(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 March 2018 (08.03.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/045078 A3

(51) International Patent Classification:
A61K 31/436 (2006.01) *G01N 33/48* (2006.01)
A61K 31/395 (2006.01) *C12Q 1/68* (2018.01)

(21) International Application Number:
PCT/US2017/049453

(22) International Filing Date:
30 August 2017 (30.08.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/382,147 31 August 2016 (31.08.2016) US

(71) Applicant: **THE CHILDREN'S HOSPITAL OF PHILADELPHIA** [US/US]; 3401 Civic Center Boulevard, Philadelphia, PA 19104 (US).

(72) Inventors: **HAKONARSON, Hakon**; 1877 Covered Bridge Road, Malvern, PA 19355 (US). **LI, Dong**; 75 Drexelbrook Drive, Apt. 6, Drexel Hill, PA 19026 (US). **TIAN, Lifeng**; 922 Morningdale Drive, Wilmington, DE 19810 (US). **NGUYEN, Kenny**; 18 Overlea Lane, Aberdeen, NJ 07747 (US). **SLEIMAN, Patrick**; 2411f Delancey Street, Philadelphia, PA 19103 (US).

(74) Agent: **RIGAUT, Kathleen, D.** et al.; Dann, Dorfman, Herrell & Skillman, 1601 Market Street, Suite 2400, Philadelphia, PA 19103-2307 (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, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, ST, 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, KM, ML, MR, NE, SN, TD, TG).

(54) Title: COMPOSITIONS AND METHODS FOR THE DIAGNOSIS AND TREATMENT OF LYMPHATIC SYSTEM DISORDERS

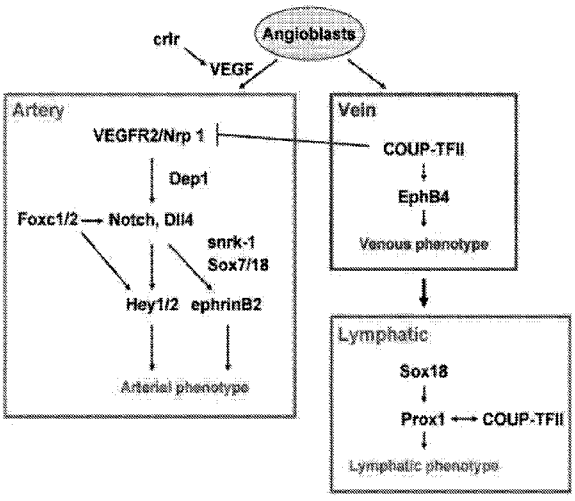


Figure 2

(57) Abstract: Compositions and methods for the diagnosis and treatment of lymphatic anomaly are disclosed.

[Continued on next page]

WO 2018/045078 A3

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:

25 April 2019 (25.04.2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/49453

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/436, 31/395; G01N 33/48; C12Q 1/68 (2017.01)

CPC - A61K 31/436, 31/395; G01N 33/48; C12Q 1/68, 1/6883, 1/6869, 1/683, 1/6827

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)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	LI, D. et al. Whole Exome Sequencing Identifies EPHB4 As A Novel Cause Of Autosomal Dominant Lymphangiomatosis. 2015 ACMG Annual Clinical Genetics Meeting. March 2015, pages 1-2; page 1, first paragraph-page 2, second paragraph.	1-2 --- 3-4, 5/3-4, 13/1, 13/3, 15/1-2, 16/15/1-2, 34-35, 44/1, 44/3, 48-51
X --- Y	ADAMS, D. et al. Efficacy And Safety Of Sirolimus In The Treatment Of Complicated Vascular Anomalies. Pediatrics. February 2016, Vol. 137, document e20153257; pages 1-10, DOI: 10.1542/peds.2015-3257; table 1; abstract; page 3, first column, third paragraph; page 8, third column, second paragraph.	8 --- 3-4, 5/3-4, 13/3, 15/1-2, 16/15/1-2, 44/3
Y	US 2010/0293130 A1 (Stephan, DA et al) 18 November 2010; figure 1; abstract; paragraphs [0001]-[0004], [0035], [0068], [0077]-[0078].	13/1, 13/3, 44/1, 44/3
Y	RICCI, K.W. et al. A Phase 2 Clinical Trial Assessing Efficacy And Safety Of The Mtor Inhibitor Sirolimus In The Treatment Of Generalized Lymphatic Anomaly, Kaposiform Lymphangiomatosis, And Gorham-Stout Disease. D23 Pediatric Rare Diseases And Lung Development. 2015, page 1; page 1, second-fourth paragraphs.	34-35
Y	KAWASAKI, J. et al. RASA1 Functions In EPHB4 Signaling Pathway To Suppress Endothelial mTORC1 Activity. The Journal of Clinical Investigation. June 2014, Vol. 124, pages 2774-2784, doi:10.1172/JCI67084; page 2778, first column, first paragraph; page 2778, secon column, second paragraph; page 2782, first column, first paragraph; page 2783, first column, third-fourth paragraphs.	48-51

☐ 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	Date of mailing of the international search report
26 December 2017 (26.12.2017)	22 JAN 2018
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/49453

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.: 6, 7, 9-12, 14, 17-33, 45-47, 52, 56
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 Page-***-

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.:
-***-Please See Supplemental Page-***-

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
Information on patent family members

International application No.
PCT/US17/49453

-***-Continued from 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.

Groups I+, Claims 1-5, 8, 13, 15, 16, 34-44, 48-51, 53-55, 57, a SNV in EPHB4 and mTOR inhibitor Rapamycin are directed toward methods for diagnosing and treating a lymphatic anomaly, vectors encoding a nucleic acid with a SNV associated with the lymphatic anomaly, and host cells and transgenic animals comprising the nucleic acid.

The methods, nucleic acid, host cells and transgenic animals will be searched to the extent they encompass a SNV in EPHB4 (gene), or SNV in linkage disequilibrium therewith; and an agent comprising mTOR inhibitor Rapamycin (agent). Applicant is invited to elect additional gene(s) and/or agent(s) to be searched. Additional gene(s) and/or agent(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 8 (in-part), 13 (in-part), 15 (in-part), 16 (in-part), 34, 35 (in-part), 44 (in-part), 48 (in-part), 49 (in-part), 50 (in-part) and 51 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass EPHB4 (gene) and Rapamycin (agent). Applicants must specify the claims that encompass any additionally elected gene(s) and/or agent(s). Applicants must further indicate, if applicable, the claims which encompass 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 gene encompassing PIK3R4 (gene).

No technical features are shared between the genes and/or agents of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a method for diagnosing and treating a lymphatic anomaly in a human patient comprising: a) obtaining a biological sample comprising nucleic acid from the patient and obtaining genotype sequence information therefrom; b) assaying the nucleic acid to determine whether i) a single nucleotide variant (SNV) is present or ii) a SNV in linkage disequilibrium with a SNV is present; c) diagnosing the patient with a lymphatic anomaly if an SNV of i) or ii) is present; and administering one or more agents suitable for treatment of said lymphatic anomaly to the patient identified as having one or more SNVs of i) or ii), thereby treating the lymphatic anomaly; a method for treating a lymphatic anomaly in a human patient comprising administering an effective amount of an agent or a combination of agents, thereby treating the lymphatic anomaly; a method for treating lymphatic anomaly in a patient having a SNV, or a SNV in linkage disequilibrium with a SNV, comprising administering one or more agents or a combination of one or more of agents, thereby improving lymph structure, decreasing chylous pleural effusions, improving respiratory function, allowing tapering of concomitant medication usage, or increasing survival; an isolated vector encoding a nucleic acid with a SNV; a host cell comprising the vector; and a transgenic animal comprising the host cell; These technical features are previously disclosed by US 2014/0349856 A1 to bioTheranostics, Inc. (hereinafter 'Biotheranostics') in view of the article 'Genome-Wide Copy Number Analysis in a Family with pG533C RET Mutation and Medullary Thyroid Carcinoma Identified Regions Potentially Associated with a Higher Predisposition to Lymph Node Metastasis' by Araujo, A.N. et al. (hereinafter 'Araujo') and the article 'Tissue-specific Carcinogenesis in Transgenic Mice Expressing the RET Proto-Oncogene with a Multiple Endocrine Neoplasia Type 2A Mutation' by Kawai, K. et al. (hereinafter 'Kawai').

Biotheranostics discloses a method for diagnosing (methods for classifying and diagnosing; paragraphs [0010], [0025]) and treating (and treating; paragraphs [0019], [0025]) a lymphatic anomaly (lymph node metastases (a lymphatic anomaly); paragraphs [0027], [0066]) in a human patient (in a human patient; paragraph [0061]) comprising: a) obtaining a biological sample comprising nucleic acid from the patient (comprising: a) obtaining a biological sample comprising nucleic acid from the patient; abstract, paragraph [0010]) and obtaining gene expression information therefrom (obtaining gene expression information therefrom; paragraph [0012]); diagnosing the patient (diagnosing the patient; paragraphs [0010], [0025]) with a lymphatic anomaly (lymph node metastases (a lymphatic anomaly); paragraphs [0027], [0066]) if a pattern of expression is present (if a pattern of expression is present; paragraphs [0012], [0025]); and administering one or more agents suitable for treatment (and administering one or more agents suitable for treatment; paragraph [0025]) of said lymphatic anomaly (of said lymph node metastases (lymphatic anomaly); paragraphs [0027], [0066]) to the patient, thereby treating the lymphatic anomaly (to the patient, thereby treating the lymph node metastases (lymphatic anomaly); paragraphs [0025], [0027]). Biotheranostics further discloses wherein the lymphatic anomaly (the lymph node metastases (lymphatic anomaly); paragraphs [0027], [0066]) is associated with medullary thyroid carcinoma (associated with medullary thyroid carcinoma; paragraphs [0014], [0116]).

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Box-***-

Biotheranostics does not disclose obtaining genotype sequence information therefrom; assaying the nucleic acid to determine whether i) a single nucleotide variant (SNV) is present or ii) a SNV in linkage disequilibrium with a SNV is present; c) diagnosing the patient with a lymphatic anomaly if an SNV of i) or ii) is present; a method for treating lymphatic anomaly in a patient having a SNV, or a SNV in linkage disequilibrium with a SNV, comprising administering one or more agents, or a combination of agents, thereby improving lymph structure, decreasing chylous pleural effusions, improving respiratory function, allowing tapering of concomitant medication usage, or increasing survival; an isolated vector encoding a nucleic acid with a SNV; a host cell comprising the vector; and a transgenic animal comprising the host cell

Araujo discloses obtaining genotype sequence information from samples from patients (obtaining SNV and CNV (genotype) sequence information from samples from patients; abstract), by assaying the nucleic acid to determine whether i) a single nucleotide variant is present (by assaying the nucleic acid to determine whether i) a single nucleotide polymorphism (variant, SNV) is present; abstract), wherein the SNV is indicative of susceptibility to lymph node metastases of medullary thyroid carcinoma (wherein the SNV is indicative of susceptibility to lymph node metastases of medullary thyroid carcinoma; abstract).

Kawai discloses wherein germline mutations in a gene, including mutations producing a specific amino acid substitution, are responsible for the development of medullary thyroid carcinoma (wherein germline mutations in a gene, including mutations producing a specific amino acid substitution, are responsible for the development of medullary thyroid carcinoma; abstract); and transgenic animals comprising a nucleic acid comprising the mutation (transgenic animals comprising a nucleic acid comprising the mutation; abstract, page 5254, second column, fifth paragraph); vectors comprising the nucleic acid (vectors comprising the nucleic acid; page 5254, second column, fourth paragraph); and animal host cells comprising the nucleic acid (animal host cells comprising the nucleic acid; page 5254, second column, fifth paragraph).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Biotheranostics to have included the detection by determination of genotype sequences, and assessment of expression of genes including SNV associated with the lyphatic anomalies, as disclosed by Araujo, in order to enable more accurate diagnosis of the susceptibility to the anomalies, as disclosed by Araujo, and for selection of appropriate treatments therefor. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Biotheranostics to have provided one or more nucleic acids comprising SNVs, as disclosed by Kawai, to animal cells in order to produce transgenic animals comprising the SNVs, as disclosed by Kawai, in order to provide animal models for the study of the effects of combinations of SNVs, as well as for the study of the effectiveness of compounds or combinations of compounds useful for treatment.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Biotheranostics, Araujo, and Kawai references, unity of invention is lacking.