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(54) Title: MATURATION OF MAMMALIAN HEPATOCYTES

(57) Abstract: The present invention relates to directed differentiation and maturation of mammalian hepatocytes, such as human hepatocytes. The hepatocyte obtained in accordance with the present invention show a phenotype which is more similar to that of primary hepatocytes than previously shown. In particular, the present invention relates to exposure of mammalian hepatocytes, such as human hepatocytes, to at least one maturation factor selected from the group consisting of Src kinase inhibitors, vitamin D including precursors, metabolites and analogs thereof, hypoxia inducing compounds, sphingosine and sphingosine derivatives, activators of peroxisome proliferator-activated receptors (PPARs), platelet-activating factor (PAF), PKC inhibitors, and combinations thereof.

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2016/062670

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N5/071 ADD.		
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) C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RICHARD SILLER ET AL: "Small-Molecule-Driven Hepatocyte Differentiation of Human Pluripotent Stem Cells", STEM CELL REPORTS, vol. 4, no. 5, 12 May 2015 (2015-05-12), pages 939-952, XP055203481, ISSN: 2213-6711, DOI: 10.1016/j.stemcr.2015.04.001 the whole document	1-40
X	----- US 2010/143313 A1 (YARMUSH MARTIN L [US] ET AL) 10 June 2010 (2010-06-10)	1-3,7, 10,11
Y	claim 2 ----- -/--	1-40
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> 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	
19 September 2016	29/11/2016	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Offermann, Stefanie	

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/062670

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>IMAN SARAMIPOOR BEHBAHAN ET AL: "New Approaches in the Differentiation of Human Embryonic Stem Cells and Induced Pluripotent Stem Cells toward Hepatocytes", STEM CELL REVIEWS AND REPORTS, HUMANA PRESS INC, NEW YORK, vol. 7, no. 3, 19 February 2011 (2011-02-19), pages 748-759, XP019927867, ISSN: 1558-6804, DOI: 10.1007/S12015-010-9216-4 the whole document page 754, right-hand column</p>	1-40
Y	<p>US 2005/037493 A1 (MANDALAM RAMKUMAR [US] ET AL) 17 February 2005 (2005-02-17) paragraph [0067] - paragraph [0071]</p>	1-40
X	<p>WO 2014/083132 A1 (CELLECTIS SA [FR]) 5 June 2014 (2014-06-05)</p>	1-11
Y	<p>claims 1-88</p>	1-40
A	<p>M P Vincenti ET AL: "v-src activation of the collagenase-1 (matrix metalloproteinase-1) promoter through PEA3 and STAT: requirement of extracellular signal-regulated kinases and inhibition by retinoic acid receptors", Molecular carcinogenesis, 7 December 1998 (1998-12-07), pages 194-204, XP055203508, UNITED STATES DOI: 10.1002/(SICI)1098-2744(199803)21:3<194::AID-MC7>3.0.CO;2-M Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pubmed/9537651 [retrieved on 2015-07-20] abstract</p>	1-40
T	<p>NIDAL GHOSHEH ET AL: "Highly Synchronized Expression of Lineage-Specific Genes during In Vitro Hepatic Differentiation of Human Pluripotent Stem Cell Lines", STEM CELLS INTERNATIONAL, vol. 2016, 1 January 2016 (2016-01-01), pages 1-22, XP055303667, US ISSN: 1687-966X, DOI: 10.1016/s0925-4773(02)00240-x</p>	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/062670

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.:
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:

see additional sheet

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 an 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.:

1-40(partially)

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/EP2016/062670

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>ASPLUND ANNIKA ET AL: "One Standardized Differentiation Procedure Robustly Generates Homogenous Hepatocyte Cultures Displaying Metabolic Diversity from a Large Panel of Human Pluripotent Stem Cells", STEM CELL REVIEWS, HUMANA PRESS INC, US, vol. 12, no. 1, 19 September 2015 (2015-09-19), pages 90-104, XP035951839, ISSN: 1550-8943, DOI: 10.1007/S12015-015-9621-9 [retrieved on 2015-09-19] -----</p>	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-40(partially)

method for promoting the maturation of or producing mammalian hepatocytes by exposing hepatocytes to a Src kinase inhibitor, such as e.g. PP1, PP2, 1-NA PP1 etc (see claim 12).

2. claims: 1-40(partially)

method for promoting the maturation of or producing mammalian hepatocytes by exposing hepatocytes to vitamin D or any precursor, metabolite or analogue thereof.

3-7. claims: 1-40(partially)

method for promoting the maturation of or producing mammalian hepatocytes by exposing hepatocytes to a hypoxia inducing compound, sphingosine, an activator of PPARs, platelet-activating factor or a PKC inhibitor (see claims 1, 3).

8-14. claims: 41-73(partially)

composition/culture medium/kit comprising a factor selected from a Src kinase inhibitor, vitamin D or any precursor, metabolite or analogue thereof, a hypoxia inducing compound, sphingosine, an activator of PPARs, platelet-activating factor or a PKC inhibitor.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2016/062670

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010143313	A1	10-06-2010	NONE
US 2005037493	A1	17-02-2005	US 2005037493 A1 17-02-2005
			US 2009136955 A1 28-05-2009
			US 2014106343 A1 17-04-2014
WO 2014083132	A1	05-06-2014	CN 105143445 A 09-12-2015
			EP 2925859 A1 07-10-2015
			JP 2016503304 A 04-02-2016
			US 2015307839 A1 29-10-2015
			WO 2014083132 A1 05-06-2014