Title: METHODS AND COMPOSITIONS FOR IDENTIFYING PEPTIDE APTAMERS CAPABLE OF ALTERING A CELL PHENOTYPE

Abstract: The invention provides methods and compositions for screening and identifying of peptide aptamers that can modulate a cell phenotype and further, can be used for the treatment of a disease involving a misregulated cell phenotype, such as, for example, a cancer. The invention encompasses methods and compositions for producing cyclic peptide aptamers, including peptide aptamers comprising a conotoxin sequence, having improved stability and bioactivity. The invention also provides methods and compositions for improved gene delivery and expression of a peptide aptamer in cell.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 33/33; C12Q 1/68, 1/70
US CL : 435/7.1, 6, Dig. 6, Dig. 2; 550/300; 556/23.4

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. : 435/7.1, 6, Dig. 6, Dig. 2; 550/300; 556/23.4

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)

West, Cas

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>Y</td>
<td>US 6,025,455 A (KAMB et al.) 15 February 2000 (15.02.2000), column 4, line 13 up to column 5, line 26; column 13, Example 1.</td>
<td>1-3, 16-17, 24-25, 28, 31, 34, 42-43, 51-53</td>
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</table>

* Further documents are listed in the continuation of Box C. ** See patent family annex.

* document defining the general state of the art which is not considered to be of particular relevance

** earlier application or patent published on or after the international filing date

*L* document which may throw doubt 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 y date claimed

Date of the actual completion of the international search

22 June 2004 (22.06.2004)

Date of mailing of the international search

06 AUG 2004

Name and mailing address of the ISA/US

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Form PCT/ISA/210 (second sheet) (July 1998)
**INTERNATIONAL SEARCH REPORT**

<table>
<thead>
<tr>
<th>Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)</th>
</tr>
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<tr>
<td>This international report has not been established in respect of certain claims under Article 17(2)(e) for the following reasons:</td>
</tr>
<tr>
<td>1. ☐ Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:</td>
</tr>
<tr>
<td>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:</td>
</tr>
<tr>
<td>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).</td>
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<tr>
<th>Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)</th>
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<tr>
<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
</tr>
<tr>
<td>Please See Continuation Sheet</td>
</tr>
<tr>
<td>1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
</tr>
<tr>
<td>2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.</td>
</tr>
<tr>
<td>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.:</td>
</tr>
<tr>
<td>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.: 1-3, 16-17, 24-31, 34-36, 42-43, 51-53</td>
</tr>
</tbody>
</table>

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**

**BOX III. 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 examined, the appropriate additional examination fees must be paid.

**Group I**, claim(s) 1-3, 16, 17, 24-31, 34-36, 42, 43, 51-53, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype.

**Group II**, claim(s) 4-6 and 14-15, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising genetically modifying the first sample of cells.

**Group III**, claim(s) 7-8 and 11-13, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers in a second sample of cells.

**Group IV**, claim(s) 9, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers in a second sample of cells and contacting with pathogens.

**Group V**, claim(s) 10, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers in a second sample of cells and contacting with an agent.

**Group VI**, claim(s) 18-23, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers wherein the altered phenotype is associated with a change in levels of signal transduction.

**Group VII**, claim(s) 32, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers wherein the peptide aptamer comprises a conodite amino acid sequence.

**Group VIII**, claim(s) 33, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers wherein the peptide aptamer comprises a predetermined sequence.

**Group IX**, claim(s) 37-41, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers wherein the peptide aptamer comprises a fusion moiety with intein that linked to either end of the aptamer that forms a cyclic peptide aptamer.

**Group X**, claim(s) 44, drawn to a use of the peptide aptamer.

**Group XI**, claim(s) 45-50, drawn to a pharmaceutical composition.

**Group XII**, claim(s) 54-56, drawn to a viral vector.

**Group** : claim(s) 57-58, drawn to a kit.

**Group XV**, claim(s) 59-67, drawn to a method of identifying a peptide aptamer capable of modifying a cell phenotype comprising expressing the peptide aptamers wherein the cells contacted with a library of expressible nucleic acid.

**Group XV**, claim(s) 68-72, 79-85, drawn to a method of producing a random peptide aptamer library.

**Group XVI**, claim(s) 73-78, drawn to a method of producing a random peptide aptamer library further comprising selecting one or more random peptide aptamers from the library.

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Group XVII, claim(s) 86, drawn to a nucleic acid encoding a peptide aptamer linked to an intein sequence and a GFP.

Group XVIII, claim(s) 87-90, drawn to a nucleic acid encoding a peptide aptamer linked to hedgehog polypeptide sequence.

Group XIX, claim(s) 91-92, drawn to a random peptide aptamer linked to a fusion moiety.

Group XX, claim(s) 93, drawn to a nucleic acid encoding the peptide aptamer.

Group XXI, claim(s) 94-96, drawn to a method of identifying a conotide.

Group XXII, claim(s) 99, drawn to a method of identifying a conotide comprising contacting the second sample of cells with a pathogen.

Group XXIII, claim(s) 100-103, drawn to a method of identifying a conotide and further contacting the second sample of cells with an agent.

Group XXIV, claim(s) 104, drawn to a use of conotide.

Group XXV, claim(s) 105, drawn to a pharmaceutical composition.

Group XXVI, claim(s) 106-117, drawn to a conotide having formula I.

Group XXVII, claim(s) 118-128, drawn to a conotide of formula II.

Group XXVIII, claim(s) 129-139, drawn to a conotide of formula III.

Group XXIX, claim(s) 140-150, drawn to a conotide of formula IV.

Group XXX, claim(s) 151-162, drawn to a conotide of formula V.

Group XXXI, claim(s) 163-173, drawn to a conotide of formula VI.

Group XXXII, claim(s) 174-181, drawn to a conotide of formula VII.

Group XXXIII, claim(s) 182-189, drawn to a conotide of formula VIII.

Group XXXIV, claim(s) 190-192, drawn to a conotide of formula IX.

The Inventions listed as Groups I-X, XIV-XVI and XXI-XXIV 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 methods in each groups lack the corresponding special technical features as each of the groups recite different and/or additional process steps and/or components to practice the method. For example, Group II recites genetic modification of the sample cells to express a receptor. Group I does not require such genetic modification.

The Inventions listed as Groups XI, XII-XIII, XVII-XX and XXV-XXXIV 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 compounds lack a corresponding special technical features as each of the compounds are structurally different comprising different base units. For example, the conotide of Group XXVI recites a bi-cyclic peptide shown by formula I. The conotide of Group XVII recites more than two cyclic or ring structures.

The Inventions listed as Groups (I-X, XIV-XVI and XXI-XXIV) and (XI, XII-XIII, XVII-XX and XXV-XXXIV) 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 methods and compounds in each groups lack a corresponding special technical features as each of the groups recite different methods and different compounds.
This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

For groups I-IX:

1. Change in phenotype of the cells (as recited in claim 12):
   a. Apoptosis 
   b. Signal transduction 
   c. Protein trafficking 
   d. Cell adhesion 
   e. Membrane transport 
   f. Cell motility 
   g. Viral resistance 
   h. Metabolic state  
   i. Cellular differentiation

For signal transduction as mediated by a receptor, the following are receptor species:

   a. Erythropoietin 
   b. Kinase 
   c. G-protein coupled receptor

2. Fusion moiety as recited in claim 34.

   a. Intein 
   b. GFP 
   c. Hedgehog 
   d. Thioredoxin 
   e. Regulatory polypeptide 
   f. Bcl-2 
   g. p53 
   h. Nfkbeta-related polypeptide 
   i. Caspase 
   j. PTE 
   k. Myc 
   l. BH3 
   m. Death domain 
   n. BIR3 domain 
   o. Nuclear localization signal 
   p. Membrane localization signal 
   q. Farnesylation 
   r. Transcriptional activation domain 
   s. Rac 
   t. Raf

For Group XIV:

1. Pathogenic agent as recited in claim 61. For Viruses (as recited in claim 63).

For Group XVI:

1. Phenotypic change as recited in claim 77
2. Cellular protein as recited in claim 78

For Groups XXVI-XXXIV:

A single species of conotides from formula 1-formula IX i.e., species, as the formula is a genus.
The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the species recited, for example, in Group 1, in reference to changes in the phenotype of cells lack the corresponding special technical features as the changes are physically or chemically different and measured by different means.