Title: PEPTIDE-BASED PASSIVE IMMUNIZATION THERAPY FOR TREATMENT OF ATHEROSCLEROSIS

ELISA results from screen II on LDL of IEI scFv clones

![Graph showing ELISA results for various IEI scFv clones on MDA-LDL and native LDL](image)

(57) Abstract: The present invention relates to passive immunization for treating or preventing atherosclerosis using an isolated human antibody directed towards at least one oxidized fragment of apolipoprotein B in the manufacture of a pharmaceutical composition for therapeutic and prophylactic treatment of atherosclerosis by means of passive immunization, as well as method for preparing such antibodies, and a method for treating a mammal, preferably a human using such an antibody to provide for passive immunization.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2003/001547

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 39/395, C07K 16/18, A61P 9/10
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)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, EPO-INTERNAL, PAJ, MEDLINE, EMBASE, BIOSIS, CHEM.ABS.DATA, REGISTRY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>X</td>
<td>WO 9908109 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 18 February 1999 (18.02.1999), page 2, line 9 - line 11; page 7, line 4 - line 12; page 7, line 21 - line 23; page 9, table I; page 23, line 25 - page 24, line 24; claim 18; page 25, line 21 - page 26, line 2</td>
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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 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 citon 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: 28 January 2004

Date of mailing of the international search report: 30-01-2004

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
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Form PCT/ISA/210 (second sheet) (January 2004)
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<td>US 5972890 A (LEES ET AL), 26 October 1999 (26.10.1999)</td>
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<td>A</td>
<td>WO 0164008 A2 (SMITHKLINE BEECHAM BIOLOGICALS S.A.), 7 Sept 2001 (07.09.2001), page 5, line 13 - page 6, line 13</td>
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<td>A</td>
<td>US 5494791 A (COHEN), 27 February 1996 (27.02.1996), column 3, line 65 - line 67; column 7, line 15 - line 18, abstract</td>
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<td>A</td>
<td>US 5861276 A (KWAK ET AL), 19 January 1999 (19.01.1999), column 1, line 13 - line 14</td>
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1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
   a. type of material
      - [x] a sequence listing
      - [ ] table(s) related to the sequence listing
   b. format of material
      - [x] in written format
      - [x] in computer readable form
   c. time of filing/furnishing
      - [x] contained in the international application as filed
      - [x] filed together with the international application in computer readable form
      - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
INTERNATIONAL SEARCH REPORT

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.: 46-66
   because they relate to subject matter not required to be searched by this Authority, namely:
   see extra sheet

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 next 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.:

Remark on Protest

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

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)
INTERNATIONAL SEARCH REPORT

Box II.1

Claims 44-46 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

Box III

The International Searching Authority found multiple inventions in this international application, as follows:

Inventions 1-37: claims 1-4 (partly), 67 (partly). Use of antibodies directed towards the respective fragment in claim 2 in the manufacture of a pharmaceutical composition for therapeutical or prophylactical treatment of atherosclerosis by means of passive immunisation.

An antibody according to SEQ ID NO:1 and applications thereof.

An antibody according to SEQ ID NO:2 and applications thereof.

An antibody according to SEQ ID NO:3 and applications thereof.

An antibody according to SEQ ID NO:4 and applications thereof.

42. Claims: 1-5 (partly), 7 (partly), 10 (partly), 24 (partly), 26 (partly), 29 (partly), 43 (partly), 46-48 (partly), 50 (partly), 53 (partly), 67 (partly).
An antibody according to SEQ ID NO:5 and applications thereof.

An antibody according to SEQ ID NO:6 and applications thereof.

Form PCT/ISA/210 (extra sheet) (January 2004)
44. Claims: 1-5 (partly), 7 (partly), 11 (partly), 24 (partly), 26 (partly), 30 (partly), 43 (partly), 46-48 (partly), 50 (partly), 54 (partly), 67 (partly).
An antibody according to SEQ ID NO:7 and applications thereof.

An antibody according to SEQ ID NO:8 and applications thereof.

An antibody according to SEQ ID NO:9 and applications thereof.

An antibody according to SEQ ID NO:10 and applications thereof.

An antibody according to SEQ ID NO:11 and applications thereof.

An antibody according to SEQ ID NO:12 and applications thereof.

An antibody according to SEQ ID NO:19 and applications thereof.

An antibody according to SEQ ID NO:20 and applications thereof.

An antibody according to SEQ ID NO:21 and applications thereof.
An antibody according to SEQ ID NO:22 and applications thereof.

54. Claims: 1-5 (partly), 7 (partly), 16 (partly), 24 (partly), 26 (partly), 35 (partly), 43 (partly), 46-47 (partly), 49-50 (partly), 59 (partly), 67 (partly).
An antibody according to SEQ ID NO:23 and applications thereof.

An antibody according to SEQ ID NO:24 and applications thereof.

56. Claims: 1-5 (partly), 7 (partly), 17 (partly), 24 (partly), 26 (partly), 36 (partly), 43 (partly), 46-47 (partly), 49-50 (partly), 60 (partly), 67 (partly).
An antibody according to SEQ ID NO:25 and applications thereof.

An antibody according to SEQ ID NO:26 and applications thereof.

An antibody according to SEQ ID NO:27 and applications thereof.

An antibody according to SEQ ID NO:28 and applications thereof.

An antibody according to SEQ ID NO:29 and applications thereof.

An antibody according to SEQ ID NO:30 and applications thereof.
An antibody according to SEQ ID NO:31 and applications thereof.

An antibody according to SEQ ID NO:32 and applications thereof.

64. Claims: 1-5 (partly), 7 (partly), 21 (partly), 24 (partly), 26 (partly), 40 (partly), 43 (partly), 46-47 (partly), 49-50 (partly), 64 (partly), 67 (partly).
An antibody according to SEQ ID NO:33 and applications thereof.

An antibody according to SEQ ID NO:34 and applications thereof.

An antibody according to SEQ ID NO:35 and applications thereof.

An antibody according to SEQ ID NO:36 and applications thereof.

68. Claims: 1-5 (partly), 7 (partly), 23 (partly), 24 (partly), 26 (partly), 42 (partly), 43 (partly), 46-47 (partly), 49-50 (partly), 66 (partly), 67 (partly).
An antibody according to SEQ ID NO:37 and applications thereof.

An antibody according to SEQ ID NO:38 and applications thereof.

The present application has been considered to contain 69 inventions which are not linked such that they form a single general inventive concept, as required by Rules 13.1, 13.2 and 13.3 PCT for the following reasons:
Claims 1-67 describe the use of antibodies wherein the antibodies are defined by being directed towards at least one oxidized fragment of apolipoprotein B or the claims pertain to the antibodies per se defined by nucleic acid sequences. The technical feature that these nucleic acid sequences have in common is that they encode antibodies which are directed towards at least one oxidized fragment of apolipoprotein B and which are useful in the treatment of atherosclerosis. The different antibodies (nucleic acid sequences) have not been shown to have any structural feature in common.

The prior art has been identified as: WO 9908109 A2 (D1) and WO 0132070 A2 (D2).

D1 discloses monoclonal antibodies that bind to one or more oxidation-specific epitopes on oxidized blood lipoproteins, e.g., oxidatively modified LDL such as malondialdehyde (MDA)-LDL (bound to lysine residues on apolipoprotein B (apoB)) (see page 2, lines 9-11 and page 7, lines 4-12, 21-23). The antibodies are useful in the prophylactic treatment of atherosclerosis (page 23, line 25-page 24, line 24 and claim 18). Antibody binding domains which exhibit binding with a peptide according to D1 can be identified and isolated with the aid of a vector system which has been used to express a combinatorial library of Fab fragments from the human antibody repertoire (see page 25, line 21-page 26, line 2). ApoB-100 is the major protein moiety of LDL (see US 5861276 column 1, lines 13-14). It is considered that antibodies against oxidized fragments of apoB according to the present application may be the same antibodies as those who bind differentially to one or more oxidation-specific epitopes on oxidized blood lipoproteins according to D1.

D2 relates to a human monoclonal antibody fragment (Fab) that binds specifically to both OxLDL and MDA-LDL, but not native LDL. The antibody, which was cloned from a combinatorial Fab library, is useful in the treatment of atherosclerosis (see page 6, lines 5-19 and claims 11,16).

The special technical feature of invention 1 that makes a contribution over this prior art (Rule 13.2 PCT) is an antibody directed towards the specific oxidized fragment of apoB first mentioned in claim 2. From this special technical feature the objective problem to be solved by this and all further inventions is to provide alternative antibodies (defined by being directed towards other oxidized apoB fragments or by being encoded by their nucleic acid sequences or parts of their nucleic acid sequences).
A substantial number of different solutions to this problem are provided, comprising many alternative antibodies/nucleic acid sequences. No novel common concept has been found linking the different antibodies/sequences. The above analysis shows that the special technical features of inventions 1 (claims 1-4 (partly) and 67 (partly)) is neither the same as nor corresponding to that of any of the inventions 2-69.

In conclusion, therefore, the inventions are not linked by common or corresponding special technical features and define different inventions not linked by a single general inventive concept. The application, hence, does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT.
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