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- (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, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
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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.*



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(54) Title: MODIFICATION OF HUMAN VARIABLE DOMAINS

(57) Abstract: The present invention relates to a method for the optimization of isolated human immunoglobulin variable heavy (V_H) and light (V_L) constructs.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/08094

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/00 C07K16/32 C12N5/10 C12N15/63

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)

IPC 7 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)

BIOSIS, EPO-Internal, WPI Data, MEDLINE, PAJ, EMBASE, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06213 A (CAMBRIDGE ANTIBODY TECH ;MEDICAL RES COUNCIL (GB)) 1 April 1993 (1993-04-01) figure 11	1,7-18
X	WO 93 17105 A (SCOTGEN LTD) 2 September 1993 (1993-09-02) abstract figure 4A; example 3	1,7-13
A	WORN ARNE ET AL: "Stability engineering of antibody single-chain Fv fragments." JOURNAL OF MOLECULAR BIOLOGY, vol. 305, no. 5, 2001, pages 989-1010, XP002249286 ISSN: 0022-2836 cited in the application	

Further documents are listed in the continuation of box C.

Patent family members are listed in 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 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.
- *Z* document member of the same patent family

Date of the actual completion of the international search

30 July 2003

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/08094

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>JUNG SABINE ET AL: "Selection for improved protein stability by phage display." JOURNAL OF MOLECULAR BIOLOGY, vol. 294, no. 1, 19 November 1999 (1999-11-19), pages 163-180, XP002249461 ISSN: 0022-2836 cited in the application</p> <p style="text-align: center;">---</p>	
A	<p>NIEBA LARS ET AL: "Disrupting the hydrophobic patches at the antibody variable/constant domain interface: Improved in vivo folding and physical characterization of an engineered scFv fragment." PROTEIN ENGINEERING, vol. 10, no. 4, 1997, pages 435-444, XP002249462 ISSN: 0269-2139 cited in the application</p> <p style="text-align: center;">---</p>	
A	<p>WILLUDA JORG ET AL: "High thermal stability is essential for tumor targeting of antibody fragments: Engineering of a humanized anti-epithelial glycoprotein-2 (epithelial cell adhesion molecule) single-chain Fv fragment." CANCER RESEARCH, vol. 59, no. 22, 15 November 1999 (1999-11-15), pages 5758-5767, XP002249463 ISSN: 0008-5472</p> <p style="text-align: center;">-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/08094

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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:

2. Claims Nos.: 1 (partially), 2-6, 7 (partially), 8-18 (partially), 19-33
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:
see FURTHER INFORMATION sheet PCT/ISA/210

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 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:

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1 (partially), 2-6, 7 (partially), 8-18 (partially), 19-33

Present claims 1(partially), 2-6, 7 (partially), 8-18 (partially),19-33 relate to an extremely large number of possible products/methods. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the products/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Said claims refer to isolated polypeptides comprising a VH domain selected from the group consisting of VH domains belonging to VH1a-, VH1b-, VH2-, VH4-, VH5- and VH6- subclass. Experimental data are given only for the VH6 subclass domain. Since two of the substitutions do not reach the alleged effect (D72 and G76) (see p. 47 l. 8-11, p. 68 l. 9-11, and p. 68 l. 17-20) the results obtained from the VH6 subclass domain cannot be transferred to the other subclasses or even other substitutions based on the differences between the sequence of the VH3 subclass domain and VH6, i.e. V44, F78, R97+E99, E99. Furthermore, since "it could not be a priori excluded that changes in the framework might affect the orientation of the CDRs", and an unchanged antigen recognition is only shown for the examined mutations (see p. 75 l. 11-15), said results cannot be applied to any substitution in any antibody sequence. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the products/methods

1. a polypeptide comprising a VH domain belonging to the VH6 subclass (defined either by the residues disclosed in the Tables 2 and 3 of the present application or by the word "VH6") wherein said VH domain comprises at least one amino acid residue selected from the group consisting of

- a. V at position 5
- b. G at position 16
- c. I at position 58
- d. Y at position 90.

Furthermore, the specific sequence identified as SEQ ID NO 19 with the above mentioned substitutions

- a. single substitutions
- b. V5/G16
- c. V5/G16/I58
- d. V5/G16/I58/Y90

as it is disclosed in Example 2 of the present application.

2. a method for obtaining an isolated nucleic acid sequence comprising the step

substituting in a nucleic acid sequence that encodes a VH6 subclass domain (defined either by the residues disclosed in the Tables 2 and 3 of the present application or by the word "VH6") at least one codon that encodes an amino acid residue selected from the group consisting of

- a. the amino acid at position 5 to V
- b. the amino acid at position 16 to G

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

- c. the amino acid at position 58 to I
- d. the amino acid at position 90 to Y

Furthermore, the specific sequences identified as SEQ ID NO 19 with the above mentioned substitutions

- a. single substitutions

- b. V5/G16

- c. V5/G16/I58

- d. V5/G16/I58/Y90

as it is disclosed in Example 2 of the present application

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No

PCT/EP 02/08094

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