(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

REVISED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number
WO 2003/054170 A1

(51) International Patent Classification[7]: C12N 5/06, C07K 14/54, A01K 67/027

(21) International Application Number: PCT/BE2002/000196

(22) International Filing Date: 20 December 2002 (20.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/BE01/00221 21 December 2001 (21.12.2001) BE
0220145.7 30 August 2002 (30.08.2002) GB

(71) Applicant *(for all designated States except US)*: **THROMB-X NV** [BE/BE]; Leopold-I-straat 1 bus 21, B-3000 Leuven (BE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **SCHOONJANS, Luc** [BE/BE]; Pompstraat 27, B-3012 Wilsele (BE).

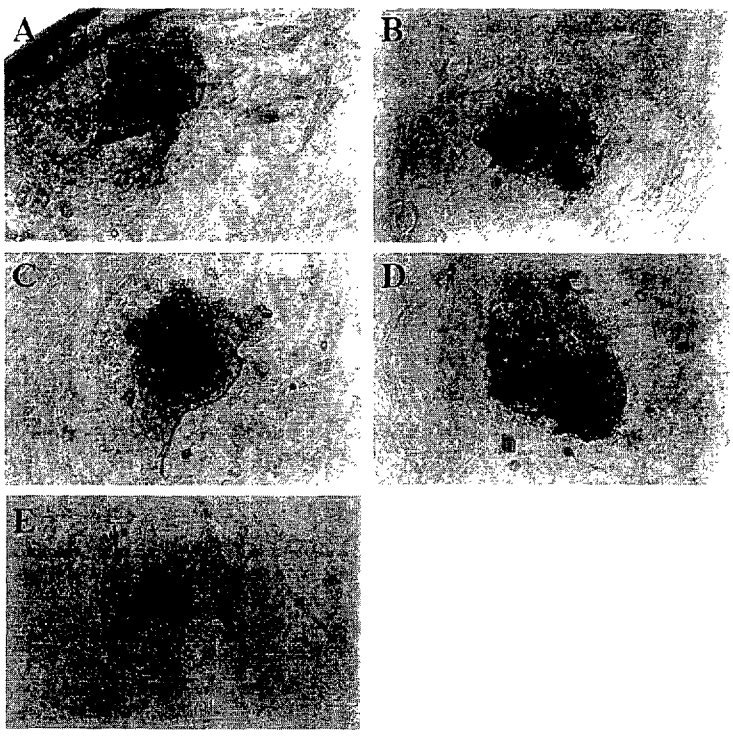
(74) Agents: **BIRD, William, E.** et al.; Bird Goën & Co, Klein Dalenstraat 42A, B-3020 Winksele (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK,

[Continued on next page]

(54) Title: COMPOSITIONS FOR THE IN VITRO DERIVATION AND CULTURE OF EMBRYONIC STEM (ES) CELL LINES WITH GERMLINE TRANSMISSION CAPABILITY AND FOR THE CULTURE OF ADULT STEM CELLS



recombinant rabbit LIF in the *Pichia pastoris* expression system.

(57) Abstract: The present invention describes novel compositions for deriving, maintaining and growing pluripotent and germ-line competent mammalian embryonic stem cells. The compositions of this invention refers to compositions comprising a 1) conditioned medium of a cell line expressing limited amounts of Leukemia Inhibitory Factor (LIF), 2) conditioned medium from a cell line transfected with mammalian LIF and 3) a medium supplemented with recombinant rabbit LIF. The present invention describes novel compositions for deriving, maintaining and growing adult human stem cells and/or adult early progenitor cells, preferably under stroma free conditions and without added LIF and/or cytokines or growth factors. The media of the present invention are used or for the generation of pluripotent and germ line competent embryonic stem cells of mammals of which these cells were not obtained up to now. The media of the present invention are used or for the generation of adult human stem cells and/or adult early progenitor cells. The present invention is also directed to a novel rabbit LIF and to nucleotides encoding the Rabbit LIF and methods for the expression of

WO 2003/054170 A1

TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *with international search report*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*

(88) Date of publication of the revised international search report: 29 April 2004

(15) Information about Correction:
see PCT Gazette No. 18/2004 of 29 April 2004, Section II

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.