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(71) Applicant: **ATOSSA GENETICS INC.** [US/US]; 2345 Eastlake Avenue East, Suite 201, Seattle, WA 98102 (US).

(72) Inventors: **CHEN, Shu-Chih**; 2345 Eastlake Avenue East, Suite 201, Seattle, WA 98102 (US). **QUAY, Steven, C.**; 2345 Eastlake Avenue East, Suite 201, Seattle, WA 98102 (US).

(74) Agent: **HARWOOD, Melissa**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304 (US).

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— *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))*

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21 January 2016

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(54) Title: MOLECULAR MAMMOGRAPHY

(57) Abstract: The disclosure relates to methods of screening and diagnosing cancer in patients undergoing mammography.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/33827

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/574 (2015.01)
CPC - G01N 33/57415
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)
IPC(8): G01N 33/574 (2015.01)
CPC: G01N 33/57415

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: G01N 33/57415 (text search)
USCP: 435/7.23, 287.2, 422/69,436/501 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Patents; Google Scholar
Search terms: Mammography, nipple aspirate fluid, biomarker (e.g. CK5, CK7, CK14, CK18, antibody assay, collection device (breast pump), triple negative breast cancer, basal-like, treatment options, BI-RADS lesion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>----<br>Y | 2013/0115629 A1 (QUAY et al.) 9 May 2013 (09.05. 2013). Especially para [0007], [0009], [0046], [0057], [0065] | 1-6, 9, 10, 22, 23<br>----------<br>7, 8, 21, 24, 25, 29-38, 49-52 |
| Y | PERNICK. Breast-nonmalignant [online] 2 April 2014 [retrieved 29 September 2015]. Available on the internet: <URL: https://web.archive.org/web/20140402042105/http://www.pathologyoutlines.com/topic/breastnormal.html>Especially pg 2 para 1 | 7, 35 |
| Y | SUIJKERBUIJK et al. Oxytocin: bringing magic into nipple aspiration. Annal Oncol Oct 2007 Vol 18 No 10 Pages 1743-1744. Especially pg 1743 col 1 para 2-3. | 8, 36 |
| Y | US 2009/0171236 A1 (Davies et al.) 2 July 2009 (02.07.2009). Especially para [0118]. | 21, 29-38, 49-52 |
| Y | GELMON et al. Targeting triple-negative breast cancer: optimizing therapeutic outcomes. Annals Oncol September 2012 Vol 23 No 9 Pages 2223-2234. Especially pg 2226 col 1 para 3, pg 2230 col 2 para 2. | 24, 25, 51, 52 |

☐ 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 | Date of mailing of the international search report |
|---|---|
| 30 September 2015 (30.09.2015) | 24 NOV 2015 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# INTERNATIONAL SEARCH REPORT

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**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:
------Go to Extra Sheet for continuation--------

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.:
Claims 1-55 limited to CK5, doxorubicin HCl (Claims 1-10, 21-25, 29-38, 49-52)

**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.

INTERNATIONAL SEARCH REPORT

International application No.
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------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.

Group I+: Claims 1-55, drawn to a method of diagnosing, prognosing or classifying a breast disorder in an individual in need thereof. The method of diagnosis, prognosis or classification of a breast disorder will be searched to the extent that the at least one intraductal fluide biomarker encompasses the first named biomarker, CK5 (claims 10 and 38), and the treatment regimen is limited to the first named therapeutic regimen, the anthracycline therapeutic agent doxorubicin HCl (claims 25 and 52). It is believed that claims 1-10, 21-25, 29-38, 49-52 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass the biomarker CK5 and the therapeutic agent doxorubicin HCl. Additional biomarkers and therapeutic regiments will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected target gene(s). Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a miRNA biomarker miR-21 (Table 3) and a therapeutic regiment comprising the agent paclitaxel (claims 1-9, 11-19, 21-25, 29-37, 39-47, 49-52).

The inventions listed as Group 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:

Special Technical Features:

Among the inventions listed as Groups I+ are the specific biomarkers (proteins and miRNAs) and therapeutic regiments recited therein. Each invention requires a specific biomarker and therapeutic regiment, not required in any other inventions.

Common Technical Feature:

The common technical feature of Group I+ is diagnosing, prognosing or classifying a breast disorder comprising screening intraductal fluid obtained from a nipple of the individual during mammography for at least one biomarker associated with a breast disorder.

However, said common technical feature does not represent a contribution over the prior art, and is obvious over US 2010/0010072 Al to DMITROVSKY et al. (hereinafter "Dmitrovsky"), in view US 2009/0171236 Al (DAVIES).

Concerning Group I+, claim 1, Dmitrovsky teaches screening intraductal fluid obtained from a nipple of the individual during mammography for at least one biomarker associated with a breast disorder (para [0019]; "The invention furthermore provides protocols for clinical application of miRNA detection as diagnostic and/or prognostic tools which would permit detecting these diagnostic miRNAs with less invasive techniques than biopsy, such as in ductal fluid samples (from nipple aspirates or ductal lavage)"; para [0075]; "The downregulation of hsa-miR-451 and the upregulation of hsa-miR-200b, hsa-miR-200c, hsa-miR-221 and hsa-miR-222 are of significant interest, especially in relation to breast cancer, and especially the discovery of the downregulation of hsa-miR-451 is of great importance").

Concerning Group I+, claim 17, Dmitrovsky teaches a method for classifying an individual as having a breast disorder comprising: a) obtaining intraductal fluid from a nipple of the individual during Mammography (para [0019]);
b) detecting a presence of at least one miRNA thereof in the intraductal fluid sample (para [0019]); and
c) classifying the individual as having a breast disorder if the detected miRNA has a measured value above said threshold value (para [0075]; miR-200-b; miR-221) wherein the presence of the miRNA is detected when said miRNA has a measured value above a threshold value for the miRNA (para [0075])

Concerning Group I+, claim 18, Dmitrovsky teaches a method for classifying an individual as having a breast disorder comprising:
a) obtaining intraductal fluid from a nipple of the individual during mammography (para [0019]);
b) detecting a decreased presence of at least one miRNA in the intraductal fluid sample (para [0019]); and
c) classifying the individual as having a breast disorder if the detected miRNA has a measured value below said threshold value (para [0075]; miR-451); wherein the decreased presence of the miRNA is detected when said miRNA has a measured value below a threshold value for the miRNA (para [0075]).

Concerning Group I+, claim 19, Dmitrovsky teaches a method a method for classifying an individual as at risk for or having IDC (para [0096]; "Breast cancers can be classified histologically based upon the types and patterns of cells that compose them. Carcinomas can be invasive; extending into the surrounding stroma, or non-invasive; confined just to the ducts or lobules. Invasive carcinomas of the breast include: Infiltrating Ductal Carcinoma [i.e. IDC]") comprising a) obtaining intraductal fluid from a nipple of the individual during mammography (para [0019]); and b) detecting an altered expression of at least one miRNA (para [0019], [0075]).

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INTERNATIONAL SEARCH REPORT

International application No.
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Concerning Group I+, claim 29, Davies teaches a method of diagnosing or prognosing a breast disorder in an individual having a BI-RADS III or a BI-RADS IV lesion (para [0118]; BI-RADS I-IV classifications indicated]. Davies does not teach screening intraductal fluid sample obtained from a nipple of the individual during mammography for at least one biomarker associated with a breast disorder. However, Dmitrovsky teaches screening intraductal fluid sample obtained from a nipple of the individual during mammography for at least one biomarker associated with a breast disorder (para [0019], [0075]). It would have been obvious for an artisan of ordinary skill in the art to combine a BI-RADS examination and classification, as taught by Davies, with screening intraductal fluid sample obtained from a nipple of the individual during mammography for at least one biomarker associated with a breast disorder, as taught by Dmitrovsky, because it would have enabled multiple independent means to characterize a breast disorder.

Concerning Group I+, claim 45, Davies teaches a method for classifying an individual having a BI-RADS III or a BI-RADS IV lesion having a breast disorder (para [0118]). Davis does not teach claim limitations a), b) or c). However, Dmitrovsky teaches a) obtaining intraductal fluid from a nipple of the individual during Mammography (para [0019]);
b) detecting a presence of at least one miRNA thereof in the intraductal fluid sample (para [0019])
and c) classifying the individual as having a breast disorder if the detected miRNA
has a measured value above said threshold value (para [0075]; miR-200-b; miR-221) wherein the presence of the miRNA is detected when said miRNA has a measured value above a threshold value for the miRNA (para [0075]). It would have been obvious for an artisan of ordinary skill in the art to combine a BI-RADS examination and classification, as taught by Davies, with screening intraductal fluid sample obtained from a nipple of the individual during mammography for at least one elevated miRNA biomarker associated with a breast disorder, as taught by Dmitrovsky, because it would have enabled multiple independent means to characterize a breast disorder.

Concerning Group I+, claim 46, Davies teaches a method for classifying an individual having a BI-RADS III or a BI-RADS IV lesion having a breast disorder (para [0118]). Davis does not teach claim limitations a), b) or c). However, Dmitrovsky teaches a) obtaining intraductal fluid from a nipple of the individual during Mammography (para [0019]);
b) detecting a presence of at least one miRNA thereof in the intraductal fluid sample (para [0019])
and c) classifying the individual as having a breast disorder if the detected miRNA has a measured value below said threshold value (para [0075]; miR-451); wherein the decreased presence of the miRNA is detected when said miRNA has a measured value below a threshold value for the miRNA (para [0075]). It would have been obvious for an artisan of ordinary skill in the art to combine a BI-RADS examination and classification, as taught by Davies, with screening intraductal fluid sample obtained from a nipple of the individual during mammography for at least one decrease miRNA level biomarker associated with a breast disorder, as taught by Dmitrovsky, because it would have enabled multiple independent means to characterize a breast disorder.

Concerning Group I+, claim 47, Davies method for classifying an individual having a BI-RADS III or a BI-RADS IV lesion (para [0118]) as at risk for or having ADH or DCIS (para [0186]). Davies does not specifically teach claim limitation a) or b). However, Dmitrovsky further teaches a) obtaining intraductal fluid from a nipple of the individual during mammography (para [0019]); and b) detecting an altered expression of at least one miRNA (para [0075]). Additionally, Dmitrovsky teaches correlation with high grade ductal carcinomas (para [0096], [0343]. It would have been obvious for an artisan of ordinary skill in the art to combine method for classifying an individual having a BI-RADS III or a BI-RADS IV lesion as at risk for or having ADH or DCIS, with claim limitations a) and b), as taught by Dmitrovsky, because it would have provided multiple independent means to characterize a breast disorder.

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

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