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Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

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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: PROAEROLYSIN CONTAINING PROTEASE ACTIVATION SEQUENCES AND METHODS OF USE FOR TREATMENT OF PROSTATE CANCER

Example of a Variant Proaerolysin (SEQ ID NOS: 3 and 4)

Binding Domain	Toxin	Prostate-Specific Protease Cleavage Site (e.g. HSSKLQ)	Inhibitory Peptide
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(57) Abstract: Disclosed herein are modified proaerolysin (PA) peptide. In some examples, the proteins include a prostate-specific protease cleavage site and can further include a prostate-tissue-specific binding domain which functionally replaces the native PA binding domain. In other examples, The proteins include a furin cleavage site and a prostate tissue-specific binding domain which functionally replaces the native PA binding domain. Methods of using such peptides to treat prostate cancer are also disclosed.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/27061

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/52, 15/57, 15/62; C07K 14/32, 14/33, 19/00

US CL : 435/220; 536/23.2, 23.4; 530/350

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. : 435/220; 536/23.2, 23.4; 530/350

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)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,777,078 A (BAYLEY et al) 07 July 1998 (07.07.1998), columns 2, 3, 7, 8, 13-16, 23 and 24, and Figures 9 and 10, especially column 2, lines 13-17 and column 12, lines 65-68.	1-3, 37 and 43
Y	ABRAMI. L. et al, The pore-forming toxin proaerolysin is activated by furin, The Journal of Biological Chemistry, December 1998, Vol 273. No. 49, pages 32565-32661, especially pages 32659-32660 and Figures 6-8.	1-3, 37 and 43
Y	WO 98/20135 A2 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA) 14 May 1998 (14.05.1998), pages 3-8, 22-25, 29-33, 38-42, and 48-55, and Figures 1B, 3A, 3B, 5, 6B, especially pages 28-30 and Figure 3A.	1-3, 37 and 43
A	US 5,798,218 A (BUCKLEY) 25 August 1998 (25.08.1991), columns 4-6 and Figure 6.	5
Y	BALLARD. J. et al, The primary structure of Clostridium septicum alpha-toxin exhibits similarity with that of Aeromonas hydrophila aerolysin, Infection and Immunity, January 1995, Vol 63. No. 1, pages 340-344 and Figure 4, especially pages 343-344.	37

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

☐ See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

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INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GORDON. V.M. et al, Clostridium septicum alpha-toxin is proteolytically activated by furin, Infection and Immunity, October 1997, Vol 65. No. 10, pages 4130-4134 and Figures 2 and 3, especially pages 4232-4134.	37
P,Y	WO 02/38799 A2 (UNIVERSITY OF VICTORIA INNOVATION AND DEVELOPMENT CORPORATION) 16 May 2002 (16.05.2002) pages 9-11, 27-28, 34-36, SEQ IDs NOs:13-16 and 21 and Figure 1.	1 and 43
A	AUDTHO. M. et al, Production of chymotrypsin-resistant Bacillus thuringiensis Cry2Aa1 delta-endotoxin by protein engineering, Applied and Environmental Microbiology, October 1999, Vol 65. No. 10, pages 4601-4605.	38
A	PANG. A.S.D. et al, Activation and fragmentation of Bacillus thuringiensis delta-endotoxin by high concentrations of proteolytic enzymes, Canadia Journal of Microbiology, October 1999, Vol 45. No. 10, pages 816-825.	38

INTERNATIONAL SEARCH REPORT

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The inventions listed as Groups 3 and 6 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 special technical feature of the invention of Group 3 is the absence of a furin cleavage, which special technical feature cannot be present in an invention of Group 6, as claimed.

The inventions listed as Group 3 and Groups 4, 5, 7 and 8 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 invention of Group 3 requires the special technical feature of a Clostridium septicum alpha toxin, which special technical feature cannot be present in any of the inventions of Groups 4, 5, 7 and 8, as claimed.

The inventions listed as Groups 4 and 7 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 special technical feature of the invention of Group 4 is the absence of a furin cleavage site, which special technical feature cannot be present in an invention of Group 7, as claimed.

The inventions listed as Group 4 and Groups 5, 6 and 8 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 invention of Group 4 requires the special technical feature of a Bacillus thuringensis delta toxin, which special technical feature cannot be present in any of the inventions of Groups 5, 6 and 8, as claimed.

The inventions listed as Groups 5 and 8 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 special technical feature of the invention of Group 5 is the absence of a native perforin activation site within a perforin polypeptide, requires the special technical feature cannot be present in an invention of Group 8, as claimed.

The inventions listed as Group 5 and Groups 6 and 7 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 invention of Group 4 requires the special technical feature of a perforin polypeptide, which special technical feature cannot be present in either of the inventions of Groups 6 and 7, as claimed.

The inventions listed as Groups 6 and 7 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 invention of Group 6 requires the special technical feature of a Clostridium septicum alpha toxin, which special technical feature cannot be present in the invention of Group 7, as claimed.

The inventions listed as Groups 7 and 8 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 invention of Group 7 requires the special technical feature of a Bacillus thuringensis delta toxin, which special technical feature cannot be present in the invention of Group 8, as claimed.

Continuation of B. FIELDS SEARCHED Item 3:

SEQ IDs NOs:4 and 24 searched in SPTREMBL23, PIR76, SwissProt41, A-GeneSeq19June03, and US Patent & Published Application amino acid sequence databases, and against, p2n, GenEmbl, N-GeneSeq19June03, and US Patent & Published Application nucleic acid sequence databases. SEQ ID NO:24 searched in SPTREMBL23, PIR76, SwissProt41, A-GeneSeq19June03, and US Patent & Published Application amino acid sequence databases, and against, p2n, GenEmbl, N-GeneSeq19June03, and US Patent & Published Application nucleic acid sequence databases.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/27061

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.: 20-34 and 44
because they relate to subject matter not required to be searched by this Authority, namely:
methods of treatment of a person or animal precluded by PCT Rule 39.1.
2. ☒ Claim Nos.: 13-15
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:
Claims 13-15 depend from claims 5 and 6 and depend ultimately from claim 1 but fail to refer to any feature or
limitation present in any the claims from which they depend, preventing a meaningful international search.
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.: 1-12,16-19,35-37 and 43
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

PCT/US02/27061

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Claims Nos. 13-15, 20-34 and 44 have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(b) and therefore have not been included with any invention

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. This International Search Authority has found 9 inventions claimed in the International Application covered by the claims indicated below:

Group 1, claim(s) 1-12, 13-19 and 43, drawn to a variant aerolysin lacking a furin cleavage site wherein the furin cleavage site has been substituted by a cleavage site recognized by a prostate-specific protease, as well as to a polynucleotide encoding same and a composition comprising the variant aerolysin lacking a furin cleavage site which is linked to a surface structure.

Group 2, claim(s) 35 and 36, drawn to a variant aerolysin lacking a furin cleavage site and further comprising an exogenous binding domain.

Group 3, claim(s) 37, drawn to a variant *Clostridium septicum* alpha toxin lacking a furin cleavage site wherein the alpha toxin variant further comprises a cleavage site recognized by a prostate-specific protease.

Group 4, claim(s) 38, drawn to a variant *Bacillus thuringensis* delta toxin lacking a functional wild-type activation sequence wherein the delta toxin variant further comprises a cleavage site recognized by a prostate-specific protease.

Group 5, claim(s) 39, drawn to a variant human perforin amino acid sequence lacking a functional wild-type activation sequence wherein the perforin variant further comprises a cleavage site recognized by a prostate-specific protease.

Group 6, claim(s) 40, drawn to a variant *Clostridium septicum* alpha toxin that comprises a furin cleavage site wherein the alpha toxin variant also comprises a functional deletion of the alpha toxin binding domain and further comprises a binding domain specific for prostate tissue.

Group 7, claim(s) 41, drawn to a variant *Bacillus thuringensis* delta toxin that comprises a wild-type activation site wherein the delta toxin variant also comprises a functional deletion of the delta toxin binding domain and further comprises a binding domain specific for prostate tissue.

Group 8, claim(s) 42, drawn to a variant human perforin amino acid sequence that comprises a wild-type activation site wherein the perforin variant also comprises a functional deletion of the perforin binding domain and further comprises a binding domain specific for prostate tissue.

The inventions listed as Groups 1 and 2 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 special technical feature of the invention of Group 1 is the absence of a furin cleavage site and its substitution by an alternative cleavage site, which special technical cannot be present in an invention of Group 2 as claimed..

The inventions listed as Group 1 and Groups 3-8 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 invention of Group 1 requires the special technical feature of an aerolysin protease, which special technical feature cannot be present in any of the inventions of Groups 3-8, as claimed.

The inventions listed as Group 2 and Groups 3-8 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 invention of Group 1 requires the special technical feature of an aerolysin protease, which special technical feature cannot be present in any of the inventions of Group 3-8, as claimed.