

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
26 February 2004 (26.02.2004)

PCT

(10) International Publication Number
WO 2004/016764 A3

(51) International Patent Classification⁷: **A61K 38/44**,
C12Q 1/26, C12N 9/02, 1/20, 15/00, C07H 21/04, A01N
43/04, C07K 16/00

(21) International Application Number:
PCT/US2003/025745

(22) International Filing Date: 15 August 2003 (15.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/403,638 16 August 2002 (16.08.2002) US

(71) Applicant (for all designated States except US): **SCHERING AKTIENGESELLSCHAFT** [DE/DE]; Müllerstrasse 178, D-13342 Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BLASKO, Eric** [US/US]; 2353 Oak Glen Circle, Martinez, CA 94553 (US). **KAUSER, Katalin** [HU/US]; 1299 Raton Ct, El Sobrante, CA 94803 (US). **PARKINSON, John** [GB/US]; 6727 Waverly Road, Martinez, CA 94553 (US).

(74) Agent: **GIL, Anna**; Berlex Biosciences, 2600 Hilltop Drive, P.O. Box 4099, Richmond, CA 94804 (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:
19 May 2005

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.

(54) Title: eNOS MUTANTS USEFUL FOR GENE THERAPY

(57) **Abstract:** The present invention provides endothelial nitric oxide synthase (eNOS) polypeptide mutants and polynucleotides encoding such polypeptide mutants, useful for gene therapy. In particular, the invention provides eNOS polypeptide mutants having one or more mutations in an amino acid sequence corresponding to a functional domain of a mammalian eNOS. More particularly, the invention provides eNOS polypeptide mutants having at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding site that is phosphorylated in mammalian cells, where the mutation is not an amino acid substitution to Ala or Asp in an eNOS polypeptide mutant having a single mutation that is at the phosphorylation site; and to polynucleotides encoding such polypeptide mutants. The present invention further provides prophylactic, diagnostic, and therapeutic methods of using such eNOS polypeptide mutants and polynucleotides.



WO 2004/016764 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/25745

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/44; C12Q 1/26; C12N 9/02, 1/20, 15/00; C07H 21/04; A01N 43/04C07K 16/00

US CL : 424/94.4; 435/25, 189, 252.3, 320.1; 536/23.2; 514/44; 530/387.1

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)

U.S. : 424/94.4; 435/25, 189, 252.3, 320.1; 536/23.2; 514/44; 530/387.1

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)
stn and west.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	McCABE et al. Enhanced Electron Flux and Reduced Calmodulin Dissociation May Explain	1, 28,30, 32-36
---	"Calcium-independent" eNOS Activation by Phosphorylation. J. Biol. Chem. March 2000,	-----
A	Vol. 275, No. 9, pages 6123-6128. see abstract, Fig. 4, and the entire document.	2-27, 29, 31, 37-41
X	WO 00/62605 (YALE UNIVERSITY) 26 October 2000 (26.10.2000), see abstract, examples and the entire document.	1, 28,30, 32-41
A	US 6,620, 616 B1 (STUCHR et al.) 16 September 2003 (16.09.2003), see the entire document.	1-41

☐ 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 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

23 February 2005 (23.02.2005)

Date of mailing of the international search report

28 MAR 2005

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Tekchand Saidha

Telephone No. (571) 272 1600

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/25745

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim 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. ☐ Claim 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:
Please See Continuation 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.: 1-41

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

However, in the instant case, because of the delayed status of this application, all the groups listed below are being searched. (claim 28 being missing, claims 29-42 are renumbered 28-41 respectively)

Group I, claim(s) 1-27 & 30, drawn to endothelial nitric oxide synthase [eNOS or ecNOS or NOS3] mutant and composition thereof.

Group II, claim(s) 28-29, drawn to polynucleotide encoding mutant eNOS and composition comprising the mutant eNOS.

Group III, claim(s) 32-34, drawn to binding partner (antibody) to eNOS.

Group IV, claim(s) 35-36, drawn to method of modulating eNOS activity.

Group V, claim(s) 37, drawn to method of diagnosing a condition with aberrant eNOS activity.

Group VI, claim(s) 38-41, drawn to a prophylactic or therapeutic method of treating a condition associated with aberrant eNOS.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

The inventions listed as Groups I-VI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking Groups I-IV appears to be that they all relate to mutant eNOS. Claim 1, lack novelty as being anticipated by McCabe et al. (2000) or WO 00/62605 (2000). The claim is drawn to any isolated mammalian mutant eNOS polypeptide, wherein the mutation is made at the functional domain and such a limitation is taught by the cited references. Therefore, Groups I-VI share no special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art. Furthermore, the products of Groups I-III do not share a special common structural or functional feature while, the methods of Groups IV-VI do not use the same reagents or produce the same results. Accordingly, Groups I-VI are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept.