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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 January 2004 (22.01.2004)

PCT

(10) International Publication Number
WO 2004/008100 A3

(51) International Patent Classification[7]: C12N 5/00

(21) International Application Number:
PCT/US2003/021733

(22) International Filing Date: 14 July 2003 (14.07.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/396,221 15 July 2002 (15.07.2002) US

(71) Applicant *(for all designated States except US)*: **IMMUNEX CORPORATION** [US/US]; Law Department, 51 University Street, Seattle, WA 98101 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **FOLLSTAD, Brian, D.** [US/US]; 900 Queen Anne Avenue N. #106, Seattle, WA 98109 (US).

(74) Agent: **SWEENEY, Rosemary**; Immunex Corporation, Law Department, 51 University Street, Seattle, WA 98101 (US).

(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, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, 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, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
25 March 2004

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.

WO 2004/008100 A3

(54) Title: METHODS AND MEDIA FOR CONTROLLING SIALYLATION OF PROTEINS PRODUCED BY MAMMALIAN CELLS

(57) Abstract: The invention provides media and methods for culturing mammalian cells whereby the sialylation of a protein produced by the cells is increased. The medium can contain N-acetylmannosamine and, optionally, galactose. The medium may also comprise fructose and mannose. Alternatively, the medium can contain galactose and fructose and, optionally, can also comprise mannose and/or N-acetylmannosamine. The methods can be practiced along with other methods for culturing cells so as to increase the quantity or quality of a protein produced by the cells, including culturing the cells at a temperature below 37 °C.