



(51) International Patent Classification:

C12N 5/071 (2010.01) *A61P 1/16* (2006.01)
A61K 35/12 (2006.01) *C12N 5/0735* (2010.01)
A61K 35/407 (2006.01) *C12Q 1/02* (2006.01)

(21) International Application Number:

PCT/CA2014/000122

(22) International Filing Date:

18 February 2014 (18.02.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/766,002 18 February 2013 (18.02.2013) US

(71) Applicants: UNIVERSITY HEALTH NETWORK

[CA/CA]; Technology Development and Commercialization, MaRS Centre, Heritage Building, 101 College Street, Suite 150, Toronto, Ontario M5G 1L7 (CA). **THE HOSPITAL FOR SICK CHILDREN** [CA/CA]; 555 University Avenue, Toronto, Ontario M5G 1X8 (CA).

(72) Inventors: KELLER, Gordon;

1 Douglas Crescent, Toronto, Ontario M4W 2E6 (CA). **OGAWA, Shinichiro**; 10 Shallmar Blvd. #601, Toronto, Ontario M5N 1J4 (CA). **GHANEKAR, Anand**; 38 Tofino Crescent, Toronto,

Ontario M3B 1R8 (CA). **BEAR, Christine**; 77 Abbeywood Trail, Toronto, Ontario M3B 3B6 (CA). **KAMATH, Binita M.**; 13 Binscarth Road, Toronto, Ontario M4W 1Y2 (CA). **OGAWA, Mina**; 10 Shallmar Boulevard, Apt. 601, Toronto, Ontario M5N 1J4 (CA). **SURAPIS-ITCHAT, James**; 88 Erskine Ave., Apt. #2404, Toronto, Ontario M4P 1Y3 (CA).

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L., S.R.L.;

40th Floor, 40 King Street West, Scotia Plaza, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

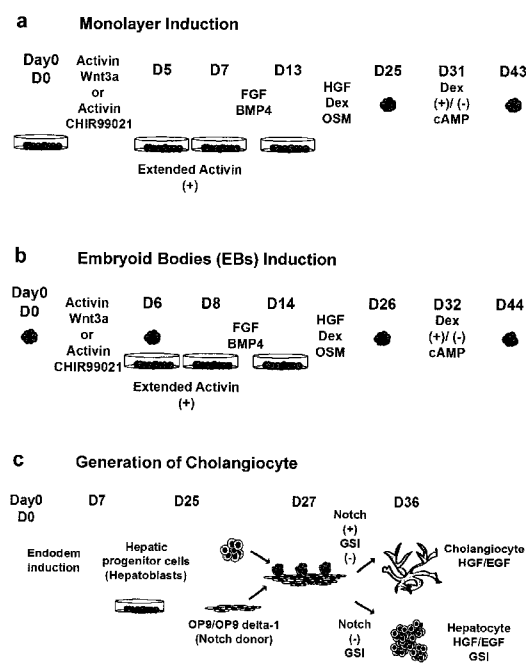
(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

[Continued on next page]

(54) Title: METHODS FOR GENERATING HEPATOCYTES AND CHOLANGIOCYTES FROM PLURIPOTENT STEM CELLS

Figure 11



(57) Abstract: Methods for producing hepatocyte and/or cholangiocyte lineage cells from pluripotent stem cells, the method comprising (a) specifying the extended nodal agonist treated induced endodermal cell population to obtain a cell population comprising hepatocyte and/or cholangiocyte progenitors by contacting the extended nodal agonist treated induced endodermal cell population with specification media comprising a FGF agonist and a BMP4 agonist and/or active conjugates and/or fragments thereof; (b) inducing maturation, and optionally further lineage specification and/or expansion of the hepatocyte and/or cholangiocyte progenitors of the cell population to obtain a population comprising hepatocyte lineage cells such as hepatoblasts, hepatocytes and/or cholangiocytes, the inducing maturation step comprising generating aggregates of the cell population. Optionally, the method also comprises activating the cAMP pathway within the aggregates and forming co-aggregates.



GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

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

Published:

— *with international search report (Art. 21(3))*