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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
03 December 2020 (03.12.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/239807 A1

(51) International Patent Classification:
C12N 5/079 (2010.01) *C12N 15/90* (2006.01)
A61K 35/30 (2015.01)

(21) International Application Number:
PCT/EP2020/064649

(22) International Filing Date:
27 May 2020 (27.05.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
19176722.7 27 May 2019 (27.05.2019) EP

(71) Applicant: **WESTFÄLISCHE WILHELMS-UNIVERSITÄT MÜNSTER** [DE/DE]; Schlossplatz 2, 48149 Münster (DE).

(72) Inventors: **PAWLOWSKI, Matthias**; Niels-Stensen-Str. 24, 48149 Münster (DE). **SPEICHER, Anna Martina**; Buersche Straße 29, 49074 Osnabrück (DE).

(74) Agent: **WEINZIERL, Gerhard** et al.; c/o Schiweck Weinzierl Koch, Ganghoferstrasse 68b, 80339 Munich (DE).

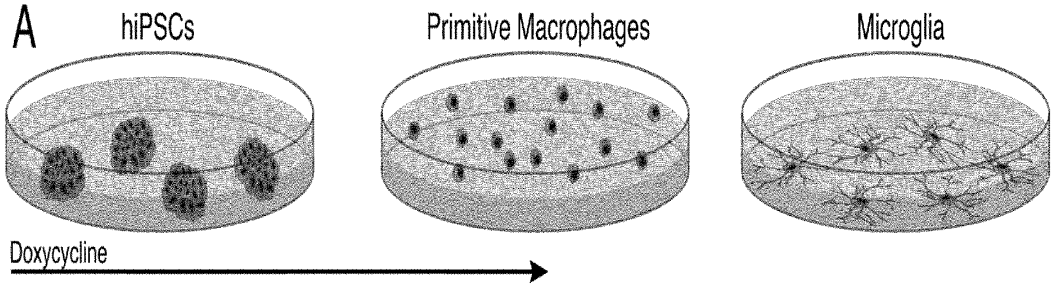
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, 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))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** RAPID AND DETERMINISTIC GENERATION OF MICROGLIA FROM HUMAN PLURIPOTENT STEM CELLS

Figure 5



(57) **Abstract:** The present invention relates to a method for the production of microglia from stem cells comprising the steps of a) targeted insertion of a nucleotide sequence encoding a transcriptional regulator protein into a first genomic safe harbour site; and b) targeted insertion of the coding sequence of the transcription factor PU.1 (SEQ ID NO: 1) into a second genomic safe harbour site, wherein the gene is operably linked to an inducible promoter, which is regulated by the transcriptional regulator protein; expression of PU.1 (SEQ ID NO: 2); and culturing the stem cells received from steps a) and b) with exposure to at least one growth factor or small molecule that mimics signaling during at least one stage of embryonic development of microglia or adult microglia proliferation, differentiation or polarization. Further, the present invention relates to the microglia obtained by the methods of the present invention and various uses thereof.

WO 2020/239807 A1