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(54) Title: MUTEINS OF TEAR LIPOCALIN AND METHODS FOR OBTAINING THE SAME

(57) Abstract: The present invention relates to novel muteins derived from human tear lipocalin. The invention also refers to a corresponding nucleic acid molecule encoding such a mutein and to a method for its generation. The invention further refers to a method for producing such a mutein. Finally, the invention is directed to a pharmaceutical composition comprising such a lipocalin mutein as well as to various uses of the mutein.



WO 2008/015239 A3

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2007/057971

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. C07K14/435		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) C07K		
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, EMBASE, BIOSIS, Sequence Search		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/019256 A (PIERIS PROTEOLAB AG [DE]; SKERRA ARNE [DE]; SCHLEHUBER STEFFEN [DE]) 3 March 2005 (2005-03-03) abstract page 2, line 33 - page 3, line 3 page 4, line 8 - page 6, line 5 page 9, line 22 - page 10, line 13 page 12, lines 6-11 page 13, lines 18-26 page 15, line 8 - page 17, line 26 examples 3-31	1-147
X	EP 1 270 725 A (PIERIS PROTEOLAB AG [DE]) 2 January 2003 (2003-01-02) abstract paragraph [0001] examples 1-8	1-147
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <span style="margin-left: 200px;"><input checked="" type="checkbox"/> See patent family annex.</span>		
* 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	Date of mailing of the international search report	
10 June 2008	01/07/2008	
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  Chavanne, Franz	

## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/057971

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/056464 A (PIERIS PROTEOLAB AG [DE]; MATSCHINER GABRIELE [DE]; HOHLBAUM ANDREAS [ ]) 1 June 2006 (2006-06-01)	1-3,5,6, 8-16,18, 20-50, 53-64, 68-76, 80-116, 119-131, 135-144
Y	abstract page 11, lines 18-33 page 13, lines 1-4 page 16, lines 5-7 page 20, line 25 - page 29, line 35 tables 1,3,4	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147
X	----- WO 99/16873 A (SKERRA ARNE [DE]; BESTE GERALD [DE]; SCHMIDT FRANK [DE]; STIBORA THOMA) 8 April 1999 (1999-04-08)	1-3,5,6, 8-16,18, 20-50, 53-64, 68-76, 80-116, 119-131, 135-144
Y	abstract page 8, lines 4-16 table 1	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147
X	----- GASYMOV O K ET AL: "Site-directed tryptophan fluorescence reveals the solution structure of tear lipocalin: Evidence for features that confer promiscuity in ligand binding" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 40, no. 49, 11 December 2001 (2001-12-11), pages 14754-14762, XP002269552 ISSN: 0006-2960 the whole document	1,2,6, 8-14, 18-43, 45-48, 53-62, 68-74, 80-87, 97-99

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/057971

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SKERRA A: "'ANTICALINS': A NEW CLASS OF ENGINEERED LIGAND-BINDING PROTEINS WITH ANTIBODY-LIKE PROPERTIES" REVIEWS IN MOLECULAR BIOTECHNOLOGY, ELSEVIER, AMSTERDAM, , NL, vol. 74, no. 4, June 2001 (2001-06), pages 257-275, XP001079016 ISSN: 1389-0352 the whole document	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147
Y	SCHLEHUBER S ET AL: "TUNING LIGAND AFFINITY, SPECIFICITY, AND FOLDING STABILITY OF AN ENGINEERED LIPOCALIN VARIANT - A SO-CALLED 'ANTICALIN' - USING A MOLECULAR RANDOM APPROACH" BIOPHYSICAL CHEMISTRY, NORTH-HOLLAND, AMSTERDAM, NL, vol. 96, no. 2/03, 2 May 2002 (2002-05-02), pages 213-228, XP001172431 ISSN: 0301-4622 the whole document	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147
Y	BESTE GERALD ET AL: "Small antibody-like proteins with prescribed ligand specificities derived from the lipocalin fold" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, vol. 96, no. 5, 2 March 1999 (1999-03-02), pages 1898-1903, XP002150337 ISSN: 0027-8424 the whole document	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147
Y	SCHLEHUBER STEFFEN ET AL: "Anticalins in drug development" BIODRUGS, AUCKLAND, NZ, vol. 19, no. 5, 1 January 2005 (2005-01-01), pages 279-288, XP009101206 ISSN: 1173-8804 the whole document	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147
Y	SCHLEHUBER S ET AL: "Anticalins as an alternative to antibody technology" EXPERT OPINION ON BIOLOGICAL THERAPY, ASHLEY, LONDON, GB, vol. 5, no. 11, 1 January 2005 (2005-01-01), pages 1453-1462, XP009101205 ISSN: 1471-2598 the whole document	4,7,17, 19,51, 52, 65-67, 77-79, 117,118, 132-134, 145-147

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/057971

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 2007/107563 A (TECH UNI MUENCHEN [DE]; SKERRA ARNE [DE]; NASREEN AMBER [DE]) 27 September 2007 (2007-09-27)</p> <p>abstract page 2, lines 19-26 page 4, line 17 - page 5, line 3 page 6, line 26 - page 10, line 15 page 14, lines 19-24 page 16, lines 4-16 examples 1-10</p>	<p>1-65, 68-77, 80-132, 135-145</p>

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2007/057971

## 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:  
  
Although claims 88-96, 100 and 101 are 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 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.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

## Invention 1

Claims 36-55 and 102-121 (completely); 1-35 and 80-101 (partially)

Mutein of human tear lipocalin comprising at least one mutated residue at at least one of positions 26-34, 56-58, 80, 83, 104-106 and 108, and at at least one of positions 61 and 153, or comprising at least one mutated residue at at least two of positions 24-36, 53-66, 79-84 and 103-110, wherein this mutein binds to IL-4 receptor alpha, nucleic acid molecule encoding said mutein, host cell comprising said nucleic acid molecule, composition comprising said mutein, methods for the production of said mutein, use of said mutein, and treatment methods based on the use of said mutein.

## Invention 2

Claims 56-67 and 122-134 (completely); 1-35 and 80-101 (partially)

Mutein of human tear lipocalin comprising at least one mutated residue at at least one of positions 26-34, 56-58, 80, 83, 104-106 and 108, and at at least one of positions 61 and 153, or comprising at least one mutated residue at at least two of positions 24-36, 53-66, 79-84 and 103-110, wherein this mutein binds to VEGF-R2, nucleic acid molecule encoding said mutein, host cell comprising said nucleic acid molecule, composition comprising said mutein, methods for the production of said mutein, use of said mutein, and treatment methods based on the use of said mutein.

## Inventions 3-15

Claims 1-35, 68-101 and 135-147 (partially)

Mutein of human tear lipocalin of SEQ ID No.26-33, 44-46 or 47 (one specific mutein, one invention), wherein this mutein binds to VEGF, nucleic acid molecule encoding said mutein, host cell comprising said nucleic acid molecule, composition comprising said mutein, methods for the production of said mutein, use of said mutein, and treatment methods based on the use of said mutein.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2007/057971

Patent document cited in search report	A	Publication date	Patent family member(s)	Publication date
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