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(71) Applicant (for all designated States except US): **MERCK SERONO S.A.** [IL/CH]; Centre industriel, CH-1267 Coin-sins, Vaud (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HELMAN, Daniel** [IL/IL]; 37 Hatzivoni St., 55525 Kiryat Ono (IL). **BAR-SHIMON, Meirav** [IL/IL]; 3 Zvi Harkin St., 70700 Gedera (IL). **TOISTER-ACHITUV, Mira** [IL/IL]; 6 Taran St., 76248 Rehovot (IL).(74) Agent: **EINAV, Henry**; Inter-Lab Ltd., 18 Hakishon St., 81220 Yavne (IL).

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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, IS, JP, KE, KG, KM, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, 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.

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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

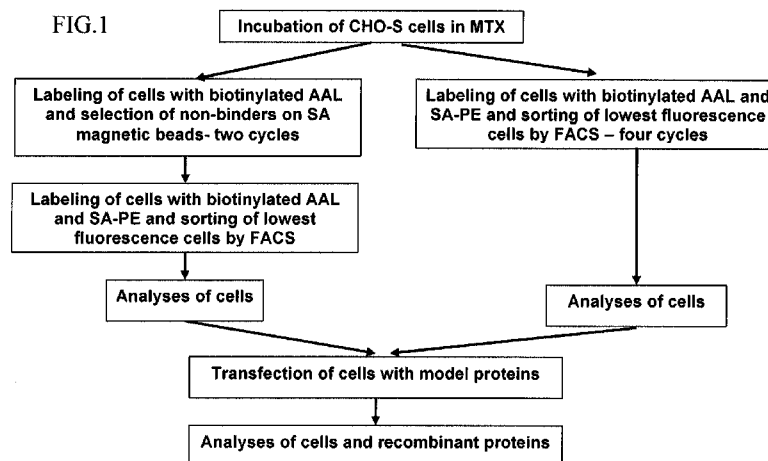
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- with international search report (Art. 21(3))
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- with sequence listing part of description (Rule 5.2(a))

[Continued on next page]

(54) Title: LOW FUCOSE CELL LINES AND USES THEREOF

FIG.1



(57) **Abstract:** A method of selecting cells having zero fucose level useful as host cells for expressing recombinant proteins is disclosed. The method comprises: (d) introducing genetic mutations into a population of CHO cells by contacting the cells with a methotrexate (MTX), (e) contacting the population of CHO cells comprising mutated cells with a non-toxic fucose binding agent for an amount of time that allows binding of the fucose binding agent to a fucose moiety on a cell membrane of the population of cells, wherein the amount of time does not allow killing of the cells; and (f) depleting from the population of cells comprising mutated cells, a subpopulation of cells which bind the fucose binding agent, thereby selecting cells useful as host cells for expressing recombinant proteins, the selected cells having zero fucose content. There are also disclosed cells and cell lines useful as host cells for expressing recombinant proteins.



— *with information concerning incorporation by reference
of missing parts and/or elements (Rule 20.6)*

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2012/000109

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/10 C12N9/10 C12N15/01
ADD.

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)
C12N

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 practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/036443 A1 (EUREKA THERAPEUTICS INC [US]; LIU CHENG [US]; XIANG JINGYI [US]; YAN S) 1 April 2010 (2010-04-01) paragraphs [0015] - [0035] paragraphs [0038] - [0040] paragraphs [0071], [0077], [0078] paragraphs [00167] - [00177] paragraphs [00202] - [00203]; figure 2 ----- -/--	1-14,18, 19,21-38

☒ Further documents are listed in the continuation of Box C.

☒ See patent family 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 application or patent 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

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European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Seroz, Thierry

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2012/000109

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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 No. III Observations where unity of invention is lacking (Continuation of item 3 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 fees, this Authority did not invite payment of additional fees.
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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2012/000109

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHOW MING ET AL: "Random population-wide genetic damage induced in replicating cells treated with methotrexate", 30 March 1998 (1998-03-30), MUTATION RESEARCH, VOL. 413, NR. 3, PAGE(S) 251-264, XP002677215, ISSN: 0027-5107 page 262, column 1, last paragraph - page 262, column 2, paragraph 1 page 252, column 1, paragraph 2 - page 252, column 2, paragraph 2 -----	1-14,18, 19,21-38
Y	HOFFMANN S ET AL: "RAPID ISOLATION OF CHORIOCAPILLARY ENDOTHELIAL CELLS BY LYCOPERSICON ESCULENTUM-COATED DYNABEADS", GRAEFE'S ARCHIVE FOR CLINICAL AND EXPERIMENTAL OPHTHALMOLOGY, SPRINGER VERLAG, XX, vol. 236, no. 10, 1 October 1998 (1998-10-01), pages 779-784, XP001025139, ISSN: 0721-832X, DOI: 10.1007/S004170050158 page 780, column 1, paragraph 2 - page 780, column 2, paragraph 1 -----	1-14,18, 19,21-38
Y	JACKSON C J ET AL: "Binding of human endothelium to ulex europaeus I-coated dynabeads: application to the isolation of microvascular endothelium", JOURNAL OF CELL SCIENCE, CAMBRIDGE UNIVERSITY PRESS, LONDON, GB, vol. 96, 1 January 1990 (1990-01-01), pages 257-262, XP002263448, ISSN: 0021-9533 page 257, column 2, last paragraph - page 258, column 1, paragraph 3 -----	1-14,18, 19,21-38
X	RIPKA J ET AL: "Two chinese hamster ovary glycosylation mutants affected in the conversion of GDP-mannose to GDP-fucose", ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, ACADEMIC PRESS, US, vol. 249, no. 2, 1 September 1986 (1986-09-01), pages 533-545, XP024759128, ISSN: 0003-9861, DOI: 10.1016/0003-9861(86)90031-7 [retrieved on 1986-09-01] page 534, column 1, paragraph 2; table 2 page 544, column 1, last paragraph - page 544, column 2, paragraph 1 Abstract ----- -/--	14-38

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2012/000109

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KANDA ET AL: "Establishment of a GDP-mannose 4,6-dehydratase (GMD) knockout host cell line: A new strategy for generating completely non-fucosylated recombinant therapeutics", JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 130, no. 3, 19 June 2007 (2007-06-19), pages 300-310, XP022119693, ISSN: 0168-1656, DOI: 10.1016/J.JBIOTEC.2007.04.025 page 301, column 1, last paragraph - page 301, column 2, paragraph 1 page 303, column 1, paragraph 2 page 304, column 1, paragraph 1 - page 305, column 2, paragraph 1; figures 4,6; table 1 page 309, column 1, paragraph 1 -----	15-23, 25-38
A	OHYAMA C ET AL: "Molecular cloning and expression of GDP-D-mannose-4,6-dehydratase, a key enzyme for fucose metabolism defective in Lec13 cells", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 273, no. 23, 5 June 1998 (1998-06-05), pages 14582-14587, XP002328593, ISSN: 0021-9258, DOI: 10.1074/JBC.273.23.14582 the whole document -----	15-23, 25-38
A	MASUDA ET AL: "Enhanced binding affinity for FcγRIIIa of fucose-negative antibody is sufficient to induce maximal antibody-dependent cellular cytotoxicity", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 44, no. 12, 17 April 2007 (2007-04-17), pages 3122-3131, XP022031617, ISSN: 0161-5890, DOI: 10.1016/J.MOLIMM.2007.02.005 the whole document -----	1-14,18, 19,21-38
A	KANDA YUTAKA ET AL: "Comparison of cell lines for stable production of fucose-negative antibodies with enhanced ADCC", BIOTECHNOLOGY AND BIOENGINEERING, WILEY & SONS, HOBOKEN, NJ, US, vol. 94, no. 4, 1 July 2006 (2006-07-01), pages 680-688, XP002415543, ISSN: 0006-3592, DOI: 10.1002/BIT.20880 the whole document ----- -/--	1-14,18, 19,21-38

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2012/000109

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATSUME A ET AL: "Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 306, no. 1-2, 30 November 2005 (2005-11-30), pages 93-103, XP027659257, ISSN: 0022-1759 [retrieved on 2005-11-30] the whole document -----	1-14,18, 19,21-38

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2012/000109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010036443 A1	01-04-2010	CN 102216452 A	12-10-2011
		EP 2340305 A1	06-07-2011
		JP 2012503656 A	09-02-2012
		KR 20110084196 A	21-07-2011
		US 2010081150 A1	01-04-2010
		US 2010081172 A1	01-04-2010
		US 2010081195 A1	01-04-2010
		US 2010081794 A1	01-04-2010
		US 2012107874 A1	03-05-2012
		WO 2010036443 A1	01-04-2010

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14, 24(completely); 18, 19, 21-23, 25-38(partially)

A method of selecting CHO cells having zero fucose level and cells obtained by said method.

2. claims: 15-17, 20(completely); 18, 19, 21-23, 25-38(partially)

An isolated CHO cell genetically modified to express a mutated GMD protein.
