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D E C I S I O N
of 28 May 2003

Case Number: T 1097/99 - 3.3.4

Application Number: 89400221.1

Publication Number: 0326490

IPC: C07K 7/00

Language of the proceedings: EN

Title of invention:
Synthetic peptides and mixtures thereof for detecting HIV antibodies

Patentee:
ADALTIS INC.

Opponents:
Abbott Laboratories
United Biomedical Corp.
Institut Pasteur
Bio-Rad Pasteur

Headword:
HIV peptides/ADALTIS INC.

Relevant legal provisions:
EPC Art. 56, 114(2)

Keyword:
"New main request: inventive step (yes)"

Decisions cited:
T 0626/90

Catchword:
-

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Case Number: T 1097/99 **-** 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 28 May 2003

Appellant: (Proprietor of the patent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 14 September 1999 revoking European patent No. 0326490 pursuant to Article 102(1) EPC.

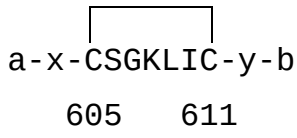
Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: R. E. Gramaglia
R. Moufang

Summary of Facts and Submissions

I. European Patent No. 0 326 490 (application No. 89 400 221.1) having the title "Synthetic peptides and mixtures thereof for detecting HIV antibodies" was granted on the basis of 24 claims for all designated Contracting States, except ES and GR, and 17 claims for the Contracting States ES and GR. Claims 1 and 9 for all designated Contracting States except ES and GR read as follows:

"1. A substantially pure peptide of the formula



where x is independently selected from the group consisting of:
G
WG
LGIWG
QLLGIWG
DQQLLGIWG
KDQQLLGIWG
LKDQQLLGIWG
YLKDQQLLGIWG
RYLKDQQLLGIWG
ERYLKDQQLLGIWG
VERYLKDQQLLGIWG
LAVERYLKDQQLLGIWG
ILAVERYLKDQQLLGIWG

RILAVERYLKDQQLLGIWG and corresponding N-terminal peptides, derived from homologous regions of other HIV-1 isolates and peptides differing from the above as a result of conservative amino acid substitutions; y, if present, is independently selected from the group consisting of:

T

TT

TTA

TTAV

TTAVP

TTAVPW

TTAVPWNA

TTAVPWNAS

TTAVPWNASW

TTAVPWNASWS

TTAVPWNASWSN

TTAVPWNASWSNK

TTAVPWNASWSNKS

TTAVPWNASWSNKSL

TTAVPWNASWSNKSLE

TTAVPWNASWSNKSLEQ

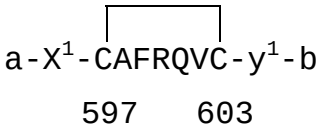
TTAVPWNASWSNKSLEQI and corresponding C-terminal peptides, derived from homologous regions of other HIV-1 isolates and peptides differing from the above as a result of conservative amino acid substitutions;

a represents an amino terminus or is selected from the group consisting of a cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;

b represents a carboxy terminus or is selected from the group consisting of cysteine residue, a tyrosine, a

glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties; and peptides differing from the above as a result of modification by terminal-NH_2 acylation, thioglycolic acid amidation, terminal-COOH amidation or in which methionine has been replaced by norleucine, which facilitates covalent linking of the peptide to solid supports and/or makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties.

9. A substantially pure peptide of the formula



wherein x^1, if present, is independently selected from the group consisting of:

G

WG

SWG

LNSWG

RLNSWG

ARLNSWG

QARLNSWG

DQARLNSWG

QDQARLNSWG

LQDQARLNSWG

YLQDQARLNSWG

KYLQDQARLNSWG

EKYLQDQARLNSWG

IEKYLQDQARLNSWG
AIEKYLQDQARLNSWG
VTAIEKYLQDQARLNSWG
RVTAIEKYLQDQARLNSWG and corresponding N-terminal peptides, derived from homologous region of other HIV-2 isolates and peptides differing from the above as a result of conservative amino acid substitutions; y^1, if present is independently selected from the group consisting of:
-H
-HT
-HTT
-HTTV
-HTTVP
-HTTVPW
-HTTVPWV
-HTTVPWVN
-HTTVPWVND
-HTTVPWVNDS and corresponding C-terminal peptides, derived from homologous regions of other HIV-2 isolates and peptides differing from the above as a result of conservative amino acid substitutions;
a represents an amino terminus or is selected from the group consisting of a cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;
b represents a carboxy terminus or is selected from the group consisting of cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;

and peptide differing from the above as a result of modification by terminal-NH_2 acylation, thioglycolic acid amidation, terminal-COOH amidation or in which methionine has been replaced by norleucine, which facilitates covalent linking of the peptide to solid supports and/or makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties."

II. Notices of opposition were filed by four opponents 01 to 04 all requesting the revocation of the European patent on the grounds of Article 100(a), (b) and (c) EPC. By a decision dated 14 September 1999 the opposition division revoked the patent because it held that the subject-matter of the claims then on file was not novel.

III. The following documents are cited in the present decision:

(D2) WO-A-87/06005;

(D3) Gnann J.W. JR. et al., Science, Vol. 237, pages 1346 to 1349 (11 September 1987);

(D7) Coulis P.A. et al., Am. Clin. Prod. Rev., pages 34 to 43 (November 1987);

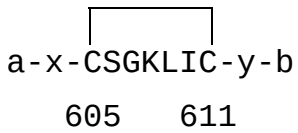
(D50) Test report "Biochem Report 2" by S. Barbeau et al. submitted by the appellant.

IV. The appellant (patentee) filed an appeal against the decision of the opposition division.

V. In two communications following the summons to oral proceedings the board expressed its preliminary non-binding opinion about some important points to be discussed at the oral proceedings.

VI. With letter of 25 April 2003 the appellant filed a main request and auxiliary requests 1 to 8. At the end of the first day of oral proceedings on 27 May 2003 the board expressed its view that the claims of the main request, while novel, did not involve an inventive step. In response to these objections, the appellant submitted a new main request together with auxiliary requests 1 to 3. On the second day of oral proceedings (28 May 2003) the appellant replaced its main request by a new main request (claims 1 to 11 for all designated Contracting States except ES and GR, and claims 1 to 8 for the Contracting States ES and GR) and successively withdrew the still pending first to third auxiliary requests. Claims 1 and 3 according to the lastly filed main request for all designated Contracting States except ES and GR read as follows:

"1. A substantially pure peptide of the formula

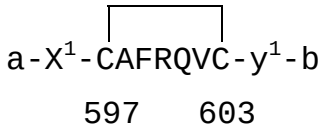


wherein x is independently selected from the group consisting of:

RILAVERYLKDQQLLGIWG and corresponding N-terminal peptides, derived from homologous regions of other HIV-1 isolates and peptides differing from the above as a result of conservative amino acid substitutions; y is independently selected from the group consisting of:

TTAVPWNAS and corresponding C-terminal peptides, derived from homologous regions of other HIV-1 isolates and peptides differing from the above as a result of conservative amino acid substitutions;
a represents an amino terminus or is selected from the group consisting of a cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;
b represents a carboxy terminus or is selected from the group consisting of cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;
and peptides differing from the above as a result of modification by terminal-NH_2 acylation, thioglycolic acid amidation, terminal-COOH amidation or in which methionine has been replaced by norleucine, which facilitates covalent linking of the peptide to solid supports and/or makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties.

3. A substantially pure peptide of the formula



wherein x^1 is independently selected from the group consisting of:

RVTAIEKYLQDQARLNSWG and corresponding N-terminal peptides, derived from homologous region of other HIV-2 isolates and peptides differing from the above as a result of conservative amino acid substitutions; y^1 is independently selected from the group consisting of:

HTTVPWVNDS and corresponding C-terminal peptides, derived from homologous regions of other HIV-2 isolates and peptides differing from the above as a result of conservative amino acid substitutions;
a represents an amino terminus or is selected from the group consisting of a cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;
b represents a carboxy terminus or is selected from the group consisting of cysteine residue, a tyrosine, a glutamic acid, an aspartic acid and a lysine, which makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties;
and peptide differing from the above as a result of modification by terminal-NH_2 acylation, thioglycolic acid amidation, terminal-COOH amidation or in which methionine has been replaced by norleucine, which

facilitates covalent linking of the peptide to solid supports and/or makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties."

VII. The submissions by the appellant, insofar as they are relevant to the present decision, can be summarized as follows:

– The closest prior art underlying the claimed subject-matter was represented by document (D3). The problem to be solved vis-à-vis this prior art was to find improved peptides capable of achieving a higher sensitivity when used in identifying HIV-1 or HIV-2 antibodies.

– The solution to the above problem was the provision of the longer cyclic peptides as claimed. Document (D3) dealing with the shorter "core" peptides did not suggest these longer peptides.

– The presently claimed peptides did not result from a selection among the peptides described in document (D3), since the latter did not disclose any broad family of peptides, from which a selection could be made.

– As for document (D7), the passage on page 39, central column, first full paragraph related to a **mixture** of peptides from the transmembrane region of the HIV-1 envelope protein. Therefore, this passage did not represent a disclosure of the cyclic, isolated peptide RILAVERYLKDQQLLGIWG-

CSGKLICTTAVPWNAS. The skilled person would also not combine the teachings of documents (D3) and (D7).

– Table 5 of the patent in suit showed the superiority of the longer cyclic peptides as claimed compared to shorter linear peptides (ie, the length of the flanking sequences also played a role).

VIII. The submissions by the respondents, insofar as they are relevant to the present decision, can be summarized as follows:

Admissibility of the new main request filed on 28 May 2003

– The late-filed main request submitted on 28 May 2003 was to be rejected under Article 114(2) EPC.

Inventive step

– That the claimed longer/cyclic peptides did not exhibit any improved properties over the shorter/linear peptides, could be derived from Tables 3 and 4 of the patent in suit (see peptides "87c" and "202") and from test report (D50).

– In the light of the passage on page 39, central column, first full paragraph of document (D7), the skilled person would have turned to the sequence RILAVERYLKDQQLLGIWGCSGKLICTTVPWNAS in order to improve the sensitivity of immunoassays for HIV-1

antibodies, whereas document (D3) suggested that taking the cyclic form thereof would have further increased the sensitivity. Therefore, the claimed subject-matter was obvious.

– The claimed subject-matter was furthermore obvious in view of document (D2) taken alone or in combination with document (D3). Document (D2) disclosed indeed a linear peptide AVERYLKDQQLLGIWGCSGKLICTTAVPWNAS exhibiting almost the same length as the claimed ones (see page 41, Table V: peptide (V)). The skilled person was thus taught to take long flanking sequences around the "core" CSGKLIC. As for the cyclic form, page 28, lines 8 to 12 of document (D2) taught that peptide (V) was converted to the cyclic form. The cyclic form could also have been obtained in the light of document (D3), suggesting an increase in sensitivity by cyclization.

– Since the cyclic peptide of claim 3 was the HIV-2 counterpart by homology of the HIV-1 peptide of claim 1, the lack of inventive step also applied to this peptide and to the mixtures of claims 5 and 6.

Adaptation of the description

– The following passages had to be deleted from the description:

(a) Page 7, lines 49-50.

(b) Page 8, lines 38 to 40.

(c) Page 10, lines 21 to 28.

(d) Page 10, lines 33 to 41.

(e) Any peptides other than peptides "87c" and "202" from the Tables.

(f) The expressions "as defined in claim 1" and "as defined in claim 3" on pages 5, 6 and 7 (counterpart of claims 1 and 3) introduced the term "substantially pure" into the description by virtue of the fact that these claims contained this expression.

IX. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of claims 1 to 11 for the Contracting States AT, BE, CH, DE, FR, GB, IT, LI, LU, NL and SE and on the basis of claims 1 to 8 for the Contracting States ES and GR (main request filed on 28 May 2003).

The respondents (opponents) requested that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

Admissibility of the new main request filed on 28 May 2003

2. As is apparent from paragraph VI above, an alternative set of claims (new main request) was submitted by the appellant on the second day of oral proceedings on 28 May 2003, which the respondents objected to as having been filed too late (Article 114(2) EPC).

In the present case, however, the board decides to admit into the proceedings this set of claims following the rationale emerging from decision T 626/90 of 2 December 1993, as the board is satisfied that the new version of the claims is a bona fide attempt to overcome the objections raised by the board in connection with the question of the inventive step of the broad group of cyclic peptides previously claimed, and that no question of the respondents being unfairly taken by surprise arises, because in this request the amendments result in a considerable limitation of the claimed subject-matter to the preferred embodiments of the invention as described in the patent in suit, namely peptides "87c", "202" and derivatives thereof (see page 8, line 45 to page 9, line 3).

Formal admissibility

3. There are no formal objections on the basis of Article 123(2) and (3) EPC to the claims of the new main request since these claims are adequately supported by the original description and, given the restriction effected (see paragraph VI *supra*), do not extend the protection conferred when compared to the

claims as granted. This has not been contested by the respondents.

Novelty

4. None of the documents considered in the present proceedings discloses cyclic peptides presenting all the features indicated in the claims in accordance with the new main request. This has not been disputed by the respondents, either. The subject-matter of said claims is thus novel.

Inventive step

5. The board agrees that the closest prior art underlying the claimed subject-matter is represented by document (D3). This document discloses immunoassays for HIV-1/HIV-2 antibodies involving *inter alia* the peptides LGLWGCSGKLIC and LNSWGCAFRQVC (see Table 2 on page 1347), ie these peptides have the same "cores" CSGKLIC and CAFRQVC as those of claims 1 and 3, respectively. On page 1347, l-h column of this document it is stated that "The minimal epitope for immune recognition is a 7-amino acid sequence (*env* amino acid 603-609) containing two essential cysteine residues linked by a disulfide bond.".

6. The appellant maintains that the problem to be solved vis-à-vis this prior art is to find improved peptides capable of achieving a higher sensitivity when used in detecting HIV-1 or HIV-2 antibodies, while the respondents, citing the experimental results of

Tables 3 and 4 of the patent in suit (see peptides "87c" and "202") and test report (D50), deny this.

7. As regards the experimental results of test report (D50), the board observes that the latter merely relates to the comparison of the linear peptide LGLWGCSGKLIC ("GNANN 1") with its cyclic form ("BCH-13205"). But since "BCH-13205" is a peptide not falling under the claims at issue, this document and any argument relying thereupon are irrelevant in the given context.

8. As for the experimental results of Tables 3 and 4 of the patent in suit invoked by the respondents, the board agrees that peptides "87c" and "202" do not appear to perform better than any other peptide reported in these Tables. However, the "% positive sera correctly identified" by a peptide at "normal" (ie not diluted) HIV-1/HIV-2 antibody concentrations (and Tables 3 and 4 deal with undiluted sera) is a poor indicator of sensitivity compared with the detection of HIV-1/HIV-2 positive sera **diluted** by a factor exceeding 500 (see page 4, lines 53 to 54 and page 5, lines 2 to 4 of the patent in suit).

9. In fact, Table 5 of the patent shows that at high serum dilutions the longer cyclic peptides as claimed perform better than the shorter linear peptides (compare eg the 1.854 optical density (OD) units at dilution 1/800 of peptide "87c (cyclic)" (length: 35 amino acids) with the 1.012 of peptide "87 linear", the 0.767 of peptide "80 cyclic" (length: 16 amino acids) and the 0.057 of peptide "77 linear" (length: 17 amino acids), taking

into account that the OD reflects the number of antibodies detected). This trend is confirmed throughout Table 5, from which the conclusion can be drawn that **both** cyclization and a longer flanking sequence contribute to increasing sensitivity.

10. Contrary to the respondents' allegation, thus, the claimed longer cyclic peptides achieve a better sensitivity in HIV antibody detection than the previously known peptides.

11. The question to be answered is now whether or not it would have been obvious for the skilled person to arrive at something falling under the terms of claim 1 (the fate of the remaining claims being tightly linked to that of claim 1: see point 16 *infra*). In a first line of argument, the respondents argue that the claimed subject-matter is obvious because the skilled person would take the sequence RILAVERYLKDQQLLGIWGCSGKLICTTVPWNAS disclosed in the first full paragraph on page 39, central column, of document (D7), in order to improve the sensitivity of immunoassays for HIV-1 antibodies, and further increase the sensitivity by taking the cyclic form thereof, as suggested by document (D3).

12. The board firstly notes that even assuming that the skilled person would take the cyclic form of the sequence RILAVERYLKDQQLLGIWGCSGKLICTTVPWNAS disclosed in the passage on page 39, central column, first full paragraph of document (D7), the so-obtained cyclic peptide would still lack an "A" (alanine) between ...CSGKLICTT and VPWNAS to become peptide "87c"

as claimed (see paragraph VI *supra*) and it is common general knowledge in peptide biochemistry that even differences in one single amino acid in a peptide may result in unpredictable changes in biological activity.

Moreover, upon a closer scrutiny, this passage ("This assay was refined further to contain a mixture of synthetic peptides corresponding to a region in the gp41 transmembrane protein (which contain the amino acid sequence RILAVERYLKDQQLLGIWGCSGKLICTTVPWNAS) and a conserved epitope contained in the HIV-1 p24 core protein.") in no way suggests that the above sequence **as such** should be taken in order to increase the sensitivity, but only that a **mixture** of synthetic peptides falling **within** this sequence (the document is silent as to where these synthetic peptides start/end) had to be added. In conclusion, this passage is neither a disclosure of the isolated RILAVERYLKDQQLLGIWG-CSGKLICTTVPWNAS peptide, nor a teaching to select long flanking sequences from the sequence RILAVERYLKDQQLLGIWGCSGKLICTTVPWNAS.

The respondents' first line of argument is therefore not convincing.

13. In a second line of argument, the respondents maintain that the claimed subject-matter is obvious because the skilled person would be taught by document (D2)(see page 41, Table V: peptide (V)) to take long flanking sequences around the "core" CSGKLIC of AVERYLKDQQLLGIWG-CSGKLICTTAVPWNAS. As to the cyclic form, in the respondents' view, page 28, lines 8 to 12 of document (D2) teaches that peptide (V) is converted

to the cyclic form. The cyclic form could also be obtained in the light of document (D3), suggesting an increase in sensitivity by cyclization.

14. In the board's judgement, though, Table V on page 41 of document (D2) lists only **one** long peptide (peptide (V)) comprising the "core" CSGKLIC and ten short peptides including the same "core" (peptides (II), (XII), (VII), (XI), (VIII), (IX), (III), (XIII) and (X)). This cannot be seen as a teaching to take long flanking sequences around the "core" CSGKLIC. If anything, it is the opposite, ie short flanking sequences are preferred.

15. As for the cyclic form, it is true that according to page 28, lines 8 to 12 of document (D2), peptide (V) undergoes oxidation to the cyclic form/dimer/polymer, however, it is also stated on page 22, lines 27 to 31 of this document that the cyclic monomer form is less efficient for ELISA, ie while polymerization is important for the reactivity, cyclization is something to be avoided. Moreover, the skilled person would also not combine two contradictory documents (document (D3): "increase in sensitivity by cyclization"; document (D7): "decrease in sensitivity by cyclization").

Therefore, the board also disagrees to the respondents' second line of argument for questioning the inventive step.

16. In view of the foregoing, the board concludes that the HIV-1 cyclic peptide according to claim 1 involves an inventive step.

The cyclic peptide of claim 3 is the HIV-2 homologous counterpart of the HIV-1 peptide of claim 1. Therefore, the above conclusion also applies to the subject-matter of claim 3. Since claims 2 and 4 to 11 as well as claims 1 to 8 for the Contracting States ES and GR directly or indirectly rely on the peptide(s) of claim 1 and/or 3, these claims also satisfy the requirements of Article 56 EPC.

Adaptation of the description

17. In addition to the amendments already effected by the appellant, the respondents further request deletion from the description of the following passages:

(a) Page 7, lines 49 to 50.

(b) Page 8, lines 38 to 40.

(c) Page 10, lines 21 to 28.

(d) Page 10, lines 33 to 41.

(e) Any peptides other than peptides "87c" and "202" from the Tables.

(f) The counterpart of claims 1 and 3 in the description "as defined in claim 1" and "as defined in claim 3" on pages 5, 6 and 7 introduced the term "substantially pure" into the description by virtue of its presence in these claims.

18. As for the requested deletions (a), (c) and (d) above, the disputed passages relate to mixtures of the peptides of claim 1 and/or claim 3 with linear peptides as "previously" defined" on page 6, lines 28 to 34. These passages are thus useful for illustrating the embodiments of claims 5 and 6. Therefore they contribute to the clarity or understanding of claims as maintained by the board and are not in contradiction therewith.

19. As for the requested deletion (b) above, the disputed passage relates to a "tail" consisting of a plurality of hydrophobic residues attached to the peptides and serving to facilitate the adsorption of the peptide to the support. This passage is thus useful for illustrating the embodiments of claims 1 and 3 (cf "...and peptide differing from the above as a result of modification by terminal-NH_2 acylation,... which... makes the peptide more useful as an immunodiagnostic reagent without changing its antigenic properties."). Therefore this passage contributes to the clarity or understanding of claims as maintained by the board and is not in contradiction therewith.

20. As regards requested deletion (e) above, the Tables are useful for comparative purposes. Moreover, the fact that the Tables illustrate how other (unclaimed) peptides behave in ELISA immunoassays is not in contradiction with the claims as maintained by the board and does not obscure the scope thereof. Requested amendment (e) above is thus not necessary for an adequate adaptation of the description to the claims maintained by the board.

21. Finally, amendment (f) above has been requested because the respondents read (and object to) the expression "substantially pure" into the description by virtue of the expressions "as defined in claim 1" and "as defined in claim 3" on pages 5, 6 and 7 (counterpart of claims 1 and 3 containing the disputed expression). However, no amendment to the description is necessary because deletions can only be made on "res scripta" and not on interpretations.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the new main request filed on 28 May 2003 and of the amended description filed on 28 May 2003 (pages 3 to 21).

The Registrar: The Chairwoman:

P. Cremona U. M. Kinkeldey