PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

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/EP2018/067397	28.06.2018	29.06.2017

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/50

Applicant
ESSER, Knud

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	Telephone No. +31 70 340-0

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2018/067397

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. II Priority

1. ☐ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 19-21

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 19-21

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-18

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	1-18
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1 Reference is made to the following documents:

D1 Henry Wu ET AL: "Reduction in lactate accumulation correlates with differentiation-induced terminal cell division of leukemia cells*", DIFFERENTIATION., Bd. 48, Nr. 1, 1. September 1991 (1991-09-01), Seiten 51-58, XP055456961

D2 Jean-Marc Blouin ET AL: "Butyrate elicits a metabolic switch in human colon cancer cells by targeting the pyruvate dehydrogenase complex", International Journal of Cancer, Bd. 128, Nr. 11, 8. Oktober 2010 (2010-10-08), Seiten 2591-2601, XP055456743

D3 Sandra Varum ET AL: "Energy Metabolism in Human Pluripotent Stem Cells and Their Differentiated Counterparts", PLoS ONE, Bd. 6, Nr. 6, 17. Juni 2011 (2011-06-17), Seite e20914, XP055457404

D4 HOFMANOVÁ JIRINA ET AL: "Lipid alterations in human colon epithelial cells induced to differentiation and/or apoptosis by butyrate and polyunsaturated fatty acids", THE JOURNAL OF NUTRITIONAL BIOCHEMISTRY, Bd. 23, Nr. 6 , Seiten 539-548, XP028918963

D5 Hui Yan ET AL: "Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells", PLOS ONE, Bd. 10, Nr. 12, 29. Dezember 2015 (2015-12-29), Seite e0145940, XP055456747

D6 Valérie Marcil ET AL: "Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate", American Journal of Physiology - Gastrointestinal and Liver Physiology, Bd. 283, Nr. 2, 1. August 2002 (2002-08-01), Seiten G340-G346, XP055457302

D7 Nívea Dias Amoêdo ET AL: "Energy Metabolism in H460 Lung Cancer Cells: Effects of Histone Deacetylase Inhibitors", PLoS ONE, Bd. 6, Nr. 7, 18. Juli 2011 (2011-07-18), Seite e22264, XP055457420

D8 AJANTA CHAKRABORTY ET AL: "Long term induction by pterostilbene results in autophagy and cellular differentiation in MCF-7 cells via ROS dependent pathway", MOLECULAR AND CELLULAR ENDOCRINOLOGY, ELSEVIER IRELAND LTD, IE, vol. 355, no. 1, 10 January 2012 (2012-01-10), pages 25-40, XP028475246

D9 PUNJ V ET AL: "EFFECT OF VITAMIN D ANALOG (1ALPHA HYDROXY D5) IMMUNOCONJUGATED TO HER-2 ANTIBODY ON BREAST CANCER", INTERNATIONAL JOURNAL OF CA, JOHN WILEY & SONS, INC, US, vol. 108, 1 January 2004 (2004-01-01), pages 922-929, XP008047279

D10 A. BELFIORE ET AL: "Insulin receptor and cancer", ENDOCRINE - RELATED CANCER, vol. 18, no. 4, 23 May 2011 (2011-05-23), pages R125-R147, XP055422259

D11 SARAH F. ANDRES ET AL: "Insulin receptor isoform switching in intestinal stem cells, progenitors, differentiated lineages and tumors: evidence that IR-B limits proliferation", JOURNAL OF CELL SCIENCE, vol. 126, no. 24, 14 October 2013 (2013-10-14), pages 5645-5656, XP055497343

D12 J. HEATH: "Appearance of functional insulin receptors during the differentiation of embryonal carcinoma cells", THE JOURNAL OF CELL BIOLOGY : JCB, vol. 91, no. 1, 1 October 1981 (1981-10-01), pages 293-297, XP055497344

Re Item II

2 Priority

The current application claims priority from EP17178817 (P1) and DE202015002198 (P2). The claimed priority is, at least in part, considered invalid because P1 does not cover the subject-matter of at least present claims 2, 4-21. The ISA does not have in its possession a copy of the priority application P2. This opinion has been established on the assumption that the filing date of P2 is the claimed priority for said claims.

Re Item IV

3 Lack of unity

This Authority considers that the application does not meet the requirements of unity of invention and that there are 3 inventions.

The technical feature of method claim 1 resides in the measurement of two biomarkers. Neither the same nor corresponding technical features (Rule 13.2 PCT) are present in the product claims 19 and 10 (Vessel for the removal of

liquids and microtiter plate). There is no single general inventive concept that links the method claim 1 to the product claims 19 and 20, as required by Rule 13.1 PCT.

Hence, the claims comprise neither the same, nor corresponding special technical features, so the technical relationship between the subject matter of the claims required by Rule 13.2 PCT is lacking and the claims are not so linked as to form a single general inventive concept as required by Rule 13.1 PCT. Thus unity of invention is lacking a priori.

Consequently the application does not meet the requirement for unity of invention.

The groups of inventions are split up us follows:

Invention 1: Claims 1-18 (all complete)

Method for the identification of compounds inducing (re-)differentiation in non- or dedifferentiated cells, comprising:
a) provision of a cell culture sample consisting of de-/ or undifferentiated tumour cells,
b) bringing the compound of interest into contact with the cell culture sample,
c) following the determination of the relative concentration of a first marker lactate in contrast to untreated cells, and
d) following the determination of the relative concentration of a second marker neutral lipids in contrast to untreated cells,
wherein steps c) and d) may be performed in reverse order if necessary

Invention 2: Claim 19 (complete)

Vessel for the removal of liquids from cells via centrifugation
characterized by comprising:
- 4 side walls (ai, a_2 , bi, b_2), where the two opposing side walls have the same length (l_a , l_b), so that a rectangular shape is obtained,
- a flat bottom (c) being connected in such a way with each of the side walls over all of the connecting area in a liquid proof way,
- each of the side walls having a protrusion (d_a , d_b) directed towards the inside of the vessel,

- 2 of the 4 side walls being opposite to each other having recesses (ei, e_2) positioned in the middle of the lengths l_a of the side wall on the upper surface of the respective side wall.

Invention 3: Claims 20 and 21 (all complete)

Microtiter plate for culturing cells enabling addition of liquid via centrifugation, said plate comprising a surface (1) and wells (2), said wells being tapered towards the bottom (3) where an opening, especially a circular opening, is present.

Complete searches for the other inventions represent substantive extra search burden. The first claimed invention mentioned in the claims has been searched (Invention 1 (claims 1-18 (all complete)).

Re Item V

4 **Inventive step**

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-18 does not involve an inventive step in the sense of Article 33(3) PCT.

4.1 Document D1 (whole document) is considered to be the closest prior art to the subject-matter of claim 1. It discloses reduced lactate production as a marker for induced differentiation in myeloid leukemia cells. It should be noted that the relationship between lactate production and stages of differentiation is well known (see also D7 (page 4, right column, §2 - page 6, left column, §1), D2 (figure 3d) and D3 (figure 3G)) ,

The subject matter of claim 1 thus differs from the known method in that the incorporation of neutral lipids is measured as an additional marker.

Since the application shows no comparative results, no effect can be deduced from this difference.

The problem to be solved by the present invention can thus be regarded as the provision of an alternative method for the identification of compounds which induce the (re-)differentiation of un-or dedifferentiated cells.

The solution proposed in claim 1 of the present application cannot be considered to involve an inventive step (Article 33(3) PCT), since the problem is not solved throughout the claimed scope.The present application shows that the incorporation of neutral lipids can only be determined under certain cell culture conditions (serum-free medium) in order to serve as a marker for the differentiation of tumor cell lines.

It should also be noted that it is known that butyrate leads to the accumulation of tirglycerides (a neutral lipid) in differentiating cells (see D4 (page 541, point 3.5); D5 (Figure 10, page 14, §1 - page 15, § 1); D6 (full document)). D8 (page 31, right-hand column, §1) additionally teaches that differentiation of MCF-7 cells is marked by the accumulation of neutral lipids. D9 (page 922, left-hand column, §3) further teaches that a vitamin D analog which is a potent cell-differentiating agent in breast cancer cells induces an increase in intracellular accumulation of neutral lipid. Hence, it is very well known that neutral lipids are a marker for differentiation in tumor cells.

4.2 Dependent claims 2-18 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, said features are either present in D1-D12 with the same context or represent standard modifications the skilled person would contemplate without exerting any inventive skill.

5 Industrial applicability

The subject-matter of claims 1-18 is found to be industrially applicable.

Re Item VIII.

6 Claim 1 lacks essential features (Article 6 PCT taken in combination with Rule 6.3(b) PCT). Indeed, the skilled person does not know, from reading the claim alone, which concentration of lactate and neutral-lipids is representative for a compound inducing differentiation in tumor cells. He does not even know whether he should look to increased or decreased values.

7 It is further apparent from the example that the claimed method is coupled to particular cell culture conditions, not all of them are recited in the claim. It is emphasized that neutral lipids only can be detected under aerobic and anabolic cell culture conditions in serum-free medium, which requires a medium change after the lactate concentration in the supernatant (not in the cells) is determined. Since independent claim 1 does not contain these features it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

8 No data has been shown in the application as filed on how insulin effects marker expression or on how the stage of differentiation correlates with the expression of insulin receptor subtypes. An objection for lack of technical support (Article 6 PCT) with regard to the subject-matter of claim 1 arises therefrom.

9 The scope of claim 5 is not clear (Article 6 PCT). The claim relates to the use of the method of claim 4. Since the claimed use and the method of claim 4 (which is dependent on claim 1 and describes further steps) do not pursue the same purpose, the scope of the claim is not clear. The method of claims 1 to 4 aim to identify means for (re) differentiation of un-or dedifferentiated cells, whereas claim 5 is aimed at determining the influence of the identified compounds on the viability and apoptotic behaviour of cells. The application seeks to combine a screening method for the identification of the above-mentioned agents with a toxicity screening of the identified agents. The determination of viability and/or adherence of the cells is independent of the determination of the metabolic markers. The method of claims 1 to 4 can thus not be used for this kind of screening.

10 Claim 16 is broadening the subject-matter of claim 1 because it states that the markers lactate and neutral lipids are used for the identification of compounds inducing (re-)differentiation in un- or dedifferentiated **cells**, whereas claim 1 is restricted to tumor cell samples. The formulation leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear, Article 6 PCT.

11 The wording of claim 1 is unclear (Article 6PCT; e.g. "following the determination", first marker lactate" etc.). A rewording of the claim is advised.