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(54) **Title:** A PROTEIN EXPRESSION-BASED CLASSIFIER FOR PREDICTION OF RECURRENCE IN ADENOCARCINOMA

(57) **Abstract:** A method for making a prognosis for a patient afflicted with a type of cancer which comprises (a) determining in a sample of a tumor from the patient a level of expression for at least three biomarkers from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta- catenin and cyclin D1; (b) calculating a score based on the levels of expression determined in step (a); (c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with a series of prognoses; so as to thereby make a prognosis for the patient.



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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/26201

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48, 33/53 (2012.01)

USPC - 436/64; 435/6.1, 7.1

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 33/48, 33/53 (2012.01)

USPC - 436/64; 435/6.1, 7.1

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

USPC - 436/501, 86, 94; 435/6.11, 6.12

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

PubWest (PGPB,USPT,USOC,EPAB,JPAB); PubMed (MEDLINE)

marker, biomarker, prognosis, ttf1, stat3, beta catenin, cyclin D1, automat pathology, image analysis

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | US 2010/0240035 A1 (JABLONS et al.) 23 September 2010 (23.09.2010) para [0005], [0029], [0076], [0090], [0097]; Table 1; Table 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 1-7, 63               |
| Y         | NOZAWA et al., Immunohistochemical alpha- and beta-catenin and E-cadherin expression and their clinicopathological significance in human lung adenocarcinoma. Pathol Res Pract. (2006, published online 14 July 2006), vol 202, no 9, pp 639-50; abstract                                                                                                                                                                                                                                                                                                                                                                                  | 1-7, 63               |
| Y         | AU et al., Evaluation of immunohistochemical markers in non-small cell lung cancer by unsupervised hierarchical clustering analysis: a tissue microarray study of 284 cases and 18 markers. J Pathol. (September 2004) Vol 204, no 1, pp 101-9; abstract                                                                                                                                                                                                                                                                                                                                                                                   | 1-7, 63               |
| Y         | KIM et al. "Clinical Impact of Phosphorylated Signal Transducer and Activator of Transcription 3, Epidermal Growth Factor Receptor, p53, and Vascular Endothelial Growth Factor Receptor 1 Expression in Resected Adenocarcinoma of Lung by Using Tissue Microarray" Cancer; 1 February 2010; Vol 116, No. 3; pg. 676-685. Especially abstract                                                                                                                                                                                                                                                                                             | 2, 4, 5               |
| L         | KIM et al. "Clinical Impact of Phosphorylated Signal Transducer and Activator of Transcription 3, Epidermal Growth Factor Receptor, p53, and Vascular Endothelial Growth Factor Receptor 1 Expression in Resected Adenocarcinoma of Lung by Using Tissue Microarray" Cancer; 1 February 2010; Vol 116, No. 3; pg. 676-685. Abstract only [online]: downloaded from <a href="http://www.ncbi.nlm.nih.gov/pubmed/20052735">http://www.ncbi.nlm.nih.gov/pubmed/20052735</a> on 23 July 2012 in order to support the full bibliographic data for Kim et al., including volume and issue number, which were not present on the publication pdf. | 2, 4, 5               |

☒ 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

"&amp;" document member of the same patent family

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/26201

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                    |                       |
|-------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------|
| Category*                                             | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A                                                     | US 2006/0024692 A1 (NAKAMURA et al.) 2 February 2006 (02.02.2006)                  | 1-7, 63               |

## INTERNATIONAL SEARCH REPORT

International application No.

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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:

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 1-7 and 63, directed to a method for making a prognosis for a patient having a tumor associated with adenocarcinoma of the lung which comprises:

- a) measuring in a sample of the patient's tumor a level of expression for each of at least three biomarkers selected from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta-catenin and cyclin D1;
- b) calculating a score based on the level of expression measured in step (a) for each of the biomarkers; and
- c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with a series of prognoses, so as to thereby make a prognosis for the patient.

- Please see 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.:  
1-7 and 63

**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.

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## Continuation of Box III: Lack of Unity of Invention

Group II: claims 8-14, directed to a method for identifying a patient having a tumor associated with adenocarcinoma of the lung as having a 40% or less chance of survival after five years if treated only by surgery comprising:

- a) measuring in a sample of the patient's tumor a level of expression for at least three biomarkers selected from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta catenin and cyclin D1;
- b) calculating a score based on the levels of expression measured in step (a); and
- c) comparing the score obtained in step (b) with a predetermined reference score associated with probability of survival after five years if treated only by surgery, wherein if the score obtained in step (b) is greater than the predetermined reference score the patient has a 40% or less chance of survival after five years if treated only by surgery.

Group III: claims 15-22, directed to a method for determining the likelihood that a patient having a tumor associated with adenocarcinoma of the lung will not survive after five years if treated only by surgery:

- a) measuring in a sample of the patient's tumor a level of expression for at each of least three biomarkers selected from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta-catenin and cyclin D1;
- b) calculating a score based on the levels of expression measured in step (a); and
- c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with survival, so as to thereby determine the likelihood that the patient will not survive after five years if treated only by surgery.

Group IV: claims 23-42, and 70-73, directed to a method for determining the likelihood that a patient having a tumor associated with adenocarcinoma of the lung will have a higher chance of survival after surgery from adjuvant therapy in addition to the surgery comprising:

- a) measuring in a sample of the patient's tumor a level of expression for each of at least three biomarkers selected from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta-catenin and cyclin D1;
- b) calculating a score based on the levels of expression determined in step (a) and
- c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with survival, so as to thereby determine the likelihood that the patient will have a higher chance of survival after surgery from adjuvant therapy in addition to surgery.

Group V: claims 43-58, directed to a method for determining the likelihood that a patient having a tumor associated with adenocarcinoma of the lung will have a higher chance of survival after surgery from adjuvant therapy in addition to the surgery comprising:

- a) measuring a total level of expression of cyclin D1 within a nuclear compartment and non-nuclear compartments combined in cells of interest in a tumor sample from the patient;
- b) measuring a total level of expression of signal transducer and activator of transcription-3 (STAT-3) within the nuclear compartment and the non-nuclear compartments combined in cells of interest in the sample from the patient;
- c) measuring a level of expression of thyroid transcription factor-1 (TTF1) in the nuclear compartment in cells of interest in the sample from the patient;
- d) measuring a level of expression of beta-catenin cytoplasmic compartment in cells of interest in a sample the from the patient;
- e) calculating a score based on the levels of expression determined in steps (a) through (d); and
- f) correlating the score obtained in step (b) with a series of predetermined reference scores associated with survival, so as to thereby determine the likelihood that the patient will have a higher chance of survival after surgery from adjuvant therapy in addition to surgery.

Group VI: claims 59-61, directed to a kit comprising at least three of the following:

- a) a stain specific for thyroid transcription factor-1 (TTF1);
- b) a stain specific for signal transducer and activator of transcription-3 (STAT-3);
- c) a stain specific for beta-catenin;
- d) a stain specific for cyclin D1; and
- e) instructions for using the kit.

Group VII: claim 62, directed to a non-transitory computer readable medium having program code recorded thereon that, when executed on a computing system, automatically processes data, the program code comprising: a) code for processing a digital microscopy image of a stained tumor specimen taken from a cancer patient to extract data related to intensity values associated with one or more stains; b) code for processing the extracted data to arrive at a value for intensity per pixel for each of the one or more stains; c) code for processing pixel intensity of at least one stain for determining pixels associated with a preselected subcompartment and determining the area of the subcompartment for use as a denominator; d) code for processing pixel intensity of a second stain for determining an expression level of a biomarker and a value for total biomarker intensity in the same preselected subcompartment for use as a numerator; e) code for calculating from the numerator and denominator a score of the biomarker expression per area; f) code for collecting the score of each of at least three of the following four biomarkers, including thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta-catenin and cyclin D1; and g) code for incorporating the scores of the at least three biomarkers into a score model for arriving at a prognostic score.

Group VIII: Claims 64 and 65, directed to a method for classifying a patient diagnosed with cancer as being at a high risk for a recurrence of cancer comprising:

- a) determining in a sample of a tumor from the patient a level of expression for at least three biomarkers from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta catenin and cyclin D1;
- b) calculating a score based on the levels of expression determined in step (a) and
- c) comparing the score obtained in step (b) with a predetermined reference score cutpoint separating high risk from low risk patients, wherein the patient is at a high risk of developing a recurrence of cancer if the score obtained in step (b) is greater than the predetermined reference score cutpoint.

- Please see next extra sheet for continuation -

## INTERNATIONAL SEARCH REPORT

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Continuation of: First Extra Sheet: Lack of Unity of Invention

Group IX: claims 66-69, directed to a method for determining the likelihood that a patient diagnosed with cancer will benefit from adjuvant therapy comprising:

a) determining in a sample of a tumor from the patient a level of expression for at least three biomarkers from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta catenin and cyclin D1;

b) calculating a score based on the levels of expression determined in step (a) and

c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with a series of high risk and low risk patients, so as to thereby determine the likelihood that the patient will benefit from adjuvant therapy.

The inventions listed as Groups I - IX 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 special technical features of the claims of Groups I-IX are indicated above in the Group descriptions.

The only common technical element shared by all of the above groups is that they are related to at least three of the four following markers: Cyclin D1, STAT-3, beta catenin and TTF1. In Groups I-V and VII-IX, the levels of expression of at least three of the markers are related to diagnosis or prognosis associated with a cancer. Groups II and III are related to prognosis of survival after 5 years. Groups IV and V share the common technical elements of being related to a method for determining the likelihood that a patient having a tumor associated with adenocarcinoma of the lung will have a higher chance of survival after surgery from adjuvant therapy in addition to the surgery comprising: a) measuring in a sample of the patient's tumor a level of expression for each of at least three biomarkers selected from the following group: thyroid transcription factor-1 (TTF1), signal transducer and activator of transcription-3 (STAT-3), beta-catenin and cyclin D1; b) calculating a score based on the levels of expression determined in step (a) and c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with survival, so as to thereby determine the likelihood that the patient will have a higher chance of survival after surgery from adjuvant therapy in addition to surgery; wherein Group V is further specific to measurement of the levels of some of the markers in specific cell compartments. Group IX shares many common technical elements with Groups IV and V, but is not specific to producing a higher chance of survival.

These common technical elements do not represent an improvement over the combined prior art of US 2010/0240035 A1 to Jablons et al. (hereinafter "Jablons") in view of the article entitled "Evaluation of immunohistochemical markers in non-small cell lung cancer by unsupervised hierarchical clustering analysis: a tissue microarray study of 284 cases and 18 markers" by Au et al. (hereinafter "Au"), further in view of the article entitled "Immunohistochemical alpha- and beta-catenin and E-cadherin expression and their clinicopathological significance in human lung adenocarcinoma" by Nozawa et al. (hereinafter "Nozawa").

In particular, Jablons teaches a method of predicting the likelihood a patient having a tumor associated with adenocarcinoma of the lung (methods for providing a prognosis for lung adenocarcinoma; abstract) will not survive after five years (para [0090]) if treated only by surgery (We identified a risk score based on the gene expression of 4-genes by quantitative PCR that is prognostic of long-term survival in patients with completely surgically resected lung adenocarcinoma. This gene expression-based score predicted survival (adjusted HR 6.7) and disease recurrence better than clinical stage and tumor size. Our model best predicted risk of death among patients with stage I disease. In our cross-validated cohort, low- and high-risk scores were associated with 5-year overall survival of 87% and 38% among stage I patients; para [0101] - without adjuvant chemotherapy; para [0097]), or is treated with adjuvant therapy (chemotherapy; para [0090] - a bioassay that is both prognostic and predictive of chemotherapy benefit; para [0097]), comprising:

a) measuring in a sample of the patient's tumor a level of expression for at each of least three biomarkers (para [0005]) and b) calculating a score based on the levels of expression measured (para [0029], [0090]) in step (a). Although Jablons does not specifically recite the method comprising:

c) correlating the score obtained in step (b) with a series of predetermined reference scores associated with survival, Jablons teaches comparing the expression with control, non-cancerous cell samples (para [0005]), as well as wherein "Prediction and prognosis involve determining the level of a panel of lung cancer biomarker polynucleotide or the corresponding polypeptides in a patient or patient sample and then comparing the level to a baseline or range. Typically, the baseline value is representative of levels of the polynucleotide or nucleic acid in a healthy person not suffering from, or destined to develop, lung cancer, as measured using a biological sample such as a lung biopsy or a sample of a bodily fluid. Variation of levels of a polynucleotide or corresponding polypeptides of the invention from the baseline range (either up or down) indicates that the patient has an increased risk of long term mortality" (para [0076]). It would have been obvious to a person skilled in the art to determine the marker levels in long-term survivors of surgical and adjuvant-therapy recipients, as taught by Jablons, in order to determine a range of markers associated with low risk of post-surgical death, or survival after 5 years, based on the teaching of Jablons.

Although Jablons includes the assessment of cyclin D1 (Table 1) and beta catenin (Table 5) these are only two of a large number of markers assessed by Jablons. However, in a related article, Au teaches the use of cyclin D1, and TTF-1 in a much smaller panel of biomarkers for non-small cell lung carcinoma (abstract), which is the broader category that includes lung adenocarcinoma. Further, in another related article, Nozawa teaches the use of beta-catenin in the assessment of lung adenocarcinoma (abstract). It would have been obvious to a person skilled in the art to use the specific publications of Au and Nozawa to narrow down the marker selections taught by Jablons to produce a method of detecting, diagnosing or prognosing adenocarcinoma, and associated parameters, such as projections for life expectancy following surgery alone, or surgery in combination with adjunct therapy that comprises the measurement of the expression of said markers, and calculation of a score based thereon, based on the teaching of Jablons.

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