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(71) Applicants *(for all designated States except US)*: **CHILDREN'S MEDICAL CENTER CORPORATION** [US/US]; 300 Longwood Avenue, Boston, MA 02115 (US). **BETH ISRAEL DEACONESS MEDICAL CENTER, INC.** [US/US]; 330 Brookline Avenue, Boston, MA 02215 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BRISCOE, David, M.** [US/US]; 6 Pleasant Street, Sharon, MA 02067 (US). **DALY, Kevin, P.** [US/US]; 14 Rustic Street, Newton, MA 02458 (US). **KARUMANCHI, Ananth** [US/US]; 117 Woodcliff Road, Chestnut Hill, MA 02467 (US). **SEIFERT, Michael** [US/US]; 7720 Shirley Drive, Unit 1E, Clayton, MO 63105 (US).

(74) Agent: **EL-HAYEK, Roque**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

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[Continued on next page]

(54) Title: NON-INVASIVE METHODS FOR DIAGNOSING CHRONIC ORGAN TRANSPLANT REJECTION

(57) Abstract: Presented herein are methods of diagnosing or assessing an individual at increased risk of developing chronic rejection, or chronic allograft vasculopathy, based on analysis the individual's biomarker profile.

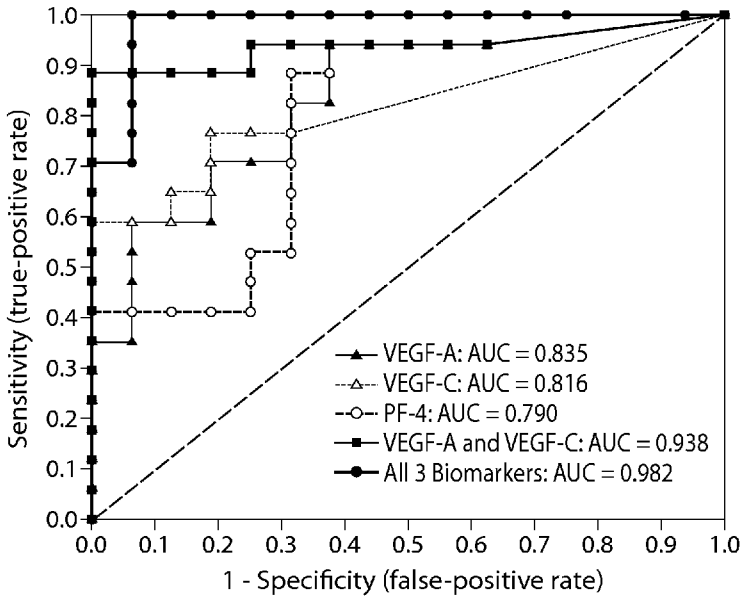

Fig. 2

WO 2012/122374 A3

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).

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— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:
10 January 2013

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/28275

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53; G01N 33/567; C12M 3/00 (2012.01)
USPC - 435/7.2
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 33/53; G01N 33/567; C12M 3/00 (2012.01)
USPC - 435/7.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/287.2
(Text Search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, USOC, EPAB, JPAB) and Google Scholar.
Search Terms: VEGF, VEGF-C, organ transplant, transplan$, rejec$, saliva, urine, sample, blood, serum, plasma.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0267956 A1 (ALITALO et al.) 30 October 2008 (30.10.2008) para [0073], [0074], [0437], [0438].	1-2, 15 and 18-21
A	Nykanen et al. 'Angiopoietin-1 Protects Against the Development of Cardiac Allograft Arteriosclerosis' Circulation vol 107 pg 1308-1314 (2003) whole doc.	1-2, 15 and 18-21

☐ 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
27 August 2012 (27.08.2012)	11 SEP 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

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/28275

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.: 5-14, 22-27, 31-47, 52-66, 70-82, 86-98, 102 and 106
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 Supplemental Boxes

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 1-4 and 15-21 limited to VEGF-C, namely, claims 1-2, 15 and 18-21

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.

PCT/US 12/28275

Continuation of

Box No. III - Observations where unity of invention is lacking:

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 1-4 and 15-21, drawn to a method, comprising:
(i) determining a level(s) of vascular endothelial growth factor-C (VEGF-C), vascular endothelial growth factor-A (VEGF-A), platelet factor-4 (PF4), Artemin, urokinase plasminogen activator (uPA), Vasohibin, or Angiopoietin-2, or any combination thereof in a sample from an organ transplant recipient individual;
(ii) comparing the level(s) of VEGF-C, VEGF-A, PF4, Artemin, uPA, Vasohibin, or Angiopoietin-2, or any combination thereof in the sample to a predetermined value(s) for VEGF-C, VEGF-A, PF4, Artemin, uPA, Vasohibin, or Angiopoietin-2, or any of the combinations thereof, respectively; and
(iii) identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level(s) of VEGF-C, VEGF-A, PF4, Artemin, uPA, Vasohibin, or Angiopoietin-2, or any of the combinations thereof in the sample is above the predetermined value(s) for VEGF-C, VEGF-A, PF4, Artemin, uPA, Vasohibin, or Angiopoietin-2, or any of the combinations thereof, respectively. The first invention is restricted to the first named biomarker, VEGF-C [namely, claims 1-2, 15 and 18-21]. Should an additional fee(s) be paid, Applicant is invited to elect an additional biomarker(s) to be searched. The exact claims searched will depend on Applicant's election.

Group II+: claims 28-30 and 48-51, drawn to a method, comprising:
(i) determining a level of vascular endothelial growth factor-C (VEGF-C) in a sample from an organ transplant recipient individual;
(ii) comparing the level of VEGF-C in the sample to a predetermined value for VEGF-C; and
(iii) identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level of VEGF-C in the sample is above the predetermined value for VEGF-C; or determining a level of a biomarker other than VEGF-C if the level of VEGF-C in the sample is at or below the predetermined for VEGF-C to identify if the individual has or is at increased risk of developing chronic organ transplant failure. The first invention is restricted to the first named biomarker, VEGF-C. Should an additional fee(s) be paid, Applicant is invited to elect an additional biomarker(s) to be searched. The exact claims searched will depend on Applicant's election.

Group III: claims 67-69 and 83-85, drawn to a method, comprising:
(i) determining a level of vascular endothelial growth factor-A (VEGF-A) in a sample from an organ transplant recipient individual;
(ii) comparing the level of VEGF-A in the sample to a predetermined value for VEGF-A;
(iii) comparing a level of Angiostatin in the sample to a predetermined value for Angiostatin if the level of VEGF-A in the sample is above the predetermined value for VEGF-A, or comparing a level of Angiopoietin-I in the sample to a predetermined value for Angiopoietin-I if the level of VEGF-A in the sample is at or below the predetermined value for VEGF-A; and
(iv) identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level of Angiostatin in the sample is at or below the predetermined value for Angiostatin or if the level of Angiopoietin-I in the sample is at or below the predetermined value for Angiopoietin-1.

Group IV: claims 99-101 and 103-105, drawn to a kit, comprising:
(i) means for measuring a protein level(s) or a nucleic acid level(s) of VEGF-C, VEGF-A, or PF4, or any combination thereof;
(ii) a predetermined value(s) for VEGF-C, VEGF-A, or PF4, or any of the combinations thereof; and
(iii) instructions for comparing the protein level(s) or the nucleic acid level(s) of VEGF-C, VEGF-A, or PF4, or any of the combinations thereof to the predetermined value(s) for VEGF-C, VEGF-A, or PF4, or any of the combinations thereof, wherein (i) (iii) are packaged into one unit.

The inventions listed as Groups I+ through IV 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 inventions of Groups IV do not include the inventive concept of identifying the individual as having or at increased risk of developing chronic organ transplant rejection, as required by Group I+-III.

The inventions of Group I+-II+ do not include the inventive concept of the use of a combination biomarkers VEGF-A/angiostatin or VEGF -A/angiopoietin-1 for identifying the individual as having or at increased risk of developing chronic organ transplant rejection, as required by Group III.

The inventions of Group II+ do not include the inventive concept of the use of biomarkers VEGF-A, PF4, Artemin, uPA, Vasohibin, or Angiopoietin-2 for identifying the individual as having or at increased risk of developing chronic organ transplant rejection, as required by Group I+.

**********Continued on Next Page**********

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The inventions of Groups I+-II+ share the technical feature of the use of biomarker VEGF-C for identifying the individual as having or at increased risk of developing chronic organ transplant rejection. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2008/0267956 A1 to Alitalo et al. (hereinafter 'Alitalo'). Alitalo discloses the method of Claim 28, comprising:
--determining a level of vascular endothelial growth factor-C (VEGF-C) in a sample from an organ transplant recipient individual (para [0074]-'screening an organ transplant recipient mammal to identify elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PlGF, PDGF-A, PDGF-B, PDGF-C, and PDGF-D polypeptides. In some embodiments, the screening step comprises obtaining a serum sample, a fluid sample, or a tissue sample from the transplanted organ and detecting elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C', [0438]);
--comparing the level of VEGF-C in the sample to a predetermined value for VEGF-C (para [0074]-detecting elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C', said 'elevated level' inherently indicates the comparison); and
--identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level of VEGF-C in the sample is above the predetermined value for VEGF-C (para [0073]-[0074], [0437]-[0438]-'The density of VEGF-C.sup.+ cells was similar in non-transplanted hearts, acutely rejecting cardiac allografts, and syngenic controls, whereas myocardial VEGF-C+density was two times higher in cardiac allografts undergoing chronic rejection'). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Groups I+-III share the technical feature of the use of a combination of biomarkers for identifying the individual as having or at increased risk of developing chronic organ transplant rejection. However, this shared technical feature does not represent a contribution over prior art as being obvious over Alitalo in view of the article titled 'Angiopoietin-1 Protects Against the Development of Cardiac Allograft Arteriosclerosis' (published in Circulation, 17 February 2003, Vol 107, pages 1308-1314) by Nykanen, A.I, et al. (hereinafter 'Nykanen'). Alitalo teaches a method of Claim 67, comprising:
--determining a level of vascular endothelial growth factor-A (VEGF-A) in a sample from an organ transplant recipient individual (para [0074]-'screening an organ transplant recipient mammal to identify elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PlGF, PDGF-A, PDGF-B, PDGF-C, and PDGF-D polypeptides. In some embodiments, the screening step comprises obtaining a serum sample, a fluid sample, or a tissue sample from the transplanted organ and detecting elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C', [0438]);
--comparing the level of VEGF-A in the sample to a predetermined value for VEGF-C (para [0074]-detecting elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C', said 'elevated level' inherently indicates the comparison); and
--identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level of VEGF-A in the sample is above the predetermined value of VEGF-A (para [0073]-[0074]-'a method for inhibiting allograft rejection or graft-related arteriosclerosis comprising administering to a mammalian subject in need of said inhibition a binding construct according to the invention, in an amount effective to inhibit the allograft rejection or the arteriosclerosis', 'The method may also comprise the step of screening an organ transplant recipient mammal to identify elevated levels of at least one growth factor selected from the group consisting of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PlGF, PDGF-A, PDGF-B, PDGF-C, and PDGF-D polypeptides', para [0362]).

Alitalo does not expressly disclose that the method further comprises comparing a level of angiopoietin-1 in the sample to a predetermined value for Angiopoietin-1 if the level of VEGF-A in the sample is at or below the predetermined value for VEGF-A; and identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level of Angiopoietin-1 is at or below the predetermined value of Angiopoietin-1. However, Nykanen teaches a method comprising:
--determining the level of Angiopoietin-1 in a sample from an organ transplant recipient individual (fig 1A; abstract-'Immunohistochemistry disclosed that only a few mesenchymal cells expressed Ang1 in normal hearts and syngrafts, whereas no immunoreactivity was found in cardiac allografts undergoing chronic rejection'; pg 1309, col, para 2);
--comparing the level of angiopoietin-1 in the sample to a predetermined value for Angiopoietin-1 (fig 1A; abstract-"Immunohistochemistry disclosed that only a few mesenchymal cells expressed Ang1 in normal hearts and syngrafts, whereas no immunoreactivity was found in cardiac allografts undergoing chronic rejection'); and
-- identifying the individual as having or at increased risk of developing chronic organ transplant rejection if the level of Angiopoietin-1 is at or below the predetermined value of Angiopoietin-1 (fig 1A; abstract-"Immunohistochemistry disclosed that only a few mesenchymal cells expressed Ang1 in normal hearts and syngrafts, whereas no immunoreactivity was found in cardiac allografts undergoing chronic rejection').

Thus one of ordinary skill in the art would have found it obvious to incorporate said relationship between angiopoietin-1 level and chronic organ transplant rejection into the method disclosed by Alitalo to develop a method aiming to capture more instances of chronic organ transplant rejection, wherein an individual is determined to have or to be at increased risk of chronic organ transplant rejection if the level of VEGF-A from a sample from said individual is above a predetermined level of VEGF-A, but if said VEGF-A level from said sample is at or below said predetermined level of VEGF-A, a further test of angiopoietin-1 level is determined to further decide whether said individual has or is at increased risk of chronic organ transplant rejection, such a two-tiered method would allow for more sensitive test with reduced likelihood of missing subjects with chronic organ transplant rejection or at increased risk of said chronic organ transplant rejection. As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Groups I+-IV share the technical feature of means for measuring a protein level(s) or a nucleic acid level(s) of VEGF-C, VEGF-A, or PF4, or any combination thereof; and (ii) a predetermined value(s) for VEGF-C, VEGF-A, or PF4, or any combination thereof. However, this shared technical feature does not represent a contribution over prior art, because Alitalo teaches a mean for measuring a protein level of VEGF-C and (ii) a predetermined value for VEGF-C (para [0438]). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

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Another special technical feature of the inventions listed as Groups I+ and II+ is the specific biomarkers recited therein. The inventions do not share a special technical feature, because no significant similarities regarding the roles of biomarkers in chronic transplant rejection can readily be ascertained. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ through IV therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of Section 4:

Claims 5-14, 22-27, 31-47, 52-66, 70-82, 86-98, 102 and 106 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).