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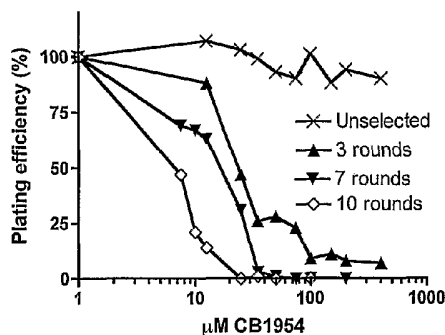
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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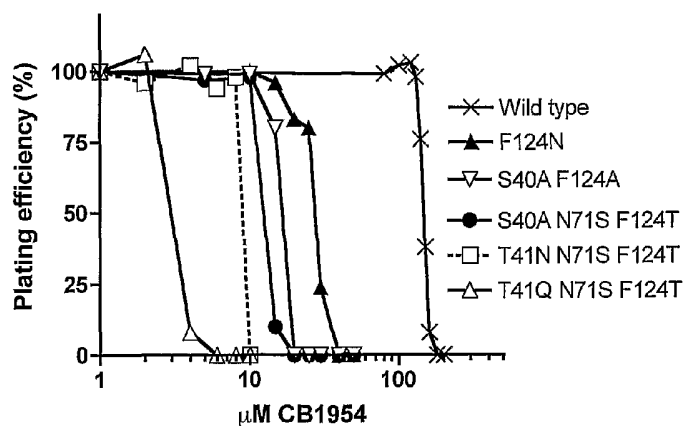
[Continued on next page]

(54) Title: IMPROVED NITROREDUCTASE ENZYMES

a



b



(57) Abstract: Novel nitroreductase mutants are disclosed, having improved converting activity for the aziridinyl prodrug CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide). The mutants have point mutations at three or more sites as compared with the *E. coli* wild-type nitroreductase and are useful for virus- or gene-directed enzyme-prodrug therapy approaches to treating diseases such as cancer. Also provided are polynucleotides and vectors encoding the improved nitroreductases of the invention.



FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments*

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

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— *with international search report*

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ning of each regular issue of the PCT Gazette.*

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2006/001180

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/02 C12N15/861 C12N5/10 C12N1/21 C12N15/52
 A61K38/44 A61K48/00 C12Q1/26

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 A61K

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)

EPO-Internal, WPI Data, PAJ, Sequence Search, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/018788 A (ML LABORATORIES PLC; GROVE, JANE, ISABEL; SEARLE, PETER, FRANCIS; LOVE) 6 March 2003 (2003-03-06) cited in the application page 4, l. 26-31; page 6 l. 31-page 10, l. 18; pages 22-24; claims 8, 11, 12, 18, 19, 23, 24, 26-52; Figure 5 ----- -/---	1-28



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 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

18 September 2006

Date of mailing of the international search report

12/10/2006

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2006/001180

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GROVE JANE I ET AL: "Generation of Escherichia coli nitroreductase mutants conferring improved cell sensitization to the prodrug CB1954." CANCER RESEARCH, vol. 63, no. 17, 1 September 2003 (2003-09-01), pages 5532-5537, XP002399200 ISSN: 0008-5472 cited in the application pages 5533, 5535 and 5536; Figures 1 and 2c	1-28
A	----- SEARLE PETER F ET AL: "Nitroreductase: A prodrug-activating enzyme for cancer gene therapy" CLINICAL AND EXPERIMENTAL PHARMACOLOGY AND PHYSIOLOGY, vol. 31, no. 11, November 2004 (2004-11), pages 811-816, XP002399201 ISSN: 0305-1870 cited in the application summary; page 814	1-28
A	----- ZENNO S ET AL: "Conversion of NfsB, a minor Escherichia coli nitroreductase, to a flavin reductase similar in biochemical properties to FRase I, the major flavin reductase in Vibrio fischeri, by a single amino acid substitution" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 178, no. 15, August 1996 (1996-08), pages 4731-4733, XP002227224 ISSN: 0021-9193 cited in the application page 4732	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/001180

Box 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:
Although claim 28 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
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 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:

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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2006/001180

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03018788	A	06-03-2003	CA 2458226 A1	06-03-2003
			EP 1419241 A2	19-05-2004
			JP 2005517386 T	16-06-2005
			NZ 531413 A	27-01-2006