

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number  
**WO 00/077236 A3**

- (51) **International Patent Classification<sup>7</sup>:** C12N 15/54, 15/67, 5/10, 5/06, 9/12, C07K 14/52, 14/54, 14/525, C12P 21/02
- (21) **International Application Number:** PCT/US00/40199
- (22) **International Filing Date:** 14 June 2000 (14.06.2000)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
60/139,313 15 June 1999 (15.06.1999) US
- (71) **Applicant:** GENETROL BIOTHERAPEUTICS [US/US]; Suite 201, 2220 Livingston Street, Oakland, CA 94606 (US).
- (72) **Inventors:** LAU, Allan, S.; 39 Rosewood Drive, San Francisco, CA 94127 (US). BROWNING, Laura; 925 Outrigger Circle, Brentwood, CA 94513 (US).
- (74) **Agents:** JUDGE, Linda, R. et al.; Iota Pi Law Group, P.O. Box 60850, Palo Alto, CA 94306-0850 (US).
- (81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, 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, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— with international search report
- (88) **Date of publication of the international search report:**  
12 September 2002
- 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 00/077236 A3

(54) **Title:** METHODS FOR ENHANCING THE PRODUCTION OF CYTOKINES IN CELL CULTURE

(57) **Abstract:** The present invention is directed to methods for enhancing the production of cytokines in mammalian cell culture by modifying the level of a cytokine regulatory factor in the cells, resulting in enhanced levels of cytokine production. The invention is further directed to cell lines which exhibit enhanced cytokine regulatory factor expression and enhanced cytokine expression.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/40199

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 C12N15/54 C12N15/67 C12N5/10 C12N5/06 C12N9/12  
 C07K14/52 C07K14/54 C07K14/525 C12P21/02

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)

IPC 7 C12N C07K C12P

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)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 08324 A (UNIV CALIFORNIA) 6 March 1997 (1997-03-06) cited in the application	1-7, 9-11
Y	the whole document	8, 12-17
Y	WO 93 17043 A (BRIGHAM & WOMENS HOSPITAL ; BETH ISRAEL HOSPITAL (US)) 2 September 1993 (1993-09-02) page 22, line 22 -page 23, line 29	8, 12, 15

Further documents are listed in the continuation of box C.

Patent family members are listed in 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

21 June 2001

Date of mailing of the international search report

09. 07. 01

Name and mailing address of the ISA  
 European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

## INTERNATIONAL SEARCH REPORT

Int ional Application No

PCT/US 00/40199

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HARANT H ET AL: "1ALPHA,25-DIHYDROXYVITAMIN D3 AND A VARIETY OF ITS NATURAL METABOLITES TRANSCRIPTIONALLY REPRESS NUCLEAR-FACTOR-KB-MEDIATED INTERLEUKIN-8 GENE EXPRESSION" EUROPEAN JOURNAL OF BIOCHEMISTRY, DE, BERLIN, vol. 282, no. 1, November 1997 (1997-11), pages 63-71, XP002915093 ISSN: 0014-2956 the whole document	13,16
Y	LINDHOLM P F ET AL: "EXTRACELLULAR TAX-1 PROTEIN STIMULATES TUMOR NECROSIS FACTOR-BETA AND IMMUNOGLOBULIN KAPPA LIGHT CHAIN EXPRESSION IN LYMPHOID CELLS" JOURNAL OF VIROLOGY, vol. 66, no. 3, 1992, pages 1294-1302, XP000993234 ISSN: 0022-538X the whole document	14,17
A	LIBERMANN T A ET AL: "ACTIVATION OF INTERLEUKIN-6 GENE EXPRESSION THROUGH THE NF-KAPPA-B TRANSCRIPTION FACTOR" MOLECULAR AND CELLULAR BIOLOGY, vol. 10, no. 5, 1990, pages 2327-2334, XP002160675 ISSN: 0270-7306 the whole document	8,12,15
A	US 5 641 670 A (HEARTLEIN MICHAEL W ET AL) 24 June 1997 (1997-06-24) claims 10,18	12,15
A	KUMAR ASEEM ET AL: "Double-stranded RNA-dependent protein kinase activates transcription factor NF-kappa-B by phosphorylating I-kappa-B." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 91, no. 14, 1994, pages 6288-6292, XP002170325 1994 ISSN: 0027-8424 the whole document	

-/--

## INTERNATIONAL SEARCH REPORT

Inter 1al Application No

PCI/US 00/40199

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BRETON J J ET AL: "THE NATURAL PRODUCT HYMENIALDISINE INHIBITS INTERLEUKIN-8 PRODUCTION IN U937 CELLS BY INHIBITION OF NUCLEAR FACTOR-KAPPA B" JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, US, AMERICAN SOCIETY FOR PHARMACOLOGY AND, vol. 282, no. 1, 1997, pages 459-466, XP002910635 ISSN: 0022-3565 the whole document</p>	13,16

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/40199

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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.:

### 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: 12,15 completely, and claims 1-11 all partially

Method for producing IL-6 using a PKR-overexpressing cell line, optionally involving priming with e.g. a phorbol ester, and/or induction with e.g. poly r(I):poly r(C), and mammalian cell line which overexpresses both PKR and IL-6.

2. Claims: 13,16 completely, and claims 1-11 all partially

Method for producing IL-8 using a PKR-overexpressing cell line, optionally involving priming with e.g. a phorbol ester, and/or induction with e.g. poly r(I):poly r(C), and mammalian cell line which overexpresses both PKR and IL-8.

3. Claims: 14,17 completely, and claims 1-11 all partially

Method for producing TNF-beta using a PKR-overexpressing cell line, optionally involving priming with e.g. a phorbol ester, and/or induction with e.g. poly r(I):poly r(C), and mammalian cell line which overexpresses both PKR and TNF-beta.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/40199

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9708324	A	06-03-1997	AU 6857696	A 19-03-1997
			AU 707130	B 01-07-1999
			AU 6860896	A 19-03-1997
			BR 9610550	A 06-07-1999
			CA 2229163	A 06-03-1997
			CA 2229405	A 06-03-1997
			CN 1200148	A 25-11-1998
			EP 0846174	A 10-06-1998
			EP 0846160	A 10-06-1998
			JP 11511324	T 05-10-1999
			JP 11514344	T 07-12-1999
			WO 9708292	A 06-03-1997
			US 6159712	A 12-12-2000
			US 2001001290	A 17-05-2001
US 2001001709	A 24-05-2001			
WO 9317043	A	02-09-1993	AU 3780593	A 13-09-1993
			CA 2130802	A 02-09-1993
			EP 0630384	A 28-12-1994
			JP 7509119	T 12-10-1995
			US 5958403	A 28-09-1999
US 5641670	A	24-06-1997	AU 709058	B 19-08-1999
			AU 2550495	A 05-12-1995
			BR 9507874	A 19-08-1997
			CA 2190289	A 23-11-1995
			CN 1119545	A 03-04-1996
			CZ 9603258	A 17-12-1997
			EP 0759082	A 26-02-1997
			FI 964536	A 09-01-1997
			HU 76844	A 28-11-1997
			JP 10500570	T 20-01-1998
			NO 964802	A 09-01-1997
			NZ 285945	A 25-03-1998
			SK 146196	A 04-02-1998
			WO 9531560	A 23-11-1995
			US 5733746	A 31-03-1998
			ZA 9503879	A 18-01-1996
			AU 689455	B 02-04-1998
			AU 5736294	A 22-06-1994
			AU 5840198	A 04-06-1998
			CA 2151032	A 09-06-1994
			EP 0672160	A 20-09-1995
			JP 8503856	T 30-04-1996
			MX 9307623	A 30-06-1994
			NZ 259165	A 26-01-1998
			NZ 329348	A 28-10-1999
			SG 46476	A 20-02-1998
			WO 9412650	A 09-06-1994
			US 5733761	A 31-03-1998
			US 5968502	A 19-10-1999
			AT 155810	T 15-08-1997
			AU 669499	B 13-06-1996
			AU 3069892	A 07-06-1993
AU 710255	B 16-09-1999			
AU 6563196	A 28-11-1996			
DE 69221168	D 04-09-1997			
DE 69221168	T 26-02-1998			

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/40199

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5641670 A		DK 649464 T	02-03-1998
		EP 0649464 A	26-04-1995
		EP 0750044 A	27-12-1996
		ES 2106891 T	16-11-1997
		GR 3025054 T	30-01-1998
		JP 7500969 T	02-02-1995
		MX 9206376 A	28-02-1994
		NZ 245015 A	21-12-1995
		NZ 270805 A	24-10-1997
		NZ 270864 A	24-10-1997

---