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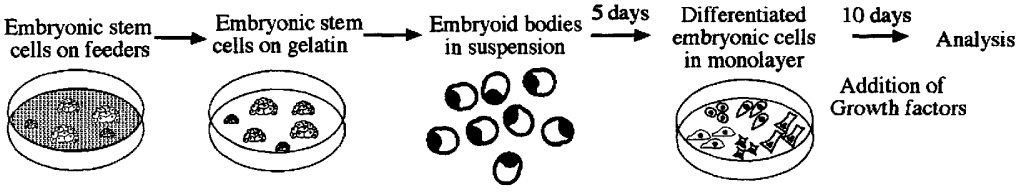
13 March 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIRECTED DIFFERENTIATION OF EMBRYONIC CELLS



(57) Abstract: Methods are described for mapping a pathway of differentiation of a population of embryonic cells which includes exposing the cells to an exogenous factor and measuring gene expression products that are characteristic of a particular cell type or lineage. Directing differentiation of human embryonic cells relies on dissociated embryoid bodies which are then exposed to one or more exogenous factors to enrich a culture for a particular cell type. The differentiated cells may be used for treating a medical condition in a human. Kits for determining differentiation pathways and screening exogenous factors for their utility in differentiation are provided.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 01/01719

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N5/08 C12N5/06 A61K48/00 C07K16/18

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)
IPC 7 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 practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOHE K K ET AL: "SINGLE FACTORS DIRECT THE DIFFERENTIATION OF STEM CELLS FROM THE FETAL AND ADULT CENTRAL NERVOUS SYSTEM" GENES AND DEVELOPMENT, COLD SPRING HARBOR, NY, US, vol. 10, no. 24, 15 December 1996 (1996-12-15), pages 3129-3140, XP000867356 ISSN: 0890-9369 abstract; figures 5,7; tables 1-4	1-7
X	-& US 5 753 506 A (JOHE KARL K.) 19 May 1998 (1998-05-19) the whole document	1-7
X	US 5 736 396 A (HAYNESWORTH STEPHEN E ET AL) 7 April 1998 (1998-04-07) abstract; figure 1; examples 1-9; tables 1-6	1-7

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

[X] 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

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2002	11.09.02

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Novak, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	VISGER VON J R ET AL: "DIFFERENTIATION AND MATURATION OF ASTROCYTES DERIVED FROM NEUROEPITHELIAL PROGENITOR CELLS IN CULTURE" EXPERIMENTAL NEUROLOGY, SAN DIEGO, CA, US, vol. 128, 1994, pages 34-40, XP002037766 abstract	1-7
Y	WOBUS ANNA M ET AL: "Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes." JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY, vol. 29, no. 6, 1997, pages 1525-1539, XP002198708 ISSN: 0022-2828 abstract; figures 1-5; table 1	1-7
Y	MUMMERY C L ET AL: "EXPRESSION OF TRANSFORMING GROWTH FACTOR SS2 DURING THE DIFFERENTIATION OF MURINE EMBRYONAL CARCINOMA AND EMBRYONIC STEM CELLS" DEVELOPMENTAL BIOLOGY, ACADEMIC PRESS, NEW YORK, NY, US, vol. 137, no. 1, January 1990 (1990-01), pages 161-170, XP000891381 ISSN: 0012-1606 abstract; table 1	1-7
X	KAUFMAN D S ET AL: "DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO HEMATOPOIETIC COLONY FORMING CELLS" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 94, 15 November 1999 (1999-11-15), page 34A XP000942815 ISSN: 0006-4971 abstract	8-17
X	PERA MARTIN F ET AL: "Human embryonic stem cells." JOURNAL OF CELL SCIENCE, vol. 113, no. 1, January 2000 (2000-01), pages 5-10, XP002209790 ISSN: 0021-9533	8-17
Y	abstract; figure 1; table 1	42-47

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BRUESTLE O ET AL: "EMBRYONIC STEM CELL-DERIVED GLIAL PRECURSORS: A SOURCE OF MYELINATING TRANSPLANTS" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 285, 30 July 1999 (1999-07-30), pages 754-756, XP002906546 ISSN: 0036-8075 the whole document	18-37
Y	-& DE 197 56 864 C (BRÜSTLE, OLIVER) 29 April 1999 (1999-04-29) the whole document	18-37
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X	RATHJEN P D ET AL: "PROPERTIES AND USES OF EMBRYONIC STEM CELLS: PROSPECTS FOR APPLICATION TO HUMAN BIOLOGY AND GENE THERAPY" REPRODUCTION, FERTILITY AND DEVELOPMENT, CSIRO, EAST MELBOURNE, AU, vol. 10, no. 1, 1998, pages 31-47, XP000916997 ISSN: 1031-3613	38-41
Y	abstract; figures 1,2; table 1	18-37
X	HEATH J K ET AL: "REGULATORY FACTORS OF EMBRYONIC STEM CELLS" JOURNAL OF CELL SCIENCE, ESSEX, GB, no. SUPPL 10, 1988, pages 257-266, XP000569976 the whole document	38-41
X	WILES MICHAEL V ET AL: "Embryonic stem cell development in a chemically defined medium." EXPERIMENTAL CELL RESEARCH, vol. 247, no. 1, 25 February 1999 (1999-02-25), pages 241-248, XP002209791 ISSN: 0014-4827 the whole document	38-41

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ITSKOVITZ-ELDOR JOSEPH ET AL: "Differentiation of human embryonic stem cells into embryoid bodies comprising the three embryonic germ layers." MOLECULAR MEDICINE (NEW YORK), vol. 6, no. 2, February 2000 (2000-02), pages 88-95, XP000953284 ISSN: 1076-1551 the whole document	42-47
X	TADMOR B ET AL: "EMBRYONAL GERM-LAYER ANTIGENS: TARGET FOR AUTOIMMUNITY" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 339, 18 April 1992 (1992-04-18), pages 975-978, XP000616025 ISSN: 0140-6736 the whole document	42-47
Y	YOUNG H E ET AL: "HUMAN PLURIPOTENT AND PROGENITOR CELLS DISPLAY CELL SURFACE CLUSTERDIFFERENTIATION MARKERS CD10, CD13, CD56, AND MHC CLASS-1 (44385)" PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY & MEDICINE, ACADEMIC PRESS INC. NEW YORK, US, vol. 221, no. 1, 1999, pages 63-71, XP000982447 ISSN: 0037-9727 page Y	42-47
A	LAKE JULIE-ANNE ET AL: "Reversible programming of pluripotent cell differentiation." JOURNAL OF CELL SCIENCE., vol. 113, no. 3, February 2000 (2000-02), pages 555-566, XP002209792 ISSN: 0021-9533	
A	MOUNTFORD PETER ET AL: "Maintenance of pluripotential embryonic stem cells by stem cell selection." REPRODUCTION FERTILITY AND DEVELOPMENT, vol. 10, no. 7-8, 1998, pages 527-533, XP001070570 ISSN: 1031-3613	
A	KLUG M G ET AL: "GENETICALLY SELECTED CARDIOMYOCYTES FROM DIFFERENTIATING EMBRYONIC STEM CELLS FORM STABLE INTRACARDIAC GRAFTS" JOURNAL OF CLINICAL INVESTIGATION, NEW YORK, NY, US, vol. 98, no. 1, 1 July 1996 (1996-07-01), pages 216-224, XP000670716 ISSN: 0021-9738	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	SCHULDINER MAYA ET AL: "Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 97, no. 21, 10 October 2000 (2000-10-10), pages 11307-11312, XP002198709 October 10, 2000 ISSN: 0027-8424	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 01/01719

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] 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 fee, this Authority did not invite payment of any additional fee.

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.

[X] 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: 1-7

   A method of mapping a pathway of differentiation of a population of embryonic cells.

2. Claims: 8-17

   A method of directing differentiation of human embryonic cells to a specific cell

3. Claims: 18-37

   A method of treating a subject suffering from a condition associated with degeneration of cells or malfunction of cells.

4. Claims: 38-40

   A kit for determining differentiation pathways.

5. Claim : 41

   A method of screening an exogenous factor to determine whether the factor is capable of causing directed differnetiation in a population of human embryonic cells.

6. Claims: 42-47

   A panel of cell type differentiation determining markers.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Rule 39.1(ii) PCT - Essentially biological process for the production of animals

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
Although claims 18-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 01/01719

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5736396 A	07-04-1998	AU 719098 B2	04-05-2000
		AU 4746996 A	14-08-1996
		CA 2211120 A1	01-08-1996
		EP 0805853 A1	12-11-1997
		JP 10512756 T	08-12-1998
		WO 9623059 A1	01-08-1996
		US 5942225 A	24-08-1999