PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
dompatent von Kreisler Selting Werner -
Partnerschaft von Patent- und Rechtsanwälten mbB
Deichmannhaus am Dom
Bahnhofsvorplatz 1
50667 Köln
ALLEMAGNE

INVITATION TO PAY ADDITIONAL FEES AND, WHERE APPLICABLE, PROTEST FEE

(PCT Article 17(3)(a) and Rule 40.1 and 40.2(e))

Applicant's or agent's file reference 181564WO EBO	Date of mailing *(day/month/year)* 13 August 2018 (13-08-2018) **PAYMENT DUE** within **ONE MONTH** from the above date of mailing
International application No. PCT/EP2018/067397	International filing date *(day/month/year)* 28 June 2018 (28-06-2018)
Applicant ESSER, Knud	

1. This International Searching Authority

(i) considers that there are 3 *(number of)* inventions claimed in the international application covered by the claims indicated on an extra sheet:

(ii) therefore considers that **the international application does not comply with the requirements of unity of invention** (Rules 13.1, 13.2 and 13.3) for the reasons indicated on an extra sheet:

(iii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

see extra sheet

(iv) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid.

2. Consequently, the applicant is hereby **invited to pay,** within the time limit indicated above, the amount indicated below:

Fee per additional invention	x	number of additional inventions	=	currency/total amount of additional fees
EUR 1.775,00		2		EUR 3.550,00

3. The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive, where applicable, subject to the payment of a protest fee.

Where the applicant pays additional fees under protest, the applicant is hereby invited, within the time limit indicated above, to pay a protest fee (Rule 40.2(e)) in the amount of EUR 875,00 *(currency/amount)*

Where the applicant has not, within the time limit indicated above, paid the required protest fee, the protest will be considered not to have been made and the International Searching Authority will so declare.

4. [ ] Claim(s) Nos. _____ have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	OSTWINKEL, Nathalie Tel: +31 (0)70 340-4437

Form PCT/ISA/206 (April 2005)

Registered Mail

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/EP2018/067397

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18

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, andd) 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

---

2. claim: 19

Vessel for the removal of liquids from cells via centrifugation characterized by comprising:- 4 side walls (ai, a2 , bi, b2), where the two opposing side walls have the same length (la , lb), 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 (da , db) directed towards the inside of the vessel,- 2 of the 4 side walls being opposite to each other having recesses (ei, e2) positioned in the middle of the lengths la of the side wall on the upper surface of the respective side wall.

---

3. claims: 20, 21

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.

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 claims19 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.

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

Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
PCT/EP2018/067397

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HENRY WU ET AL: "Reduction in lactate accumulation correlates with differentiation-induced terminal cell division of leukemia cells*", DIFFERENTIATION., vol. 48, no. 1, 1 September 1991 (1991-09-01), pages 51-58, XP055456961, DE ISSN: 0301-4681, DOI: 10.1111/j.1432-0436.1991.tb00242.x the whole document	1-18

Y	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, vol. 128, no. 11, 8 October 2010 (2010-10-08), pages 2591-2601, XP055456743, US ISSN: 0020-7136, DOI: 10.1002/ijc.25599 figure 3d	1-18

	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in 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 document 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

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SANDRA VARUM ET AL: "Energy Metabolism in Human Pluripotent Stem Cells and Their Differentiated Counterparts", PLOS ONE, vol. 6, no. 6, 17 June 2011 (2011-06-17), page e20914, XP055457404, DOI: 10.1371/journal.pone.0020914 figure 3G	1-18
Y	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, vol. 23, no. 6, 2012, pages 539-548, XP028918963, ISSN: 0955-2863, DOI: 10.1016/J.JNUTBIO.2011.02.010 Punkt 3.5; page 541	1-18
Y	HUI YAN ET AL: "Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells", PLOS ONE, vol. 10, no. 12, 29 December 2015 (2015-12-29), page e0145940, XP055456747, DOI: 10.1371/journal.pone.0145940 page 14, paragraph 1 - page 15, paragraph 1; figure 10	1-18
Y	VALÉRIE MARCIL ET AL: "Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate", AMERICAN JOURNAL OF PHYSIOLOGY - GASTROINTESTINAL AND LIVER PHYSIOLOGY, vol. 283, no. 2, 1 August 2002 (2002-08-01), pages G340-G346, XP055457302, US ISSN: 0193-1857, DOI: 10.1152/ajpgi.00440.2001 the whole document	1-18

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NÍVEA DIAS AMOÊDO ET AL: "Energy Metabolism in H460 Lung Cancer Cells: Effects of Histone Deacetylase Inhibitors", PLOS ONE, vol. 6, no. 7, 18 July 2011 (2011-07-18), page e22264, XP055457420, DOI: 10.1371/journal.pone.0022264 page 4, right-hand column, paragraph 2 - page 6, left-hand column, paragraph 1	1-18
Y	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, ISSN: 0303-7207, DOI: 10.1016/J.MCE.2012.01.009 [retrieved on 2012-01-16] page 31, right-hand column, paragraph 1	1-18
Y	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, ISSN: 0020-7136, DOI: 10.1002/IJC.11590 page 922, right-hand column, paragraph 3	1-18
A	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, GB ISSN: 1351-0088, DOI: 10.1530/ERC-11-0074 the whole document	1-18

-/--

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	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, GB ISSN: 0021-9533, DOI: 10.1242/jcs.132985 the whole document --- 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, US ISSN: 0021-9525, DOI: 10.1083/jcb.91.1.293 the whole document ---	1-18 1-18

Application no:
Demande nº:
Anmelde-Nr: PCT/EP2018/067397

DISCLAIMER

The attached provisional opinion on the patentability of the first invention searched serves only as information.
A reply addressing the points raised in the opinion is **not** required and will **not** be taken into account when issuing the final search report and opinion on patentability.

AVERTISSEMENT

L'avis provisoire ci-joint sur la brevetabilité de la première invention recherchée ne sert qu'à titre d'information.
Une réponse abordant les points soulevés dans l'avis n'est **pas** nécessaire et ne sera **pas** prise en compte lors de l'établissement du rapport final de la recherche et de l'avis sur la brevetabilité.

DISCLAIMER

Die beigefügte vorläufige Stellungnahme zur Patentierbarkeit der ersten geprüften Erfindung dient lediglich zur Information.
Eine Antwort auf die erhobenen Punkte in der Stellungnahme ist **nicht** erforderlich und bleibt bei der Erstellung des endgültigen Recherchenberichts und der Stellungnahme zur Patentierbarkeit **unberücksichtigt**.

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.