



(43) International Publication Date
6 December 2012 (06.12.2012)

(10) International Publication Number
WO 2012/166700 A3

- (51) **International Patent Classification:**
G01N 33/48 (2006.01)
- (21) **International Application Number:**
PCT/US2012/039814
- (22) **International Filing Date:**
29 May 2012 (29.05.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/491,218 29 May 2011 (29.05.2011) US
61/491,219 29 May 2011 (29.05.2011) US
- (72) **Inventor; and**
- (71) **Applicant : LISANTI, Michael P.** [US/US]; 1104 Rodman Street, Philadelphia, PA 19147 (US).
- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only): SOTGIA, Federica** [US/US]; 1104 Rodman Street, Philadelphia, PA 19147 (US).
- (74) **Agents: UDAL, Robert P.** et al.; Morgan, Lewis & Bockius LLP, 1701 Market Street, Philadelphia, PA 19103-2921 (US).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- 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))

- (88) **Date of publication of the international search report:**
24 January 2013

(54) **Title:** MOLECULAR PROFILING OF A LETHAL TUMOR MICROENVIRONMENT

(57) **Abstract:** Presented herein are useful biological markers related to cancers with stromal components. These newly identified biological markers are useful as prognostic signatures for assessing cancer prognosis in subject having cancer (e.g., risk of cancer recurrence, progression of a cancer in a cancer subject, overall survival of cancer by a cancer patient). They can also be implemented as a method of monitoring progression of breast cancer in subjects undergoing treatment for cancer or as a method of stratifying a subject or a group of subjects having a cancer tumor for a clinical trial.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/39814

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48 (2012.01)

USPC - 436/64, 94; 435/6.14

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)

USPC: 436/64, 94; 435/6.14

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

USPC: 436/64, 94; 435/6.14

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

Electronic data bases: PatBase (EU, WO, US); Google Scholar

Search terms: breast cancer, stromal, caveolin (Cav1, Cav-1), cavelolin -/-, Cav-1 deficient breast cancer, biomarker, signature, transgenic, upregulated, down regulated, prognostic, progression, stratification, estrogen receptor positive (ER+)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SOTGIA et al. Caveolin-1-/- Null Mammary Stromal Fibroblasts Share Characteristics with Human Breast Cancer-Associated Fibroblasts. Am J Pathol March 2009 Vol 174 No 3 Pages 746-761. Especially abstract, pg 751 table 1, pg 752 fig 4, pg 758 fig 14, pg 759 table 5.	1, 3, 5, 6, 8-10, 12, 13, 19 - 21, 24-28, 30, 31
X,P	WITKIEWICZ et al. Molecular profiling of a lethal tumor microenvironment, as defined by stromal caveolin-1 status in breast cancers. Cell Cycle ePub 1 June 2010 Vol 10 No 11 Pages 1794-1809. Available on the internet: <URL: http://www.landesbioscience.com/journals/cc/article/15675/ >. Especially pg 1795 col 2 para , Supplemental Table 1.	1, 3, 5, 6, 8-10, 12, 13, 19 - 21, 24-28, 30, 31
A	WO 2010/096574 A1 (LISANTI et al.) 26 August 2010 (26.08.2010). Especially para [0011]-[0032].	1, 3, 5, 6, 8-10, 12, 13, 19 - 21, 24-28, 30, 31
A	WITKIEWICZ et al. "Towards a new "stromal-based" classification system for human breast cancer prognosis and therapy" Cell Cycle; June 2009; Vol. 8, No. 11; pg. 1654-1658.	1, 3, 5, 6, 8-10, 12, 13, 19 - 21, 24-28, 30, 31

☐ Further documents are listed in the continuation of Box C.

* 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

29 October 2012 (29.10.2102)

Date of mailing of the international search report

03 DEC 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/39814

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:

- Please see extra 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 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.:
1, 3, 5 (limited to up-regulated transcripts), 6, 8, 9, 10, 12, 13, 19-21, 24-26, 30 and 31

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.

Continuation of Box III: Lack of Unity of Invention

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: claims 1, and 3-31, directed to a prognostic signature (also referred to herein as a biological marker or biomarker) for assessing cancer prognosis in a subject (e.g., risk of cancer recurrence, progression of a cancer in a cancer subject, overall survival of cancer by a cancer patient), said prognostic signature comprising a tumor/cancer stromal signature, wherein the tumor/stromal stromal signature is derived from a genome-wide transcriptional profiling of a tumor/cancer stromal material derived from a biological sample obtained from a set of at least one or more subjects having cancer, said tumor stromal signature comprising a first set of gene transcripts that is up-regulated in the tumor/cancer stromal material and/or a second set of gene transcripts that is down-regulated in the tumor stromal material, further wherein the gene transcripts are a set of one or more gene transcripts up-regulated in Cav-1 deficient tumor/cancer having gene names and/or gene symbols listed in Supplemental Tables 1, 3 or combinations thereof; wherein the first invention is limited to the first gene listed in Supplemental Table 1, Homo sapiens hypothetical protein LOC100293390 (claims 1, 3, 5 (limited to the portion comprising a signature of up-regulated transcripts), 6, 8, 9, 10, 12, 13, 19-21, 24-28, 30 and 31) (applicants may opt for additional markers to be searched, including CAV-1 (i.e. CAV-1 positive cells instead of CAV-1 negative cells) by specifying the marker, and paying an additional invention search fee for each elected marker).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The common technical element shared by all of the above groups is that they are related to a prognostic signature (also referred to herein as a biological marker or biomarker) for assessing cancer prognosis in a subject (e.g., risk of cancer recurrence, progression of a cancer in a cancer subject, overall survival of cancer by a cancer patient), said prognostic signature comprising a tumor/cancer stromal signature, wherein the tumor/stromal stromal signature is derived from a genome-wide transcriptional profiling of a tumor/cancer stromal material derived from a biological sample, wherein the signature comprises genes which are up-regulated or down-regulated in Cav-1 deficient cells, or CAV-1 positive cells. These common technical elements do not represent an improvement over the prior art of "Caveolin-1-/- Null Mammary Stromal Fibroblasts Share Characteristics with Human Breast Cancer-Associated Fibroblasts" by SOTGIA et al., which teaches a prognostic signature (also referred to herein as a biological marker or biomarker) for assessing cancer prognosis in a subject (e.g., risk of cancer recurrence, progression of a cancer in a cancer subject, overall survival of cancer by a cancer patient), said prognostic signature comprising a tumor/cancer stromal signature, wherein the tumor/stromal stromal signature is derived from a genome-wide transcriptional profiling of a tumor/cancer stromal material derived from a biological sample obtained from a set of at least one or more subjects having cancer (abstract; "To establish a direct cause-and-effect relationship, mammary stromal fibroblasts (MSFs) were prepared from Cav-1-/- null mice and subjected to phenotypic analysis. Here, we provide evidence that Cav-1-/- MSFs share many characteristics with human cancer-associated fibroblasts. The Cav-1-/- MSF transcriptome significantly overlaps with human cancer associated fibroblasts; both show a nearly identical profile of RB/E2F-regulated genes that are up-regulated, which is consistent with RB inactivation. This Cav-1-/- MSF gene signature is predictive of poor clinical outcome in breast cancer patients treated with tamoxifen") said tumor stromal signature comprising a first set of gene transcripts that is up-regulated in the tumor/cancer stromal material (pg 751 table 1; "A. Genes up-regulated in both human breast CAFs and Cav-1-/-MSFs (55 genes); CENPK upregulated 2.0 fold, HMMR upregulated 3.3 fold, KIF2C upregulated 3.3 fold, MCM10 upregulated 2.4 fold, TYMS upregulated 3.5 fold). Sotgia further teaches a set of genes which are down-regulated in the same cells (Table 1b).

Therefore, the inventions of Groups I+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.