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- (71) Applicants (for all designated States except US): **THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA** [US/US]; 3160 Chestnut Street, Suite 200, Philadelphia, PA 19104 (US). **VERIDEX LLC** [US/US]; 1001 US Highway 202, Raritan, NJ 08869 (US).

Brad [US/US]; 21 Edinboro Circle, Chalfont, PA 18914 (US).

(74) Agents: **BAK, Mary, E.** et al.; Howson & Howson LLP, 501 Office Center Drive, Suite 210, Fort Washington, PA 19034 (US).

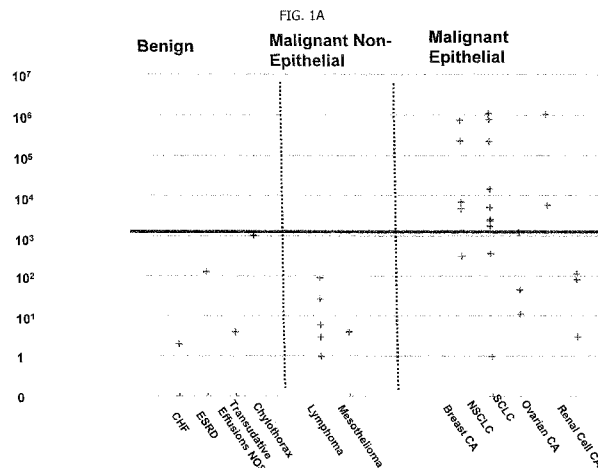
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- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **ALBELDA, Steven, M.** [US/US]; 135 S. 19th Street, Apt. 1603, Philadelphia, PA 19103 (US). **SCHWED, Daniel** [VE/US]; 210 Locust Street, #10G, Philadelphia, PA 19106 (US). **RAO, Galla, Chandra** [US/US]; 9 Haverford Road, Princeton Junction, NJ 08550 (US). **CONNELLY, Mark** [US/US]; 4770 Nottingham Way, Doylestown, PA 18902 (US). **FOULK,**

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[Continued on next page]

(54) Title: METHODS FOR DIAGNOSING CANCER BY CHARACTERIZATION OF TUMOR CELLS ASSOCIATED WITH PLEURAL OR SEROUS FLUIDS



(57) Abstract: A method for diagnosing or differentially diagnosing a cancer characterized by the presence of cancer cells in the pleural fluid of a mammalian subject, the method comprising contacting a sample of pleural fluid of the subject with colloidal magnetic particles coupled to a ligand which binds to a determinant on a cancer cell, but does not bind above a baseline threshold to other cellular and non-cellular components in pleural fluid; subjecting the pleural fluid-magnetic particle mixture to a magnetic field to produce a cell fraction enriched in ligand coupled-magnetic particle-bound cancer cells, if present in the pleural fluid; and analyzing the enriched fraction for the number of cancer cells in the pleural fluid. In certain aspects, this method involves preparing the pleural fluids for the above-noted method steps by, e.g., dilution of unprocessed pleural fluid. In certain aspect, the pleural fluid is subjected to the diagnostic method within 24 hours of withdrawal from the subject. This method has advantages to present diagnostic procedures for identifying malignant pleural effusions. The tumor cells present in pleural fluid can be characterized with cellular and molecular markers to determine prognostic and predictive factors.

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Published:

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- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

18 April 2013

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/048452

A. CLASSIFICATION OF SUBJECT MATTER
 INV. G01N33/574 G01N15/00
 ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MASAHIRO HORIKAWA ET AL: "Clinical significance of intraperitoneal CD44 mRNA levels of magnetically separated CD45-negative EpCAM-positive cells for peritoneal recurrence and prognosis in stage II and III gastric cancer patients", ONCOLOGY REPORTS, vol. 25, no. 5, 18 February 2011 (2011-02-18), pages 1413-1420, XP55040693, ISSN: 1021-335X, DOI: 10.3892/or.2011.1191	1,2, 4-10, 13-15, 18-21, 23-27
A	the whole document, in particular abstract; page 1414, column 1, 2nd and 3rd full paragraph ----- -/--	3,11,12, 16,17,22

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 February 2013

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Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040,
 Fax: (+31-70) 340-3016

Authorized officer

Weber, Peter

INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>"Medical diagnosis", Wikipedia, 30 June 2011 (2011-06-30), pages 1-7, XP55040777, Retrieved from the Internet: URL:en.wikipedia.org/wiki/Medical_diagnosi s [retrieved on 2012-10-11] the whole document</p>	1-27
X	<p>----- SAKAGUCHI M ET AL: "DEVELOPMENT OF A SENSITIVE, SPECIFIC REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION-BASED ASSAY FOR EPITHELIAL TUMOUR CELLS IN EFFUSIONS", BRITISH JOURNAL OF CANCER, vol. 79, no. 3/4, 1 February 1999 (1999-02-01), pages 416-422, XP000944490, ISSN: 0007-0920, DOI: 10.1038/SJ.BJC.6690065</p>	1-7,9, 12-15, 17,20-27
A	<p>the whole document, in particular abstract; page 417, column 1, section "patient characteristics", column 2, sections "preparation of cells from effusions" and "selection of tumour cells by ..."; table 1</p>	8,10,11, 16,18,19
X	<p>----- J. M. LLOYD ET AL: "Identification of Early-Stage Colorectal Cancer Patients at Risk of Relapse Post-Resection by Immunobead Reverse Transcription-PCR Analysis of Peritoneal Lavage Fluid for Malignant Cells", CLINICAL CANCER RESEARCH, vol. 12, no. 2, 15 January 2006 (2006-01-15), pages 417-423, XP55040805, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-05-1473</p>	1,2,4-7, 9,13-15, 20-27
A	<p>the whole document, in particular abstract; par. spanning pages 418 and 419; par. "immunobead RT-PCR" on page 419; figure 1</p>	3,8, 10-12, 16-19
X	<p>----- US 6 265 229 B1 (FODSTAD OSLASH YSTEIN [NO] ET AL) 24 July 2001 (2001-07-24) the whole document, in particular examples 1, 3, 6, 7, 9; claims 1, 2, 8-10, 15, 16, 19, 21, 22, 31-33, 37, 39 ----- -/--</p>	1-27

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/048452

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/024824 A1 (WOODSIDE STEVEN [CA] ET AL) 2 February 2006 (2006-02-02)	1-7,9, 13-15, 17,20
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INTERNATIONAL SEARCH REPORT

International application No

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEARS D ET AL: "THE CYTOLOGIC DIAGNOSIS OF MALIGNANT NEOPLASMS IN PLEURAL AND PERITONEAL EFFUSIONS", ACTA CYTOLOGICA, INTERNATIONAL ACADEMY OF CYTOLOGY, CHICAGO, IL, US, vol. 31, no. 2, 1 January 1987 (1987-01-01), pages 85-97, XP009163625, ISSN: 0001-5547 the whole document, in particular abstract -----	1-27
Y	TAYLOR IAN MITCHELL ET AL: "QUANTITATIVE ASSAY FOR CIRCULATING TUMOR STEM/PROGENITOR CELLS", JOURNAL OF CLINICAL PHARMACOLOGY, vol. 49, no. 9, September 2009 (2009-09), page 1127, XP009163529, & 38TH ANNUAL MEETING OF THE AMERICAN-COLLEGE-OF-CLINICAL-PHARMACOLOGY; SAN ANTONIO, TX, USA; SEPTEMBER 13 -15, 2009 ISSN: 0091-2700 the whole document, in particular line 8 of abstract -----	1-27
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INTERNATIONAL SEARCH REPORT

International application No

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>JAMES M REUBEN ET AL: "Chapter 73: Detection and Clinical Implications of Occult Systemic Micrometastatic Breast Cancer", 1 January 2009 (2009-01-01), THE BREAST: COMPREHENSIVE MANAGEMENT OF BENIGN AND MALIGNANT DISORDERS, 2-VOLUME SET, 4TH EDITION,, PAGE(S) 1203 - 1211, XP009163573, ISBN: 978-1-4160-5221-0 the whole document</p>	1-27
X,P	<p>J Thompson ET AL: "Use Of Circulating Tumor Cell Technology (cellsearch) For The Diagnosis Of Malignant Pleural Effusions", Am. J. Respir. Crit. Care Med., 1 May 2012 (2012-05-01), page A5336, XP55040260, Retrieved from the Internet: URL:http://ajrccm.atsjournals.org/cgi/reprint/185/1_MeetingAbstracts/A5336 [retrieved on 2012-10-08] the whole abstract</p>	1-27
X	<p>ERIC KIELHORN ET AL: "Use of magnetic enrichment for detection of carcinoma cells in fluid specimens", CANCER, vol. 94, no. 1, 1 January 2002 (2002-01-01), pages 205-211, XP055054000, ISSN: 0008-543X, DOI: 10.1002/cncr.10193</p>	1-7,9, 13-15, 18-27
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	<p style="text-align: center;">----- -/--</p>	

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International application No
PCT/US2012/048452

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>US 2007/025883 A1 (TAI YU-CHONG [US] ET AL) 1 February 2007 (2007-02-01) the whole document, in particular paragraphs [0056], [0080], [0081], [0116]-[0118], [0146]; figures 1, 2; claims 1, 4, 10, 13, 16, 18, 21-23</p> <p style="text-align: center;">-----</p>	28-31
A	<p>M FIEGL ET AL: "Sensitive detection of tumour cells in effusions by combining cytology and fluorescence in situ hybridisation (FISH)", BRITISH JOURNAL OF CANCER, vol. 91, no. 3, 29 June 2004 (2004-06-29), pages 558-563, XP055053853, ISSN: 0007-0920, DOI: 10.1038/sj.bjc.6601942 the whole document, in particular title and abstract</p> <p style="text-align: center;">-----</p>	23,29

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PCT/US2012/048452

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-27

A method for diagnosing or differentially diagnosing a cancer characterized by the presence of cancer cells in the pleural fluid or serous fluid of a mammalian subject, the method comprising: a) contacting a sample of pleural fluid or serous fluid of the subject with colloidal magnetic particles coupled to a first ligand which binds to a determinant on cancer cells, but does not bind above a baseline threshold to other cellular and non-cellular components in pleural fluid or serous fluid; and b) subjecting the pleural fluid-magnetic particle mixture or serous fluid-magnetic particle mixture to a magnetic field to produce a cell fraction enriched in ligand-coupled, magnetic particle-bound cells, if present in the pleural fluid or serous fluid.

2. claims: 28-31

A method for diagnosing or differentially diagnosing a cancer characterized by the presence of cancer cells in the pleural fluid or serous fluid of a mammalian subject, the method comprising: a) filtering a sample of pleural fluid or serous fluid of the subject with a filter comprising pores of a known essentially uniform pore size to enrich pleural effusion tumor cells (PETCs); b) analyzing the number of filtered PETCs; and c) providing a differential diagnosis of a cancer by identifying a number of filtered PETCs greater than the baseline threshold in the pleural fluid or serous fluid.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/048452

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