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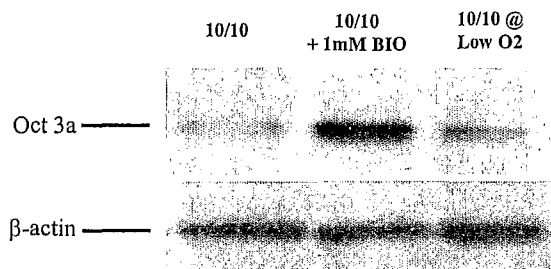
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22 March 2007

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: USE OF A GSK-3 INHIBITOR TO MAINTAIN POTENCY OF CULTURED CELLS

**Increased Expression of Oct-3a by Western Blot  
Analysis of BMC43 Extracts**



(57) Abstract: The present invention is directed to the culture of non-embryonic cells, that can differentiate into cell types of more than one embryonic lineage, in culture under conditions that maintain differentiation capacity during expansion; more particularly, culturing non-embryonic cells in the presence of at least one GKS-3 inhibitor, such as BIO.

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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2006/029736

## A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N5/02 C12N5/06 C12N5/08 A61K35/12

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 A61K

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, BIOSIS, MEDLINE, EMBASE, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                  | Relevant to claim No. |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| P,X       | WO 2006/072016 A (UNIV WASHINGTON [US];<br>ROBARTS RES INST [CA]; MOON RANDALL T<br>[US]; BHAT) 6 July 2006 (2006-07-06)<br>paragraphs [0012], [0028], [0041],<br>[0055]<br>figures<br>examples<br>claims 1,6,11,12<br>-----<br>-/- | 1-3,<br>11-29         |



Further documents are listed in the continuation of Box C.



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 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

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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2006/029736

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                       |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                          | Relevant to claim No. |
| X                                                    | <p>XU TIANSHUN ET AL: "GSK3 inhibitors inhibit adipocyte differentiation from pluripotential mesenchymal stem cells". DIABETES, vol. 53, no. Suppl. 2, June 2004 (2004-06), page A100, XP009074258<br/>&amp; 64TH ANNUAL MEETING OF THE AMERICAN-DIABETES-ASSOCIATION; ORLANDO, FL, USA; JUNE 04 -08, 2004<br/>ISSN: 0012-1797<br/>abstract</p>                                                                                                                             | 1-3,<br>12-29         |
| X                                                    | <p>WO 2005/063966 A2 (AXARON BIOSCIENCE AG [DE]; MAURER MARTIN H [DE]; FELDMANN ROBERT E [DE] 14 July 2005 (2005-07-14) page 3, paragraph 3<br/>page 5, paragraphs 2,3<br/>page 8, paragraph 1-5<br/>example 3<br/>claims 1,2,6-9</p>                                                                                                                                                                                                                                       | 1-3,11,<br>12,14-29   |
| X                                                    | <p>CUI HONG ET AL: "Inhibition of glycogen synthase kinase 3beta activity regulates proliferation of cultured cerebellar granule cells" DEVELOPMENTAL BRAIN RESEARCH, vol. 111, no. 2, 7 December 1998 (1998-12-07), pages 177-188, XP002404792<br/>ISSN: 0165-3806<br/>abstract<br/>page 178, left-hand column, lines 12-14<br/>page 183, right-hand column, lines 15-18<br/>page 185, right-hand column, paragraph 10-15<br/>page 186, right-hand column, lines 11-17</p> | 1-3,11,<br>12,16-29   |
| X                                                    | <p>MINCHATA K ET AL: "LITHIUM CHLORIDE MAINTAINS HEMATOPOIETIC PROGENITOR ACTIVITY BY INHIBITING THE PROLIFERATION AND DIFFERENTIATION OF MURINE HEMATOPOIETIC CELLS" BLOOD, W.B.SAUNDERS COMPANY, ORLANDO, FL, US, vol. 100, no. 11, 16 November 2002 (2002-11-16), page 179B, ABSTRACT NO 4216, XP009066417<br/>ISSN: 0006-4971<br/>abstract</p>                                                                                                                          | 1-3,11,<br>12,14-29   |
|                                                      | -----<br>-/--                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                       |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2006/029736

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>SATO N ET AL: "MAINTENANCE OF PLURIPOTENCY IN HUMAN AND MOUSE EMBRYONIC STEM CELLS THROUGH ACTIVATION OF WNT SIGNALING BY A PHARMACOLOGICAL GSK-3-SPECIFIC INHIBITOR"</p> <p>NATURE MEDICINE, NATURE PUBLISHING GROUP, NEW YORK, NY, US,<br/>vol. 10, no. 1,<br/>21 December 2003 (2003-12-21), pages 55-63, XP002380572<br/>ISSN: 1078-8956<br/>abstract<br/>page 55, right-hand column, lines 6-13<br/>page 57, left-hand column, line 1 - right-hand column, line 3<br/>page 58, right-hand column, paragraph 3 -<br/>page 61, left-hand column, paragraph 1<br/>page 61, right-hand column, lines 54-58<br/>page 62, left-hand column, paragraph 2<br/>page 62, right-hand column, paragraph 3</p> | 1-29                  |
| Y         | <p>MEIJER L ET AL: "Pharmacological inhibitors of glycogen synthase kinase 3"</p> <p>TRENDS IN PHARMACOLOGICAL SCIENCES, ELSEVIER, AMSTERDAM, NL,<br/>vol. 25, no. 9, September 2004 (2004-09), pages 471-480, XP004552644<br/>ISSN: 0165-6147<br/>abstract<br/>page 471, left-hand column, paragraph 2<br/>page 477, right-hand column, paragraph 6 -<br/>page 478, left-hand column, paragraph 3</p>                                                                                                                                                                                                                                                                                                    | 1-29                  |
| A         | <p>OKUDA T ET AL: "Oct-3/4 repression accelerates differentiation of neural progenitor cells in vitro and in vivo"</p> <p>MOLECULAR BRAIN RESEARCH, ELSEVIER SCIENCE BV, AMSTERDAM, NL,<br/>vol. 132, no. 1,<br/>6 December 2004 (2004-12-06), pages 18-30, XP004645592<br/>ISSN: 0169-328X<br/>page 18 - page 30</p>                                                                                                                                                                                                                                                                                                                                                                                     | 1-29                  |

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2006/029736

### Box 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 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 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.:

4-10 (completely) and 1-3, 11-29 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ 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: 4-10 (completely) and 1-3, 11-29 (partially)

A culture method comprising culturing non-embryonic cells in the presence of the GSK-3 inhibitor indirubin or various derivatives thereof; a composition comprising non-embryonic cells, the GSK-3 inhibitor indirubin or various derivatives thereof; a method of preparing said composition.

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2. claims: 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is LiCl.

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3. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is hymenialdisine.

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4. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is flavopiridol.

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5. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is kenpauillone, or alsterpauillone or azakenpauillone.

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6. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is Aloisine A or Aloisine B.

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7. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is TDZD8.

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8. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is Pyrazolopyridine 18, Pyrazolopyridine 19 or Pyrazolopyridine 34.

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9. claims: claims 1-3 and 11-29 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as invention 1 but where said GSK-3 inhibitor is  
CHIR98014 or CHIR99021.  
---

10. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is  
CHIR-637 (CT20026).  
---

11. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is  
SU9516.  
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12. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is  
ARA014418.  
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13. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is  
Staurosporine, GF109203x, Ro318220, SB216763 or SB415286.  
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14. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is  
CGP60474 or TWS119.  
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15. claims: claims 1-3 and 11-29 (all partially)

Idem as invention 1 but where said GSK-3 inhibitor is  
thiazolo 5,4-f quinazolin-9-one.  
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2006/029736

| Patent document<br>cited in search report |    | Publication<br>date | Patent family<br>member(s) |               | Publication<br>date |
|-------------------------------------------|----|---------------------|----------------------------|---------------|---------------------|
| WO 2006072016                             | A  | 06-07-2006          | US                         | 2006147435 A1 | 06-07-2006          |
| WO 2005063966                             | A2 | 14-07-2005          | AU                         | 2004309070 A1 | 14-07-2005          |
|                                           |    |                     | CA                         | 2551259 A1    | 14-07-2005          |
|                                           |    |                     | DE                         | 10361444 A1   | 21-07-2005          |
|                                           |    |                     | EP                         | 1697501 A2    | 06-09-2006          |