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61/520,569 10 June 2011 (10.06.2011) US
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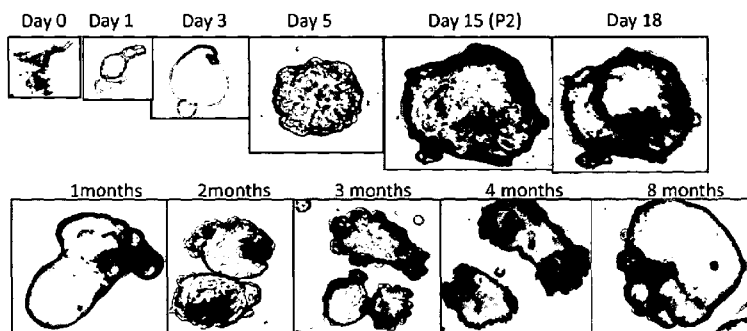
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(54) Title: LIVER ORGANOID, USES THEREOF AND CULTURE METHOD FOR OBTAINING THEM

Fig 1d



(57) Abstract: The invention relates to a liver organoid, uses thereof and method for obtaining them.



INTERNATIONAL SEARCH REPORT

International application No PCT/IB2011/002167
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A. CLASSIFICATION OF SUBJECT MATTER INV. C12N5/074 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, CHEM ABS Data, Sequence Search, WPI Data, BIOSIS				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	SNYKERS SARAH ET AL: "In Vitro Differentiation of Embryonic and Adult Stem Cells into Hepatocytes: State of the Art", STEM CELLS (MIAMISBURG), vol. 27, no. 3, 2009, pages 577-605, XP002603406, ISSN: 1066-5099	23-44, 52,53		
A	the whole document page 585 - page 590; table 2 ----- -/--	1-22		
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;"><input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.</td> <td style="width: 50%; border: none;"><input checked="" type="checkbox"/> See patent family annex.</td> </tr> </table>			<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.
<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 <div style="text-align: center; font-size: 1.2em;">22 June 2012</div>		Date of mailing of the international search report <div style="text-align: center; font-size: 1.2em;">11/07/2012</div>		
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 <div style="text-align: center; font-size: 1.2em;">Zuber Perez, C</div>		

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2011/002167

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MITAKA TOSHIHIRO: "Reconstruction of hepatic organoid by hepatic stem cells", JOURNAL OF HEPATO-BILIARY-PANCREATIC SURGERY, SPRINGER VERLAG, BERLIN, DE, vol. 9, no. 6, 1 January 2002 (2002-01-01), pages 697-703, XP002331148, ISSN: 0944-1166, DOI: DOI:10.1007/S005340200096	23-44, 52,53
A	the whole document	1-22
X	WO 2004/087896 A2 (PFIZER PROD INC [US]; ROACH MARSHA LYNN [US]; HAMBOR JOHN EDWARD [US]) 14 October 2004 (2004-10-14)	23-45, 48-50, 52,53
A	the whole document HepI and HepII mediaexample 2	1-22
X	WO 2007/127454 A2 (CYTHERA INC [US]; D AMOUR KEVIN A [US]; BAETGE EMMANUEL E [US]) 8 November 2007 (2007-11-08)	23-44, 52,53
A	the whole document page 116, paragraph 2 - page 121, paragraph 1; example 15 figure 41	1-22
X	SNYKERS S ET AL: "Differentiation of neonatal rat epithelial cells from biliary origin into immature hepatic cells by sequential exposure to hepatogenic cytokines and growth factors reflecting liver development", TOXICOLOGY IN VITRO, ELSEVIER SCIENCE, GB, vol. 30, 4 April 2007 (2007-04-04), pages 1-7, XP002453247, ISSN: 0887-2333	23-44, 52,53
A	the whole document	1-22
Y	LEMAIGRE ET AL: "Mechanisms of Liver Development: Concepts for Understanding Liver Disorders and Design of Novel Therapies", GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, vol. 137, no. 1, 1 July 2009 (2009-07-01), pages 62-79, XP026418789, ISSN: 0016-5085 [retrieved on 2009-03-27] the whole document figures 3-4; table 1 page 68 - page 69	1-53
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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2011/002167

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WILLIAMS JENNIFER M ET AL: "The Role of the Wnt Family of Secreted Proteins in Rat Oval "Stem" Cell-Based Liver Regeneration Wnt1 Drives Differentiation", AMERICAN JOURNAL OF PATHOLOGY, vol. 176, no. 6, June 2010 (2010-06), pages 2732-2742, XP002678410, the whole document page 2741 - left-hand column, paragraph 4 is required for rat oval stem cell-based liver r	1-53
X	----- WO 2009/022907 A2 (HUBRECHT INST [NL]; KONINK NL AKADEMIE VAN WETENSC [NL]; CLEVERS JOHAN) 19 February 2009 (2009-02-19)	23-25
A	the whole document page 82; example 2 page 19; table 1	1-22, 26-53
A	----- BARKER N ET AL: "Identification of stem cells in small intestine and colon by marker gene Lgr5", NATURE: INTERNATIONAL WEEKLY JOURNAL OF SCIENCE, NATURE PUBLISHING GROUP, UNITED KINGDOM, vol. 449, 25 October 2007 (2007-10-25), pages 1003-1008, XP002457164, ISSN: 0028-0836, DOI: 10.1038/NATURE06196 the whole document -----	1-53

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002167

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.:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-22, 46, 47(completely); 49, 50(partially)

a method for obtaining /culturing a liver fragment/organoid, said method comprising the culture of epithelial stem cells or tissue fragments comprising them, in the presence of an extracellular matrix, and a basal medium for animal/human cells supplemented with EGF, and a FGF able to bind FGFR2 or FGFR4, in the presence of nicotinamide, with preferably a BMP inhibitor, and/or a Wnt agonist, and/or B27, and/or N2 and/or N-Acetylcysteine.

2. claims: 23-44, 52, 53

a population of adult liver stem cells characterised by natural expression of Lgr5, Hnf1a and/or Hnf4 at significant level, a population of said cells or a liver organoid made thereof; use of said liver organoid in a drug discovery screen, toxicity assay or in regenerative medicine and for treating a liver disorder, condition or disease.

3. claims: 45, 48(completely); 49, 50(partially)

a cell culture medium comprising a basal medium supplemented with EGF, a FGF able to bind FGFR2 or FGFR4, HGF, gastrin, Nicotinamide, B27, N2 and N-Acetylcysteine with preferably a BMP inhibitor, and/or a Wnt agonist, and its use for culturing epithelial stem cells or isolated tissue fragments on an extracellular matrix.

4. claim: 51

a method for regenerating the liver or for obtaining cells expressing Lgr5 comprising stimulating Wnt signaling in the liver.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/IB2011/002167

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004087896 A2	14-10-2004	US 2004191902 A1 WO 2004087896 A2	30-09-2004 14-10-2004

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