PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2018/036084	05.06.2018	14.06.2017

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/6886

Applicant
THE UNITED STATES OF AMERICA, AS REPRESENTED BY...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	see form PCT/ISA/210	Aguilera, Miguel Telephone No. +31 70 340-0

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-7
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations

see separate sheet

Re item V.

Reasoned statement (Continuation)

1 CITATIONS:

Reference is made to the following documents:

D1 WO 2015/069790 A1 (US HEALTH [US]; BRITISH COLUMBIA CANCER AGENCY [CA]; UNIV ARIZONA STAT) 14 May 2015 (2015-05-14)

D2 WO 2005/024043 A2 (NAT INST HEALTH [US]; STAUDT LOUIS [US]; WRIGHT GEORGE [US]; TAN BRUCE) 17 March 2005 (2005-03-17)

D3 A. MOTTOK ET AL: "MOLECULAR CLASSIFICATION OF PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA USING FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SPECIMENS - AN LLMPP PROJECT", HEMATOLOGICAL ONCOLOGY, vol. 35, 7 June 2017 (2017-06-07), pages 59-60, XP055495961, ISSN: 0278-0232, DOI: 10.1002/hon.2437_46

D4 D. W. SCOTT ET AL: "Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue", BLOOD, vol. 123, no. 8, 20 February 2014 (2014-02-20), pages 1214-1217, XP055495536, ISSN: 0006-4971, DOI: 10.1182/blood-2013-11-536433

D5 ROSENWALD A ET AL: "Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma", THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 198, no. 6, 15 September 2003 (2003-09-15), pages 851-862, XP002396795, ISSN: 0022-1007, DOI: 10.1084/JEM.20031074

D6 LENZ G ET AL: "Stromal gene signatures in large-B-cell lymphomas", NEW ENGLAND JOURNAL OF MEDICINE, vol. 359, no. 22, 27 November 2008 (2008-11-27), pages 2313-2323, XP002551776, ISSN: 1533-4406, DOI: 10.1056/NEJMOA0802885

D7 M. H. VELDMAN-JONES ET AL: "Reproducible, Quantitative, and Flexible Molecular Subtyping of Clinical DLBCL Samples Using the NanoString nCounter System", CLINICAL CANCER RESEARCH, vol. 21, no. 10, 9 October 2014 (2014-10-09), pages 2367-2378, XP055495781, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-14-0357

2 NOVELTY (Art. 33(2) PCT)

2.1 D1 discloses differential diagnosis of ABC DLBCL, GCB DLBCL, PMBL, Burkitt lymphoma or mantle cell lymphoma based on expression profiling using a 297-gene signature: Lymph5Cx (paragraph [0054]; table 2; abstract; claim 1; examples 1-2). In detail:

The signature in Table 2 contains 51 out of 58 genes of the present signature.

The list of genes in Table 1 (approx. 800 genes) contains 56 out of 58 genes of the present signature.

None of them contains completely the gene expression signature required in claim 1 (Table 1).

2.2 D2 discloses differential diagnosis and sub-classification of lymphoma types based on expression profiles by microarray technology. Multiple tables with expression signatures for pairwise discrimination are described (pages 123-165; examples 13-17; tables 36-39, 45, 46, 56). In detail:

- DLBCL ABC vs GCB (Example 13): 27 genes, Table 36, page 125; Tables 37-38.

- DLBCL ABC vs GCB (Example 14): 14 genes, Table 39, page 129.

- DLBCL vs PMBCL (Example 15): 46 genes, page 133.

- DLBCL ABC vs GCB (Table 45, Example 17, page 146).

- ABC DLBCL vs PMBCL (Table 46).

- GCB DLBCL vs PMBCL (Table 56).

There are common genes between these gene sets and the present one, but the overlap is always incomplete.

2.3 D3 teaches molecular classification of PMBCL based on a gene expression signature comprising 64 genes. It is published by (some of) the present inventors shortly before priority date, but it does not disclose the list of expression markers used (see Abstract).

2.4 D4 discloses a similar method for differential diagnosis, also authored by (some of) the inventors and cited in the application. The method uses a signature of 20 genes (Lymph2Cx), all contained in the present set (abstract; figure 1A).

2.5 D5 discloses a method for molecular diagnosis of PMBCL vs DLBCL based on a gene expression signature comprising 46 genes with little overlap with the present one (abstract; figure 2A).

2.6 D6 discloses stromal gene signatures in large-B-cell lymphomas, including a GCB-specific signature, but it does not contain the present set (abstract; figure 2).

2.7 D7 discloses amethod for molecular subtyping of DLBCL samples also using the NanoString system. They expression signature contains 307 genes and does not contain the present one (Supplemental Table 1).

2.8 Moreover, none of the above documents discloses the specific decision algorithm and coefficient tables required by the claims.

2.9 Therefore, claims 1-7 are new.

3 INVENTIVE STEP (Art. 33(3) PCT)

3.1 Document D1 is considered to represent the most relevant state of the art (see above). The subject-matter of claim 1 differs in that the expression signature to be used contains only 58 genes, instead of 297 and includes 7 genes not included in D1 (Table 2), namely: BATF3, BTG2, FSCN1, MST1R, PDCD1LG2, QSOX1 and TFPI2.

The larger set of Table 1 of D1 is not shown to have discriminatory power in the diagnosis of lymphomas and it still lacks two genes, namely BATF3 and QSOX1. Thus, it is considered a less suitable starting point.

Claim 1 also differs in the specific decision algorithm and coefficient tables, which are not disclosed in D1.

3.2 In absence of comparative data, no technical effect appears to be associated with any of these differences. Hence, the problem to be solved by the subject matter of claim 1 is regarded as the provision of an alternative gene expression signature suitable for sub-typing of lymphomas. The solution proposed is the signature of Table 1 (58 genes) and the specific decision algorithm and coefficient tables.

3.3 This solution is considered as involving an inventive step because it is not suggested in any of the prior art documents found. None of them contains a pointer to the 7 missing markers, and there is no pointer to their combination for the present purpose. Also, none of them discloses the specific decision algorithm and coefficient tables.

3.4 Therefore, claim 1, and its dependent claims 2-7 are considered to involve an inventive step.