 1, soluble CEACAM1 is increased in melanoma patients (LR (Low Risk); HR (High Risk); Met (Metastatic patients); healthy donors.). Soluble CEACAM1 is quantified using a CEACAM1 specific sandwich ELISA. For example, the specific anti-CEACAM1 5F4 mAb can be used as capturing antibody. For detection, biotinylated Kat4c mAb can be used, followed by streptavidin-horseradish peroxidase (Jackson ImmunoResearch). Biotinylation of the Kat4c mAb can be performed with Sulfo-NHS-SS-Biotin (Pierce) according to the manufacturer's instructions. The quantification can then be calculated according to standard samples of CEACAM1-Ig fusion proteins.

**[0014]** Figure 2 is an schematic illustration of one aspect of the present technology. Referenced generally as 1 is a immunochromatographic strip comprising a matrix material 2 having an application (or sample loading) zone 3, a reaction zone 4, a CEACAM1 capture zone 5, a control analyte capture zone 6, and an optional wicking pad 7.

**[0015]** The test fluid (and any CEACAM1 suspended or dissolved therein) is first brought into contacted with or applied to the application zone 3 of the immunochromatographic strip 1. The immunochromatographic strip 1 comprises a matrix material 2 which facilitates the test fluid moving by capillarity action from the application zone 3 through the reaction zone 4 and past the capture zones (CEACAM1 capture zone 5 and control analyte capture zone 6) to an optional wicking pad 7. Any detectable signal in the capture zones will reveal the presence of analyte (CEACAM1 or control).

**[0016]** The matrix material comprises a membrane material, for example nitrocellulose membrane, cellulose acetate membrane, glass-fiber membrane, or any combinations or derivatives thereof. The matrix material is porous, and any test liquid or sample applied to the matrix material at application zone 3 can move by capillary into the reaction zone 4 and continue to and/or past the capture zones 4 and 5.

**[0017]** The reaction zone 4 contains a mobile phase comprising a first CEACAM1 specific antibody (or fragment thereof) conjugated to a detectable moiety (e.g. - radioactive isotopes, enzymes, dyes, fluorescent dyes, dyed microspheres, affinity tag, or any combination or derivative thereof). The reaction zone 4 will also comprise, in mobile phase, a first control analyte specific antibody conjugated to a detectable moiety. When the test sample (and any CEACAM) moves by capillary action out of the application zone 3 and into the reaction zone 4, an antigen-antibody reaction ensues resulting in any CEACAM1 becoming bound (in mobile phase) by a first CEACAM1 specific antibody (or fragment thereof) conjugated to a detectable moiety. See figure 3.

**[0018]** The antigen (CEACAM1)-antibody reaction product formed within the reaction zone then migrates with the test liquid into one or more downstream capture zones having

immobilized or stationary antibody. The CEACAM1 capture zone 5 comprises an immobilized or stationary phase second CEACAM1 binding element (e.g. – a second CEACAM1 antibody or fragment thereof) or CEACAM1 capture agent (e.g. - CEACAM1 capture antibody). The control analyte capture zone 6 also comprises an immobilized or stationary phase second control analyte binding element (e.g. – a second control analyte antibody or fragment thereof) or control analyte capture agent (e.g. – control analyte capture antibody). See figure 3.

**[0019]** As a result of capillary flow from the application zone 3 through the reaction zone 4 and into the capture zones 5 and 6, a concentration of the detectable moiety builds within the capture zones, and the concentration of CEACAM1 in capture zone 5 ( and control analyte(s) in capture zone 6) can then be assessed qualitatively or quantitatively with a suitable measuring instrument, or if dyes that are adsorbent in the visible range are used as the detectable moiety, can be perceived with the naked eye (Figure 3).

**[0020]** The detection moiety can be radioactive isotopes, enzymes, dyes, fluorescent dyes, dyed microspheres, affinity tag, or any combination or derivative thereof.

**[0021]** The CEACAM1 binding agent (or capture agent) include without limitation an antibody (or fragment thereof) specific for CEACAM1. The antibody can be either polyclonal or monoclonal. Exemplar CEACAM1 specific antibodies include: Mouse monoclonal [29H2] to CEACAM1; Mouse monoclonal [GM8G5] to CEACAM1 (GM8G5 recognizes the Human CEACAM1 A2 domain); CEACAM1 antibody number 2037.00.02 (from Strategic Diagnostics Inc) binding CEACAM1 amino acids 35-134; the CEACAM1-specific antibody 4D1/C2; anti-CEACAM1 5F4 mAb; anti-CEACAM1 Kat4c mAb; or any combination or derivative thereof. The CEACAM1 binding agent can also be a member of the CEA protein family.

TABLE 1

| | Low Risk patients | High Risk patients | Metastatic patients | Healthy donors |
|---|---|---|---|---|
| 1 | **34.9** | **49.1** | 22.2 | 7.6 |
| 2 | **33.3** | 15.8 | 24.2 | 10.6 |
| 3 | **50.4** | 18.9 | **104.3** | 18.4 |
| 4 | 20.6 | **39.8** | 17.8 | 15.8 |
| 5 | 27.0 | **41.3** | 18.1 | 7.8 |
| 6 | 3.4 | 24.4 | 21.9 | 0.4 |
| 7 | 18.2 | 18.5 | **36.1** | 15.8 |
| 8 | 8.9 | 25.2 | 18.4 | 22.6 |
| 9 | **52.0** | 19.7 | 17.6 | 22.3 |
| 10 | 23.0 | **33.5** | 21.1 | 11.2 |
| 11 | 29.4 | 0 | **30.3** | 0.0 |
| 12 | 26.0 | **75.1** | 20.4 | 0.0 |
| 13 | 28.2 | **67.6** | **32.1** | 8.0 |
| 14 | 26.5 | **51.0** | **68.8** | 9.5 |
| 15 | 21.3 | 26.1 | **91.7** | 12.0 |
| 16 | 24.6 | 18.4 | **36.6** | |
| 17 | 19.2 | **40.8** | 29.8 | |
| 18 | **41.0** | 22.2 | **50.0** | |
| 19 | 16.8 | 24.1 | 20.0 | |
| 20 | **57.5** | 12.0 | 25.8 | |
| 21 | 24.1 | | **31.0** | |
| 22 | 23.1 | | 21.8 | |
| 23 | 23.6 | | **42.5** | |
| 24 | | | 2.7 | |

CLAIMS

What is claimed is:

1. A device for the point-of-care detection of soluble CEACAM1 in a biological sample, the device comprising an immunochromatographic strip for the point-of-care detection of CEACAM1 in a biological sample.
2. The device of claim 1, wherein the biological sample is blood, blood serum, or urine.
3. The device of claim 1, wherein said immunochromatographic strip comprises a porous matrix having a sample application zone, a reaction zone, and a capture zone in communication with each other, said porous matrix supporting the capilary movement of said biological sample.
4. The device of claim 3, wherein the reaction zone comprises a mobile phase comprising a CEACAM1 specific antibody or fragment thereof conjugated to a detectable moiety
5. The device of claim 4, wherein said detectable moiety comprises a radioactive isotope, an enzyme, a fluorescent dye, a dyed microsphere, an affinity tag, or any combination or derivative thereof.

FIGURE 1

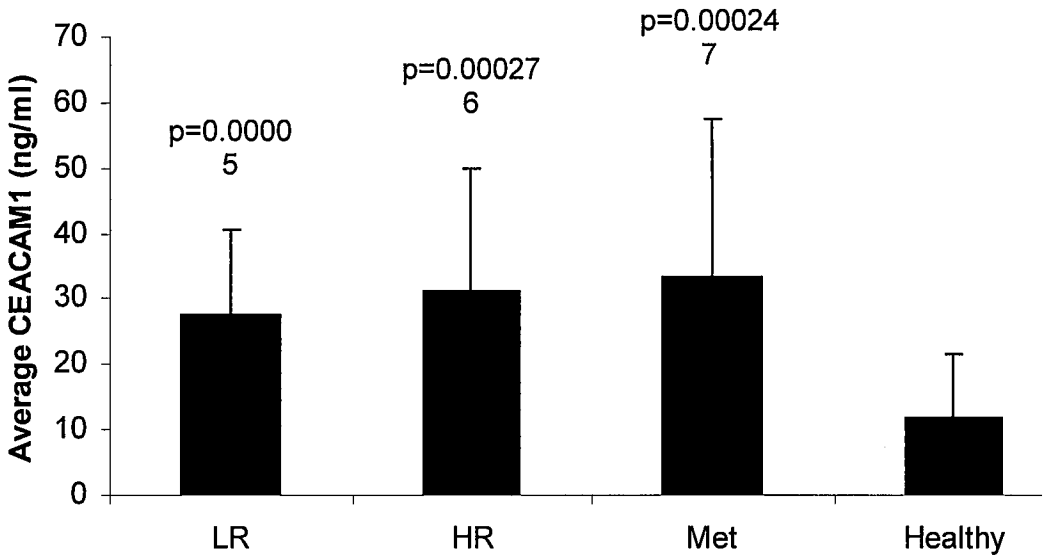

Average CEACAM1 (ng/ml)
70
60
50
40
30
20
10
0
p=0.0000
5
p=0.00027
6
p=0.00024
7
LR
HR
Met
Healthy

FIGURE 2

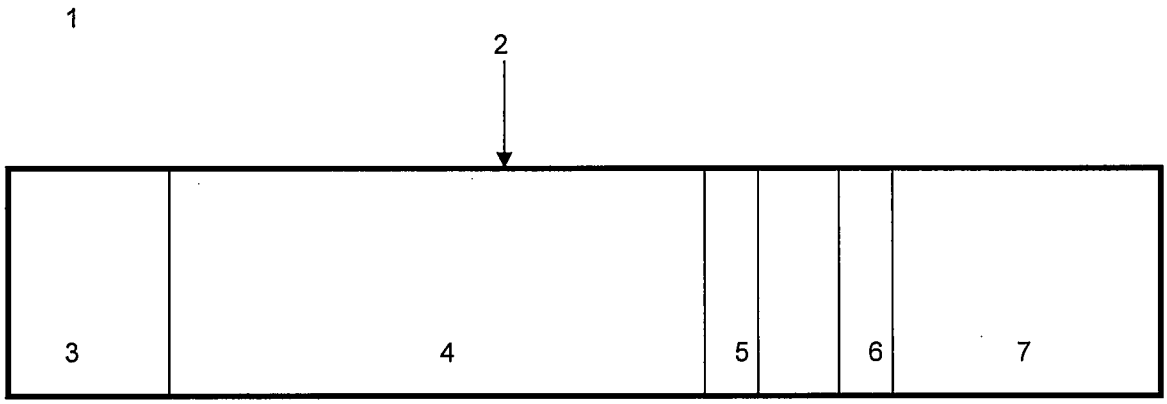
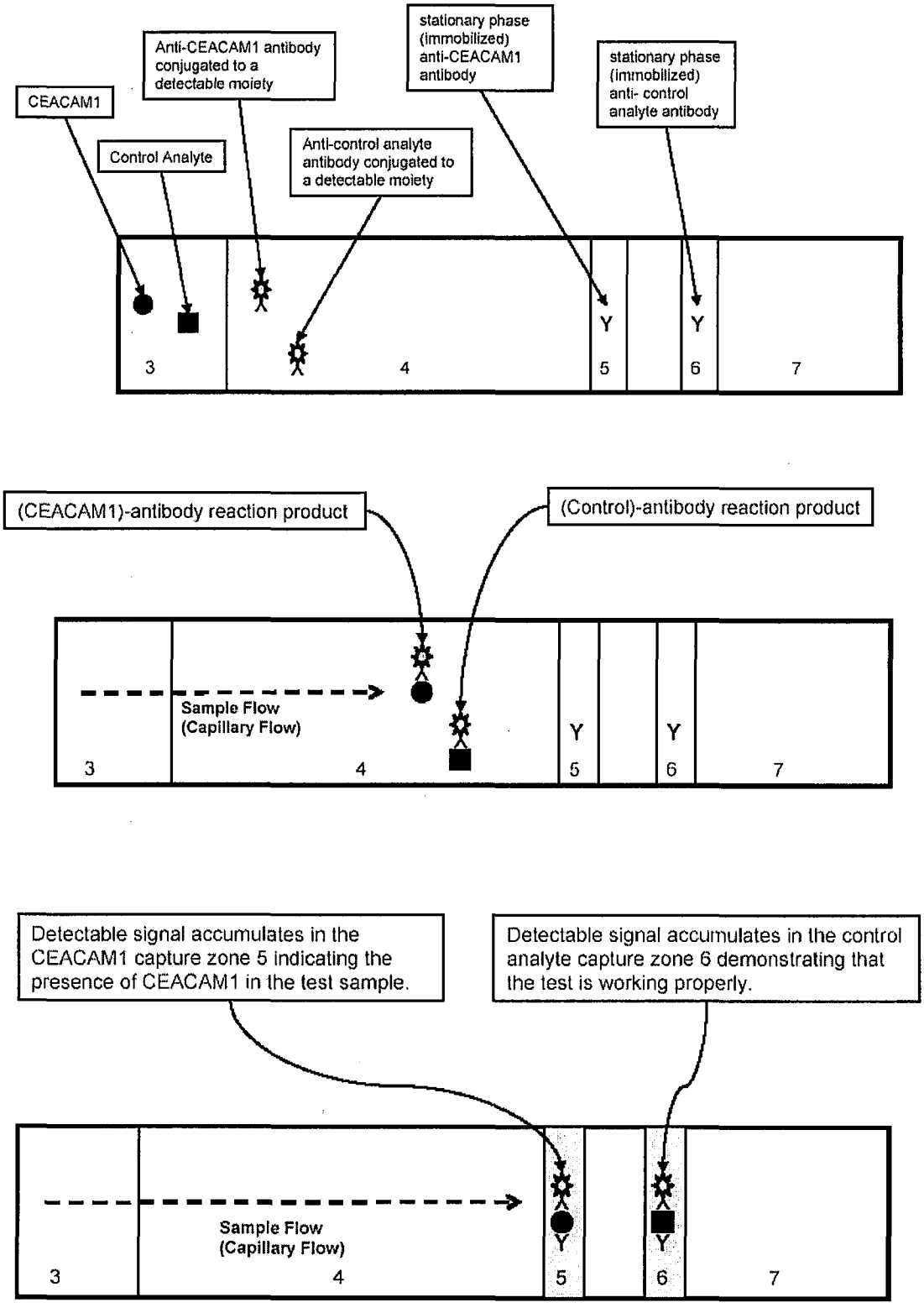


FIGURE 3


CEACAM1
Control Analyte
Anti-CEACAM1 antibody conjugated to a detectable moiety
Anti-control analyte antibody conjugated to a detectable moiety
stationary phase (immobilized) anti-CEACAM1 antibody
stationary phase (immobilized) anti- control analyte antibody
Y
Y
3
4
5
6
7
(CEACAM1)-antibody reaction product
(Control)-antibody reaction product
Sample Flow (Capillary Flow)
Y
Y
3
4
5
6
7
Detectable signal accumulates in the CEACAM1 capture zone 5 indicating the presence of CEACAM1 in the test sample.
Detectable signal accumulates in the control analyte capture zone 6 demonstrating that the test is working properly.
Sample Flow (Capillary Flow)
Y
Y
3
4
5
6
7