BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen
(D) [ ] No distribution

Datasheet for the decision of 27 July 2007

Case Number: T 1466/05 - 3.3.08

Application Number: 98932871.1

Publication Number: 0956504

IPC: G01N 33/53

Language of the proceedings: EN

Title of invention:
Antibody to and assay for peptide linked-pyridinoline as indicator of bone resorption level

Applicant:
SEREX, INC.

Opponent:
-

Headword:
Pyridinoline/SEREX

Relevant legal provisions:
EPC Art. 83, 113(1)
EPC R. 28(1), 88

Keyword:
"Main request and auxiliary request 1 - sufficiency of disclosure (no)"

Decisions cited:
T 0158/91, T 0349/91, T 0409/91, T 0510/94, T 0513/94, T 0639/95, T 0716/01

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1466/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 27 July 2007

Appellant: SEREX, INC.
230 West Passaic Street
Maywood, NJ 07607 (US)

Representative: Pilkington, Stephanie Joan
Eric Potter Clarkson LLP
Park View House
58 The Ropewalk
Nottingham NG1 5DD (GB)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 9 June 2005 refusing European application No. 98932871.1 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: M. R. Vega Laso
C. Rennie-Smith

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division posted on 9 June 2005 refusing the European patent application No. 98 932 871.1 (published as WO 99/00668) under Article 97(1) EPC. The refusal was based on the grounds that the invention as claimed in claims 1, 6 and 12 of both sets of claims on file (designated "main request" and "first auxiliary request") did not fulfil the requirements of Article 83 EPC. Furthermore, the subject-matter of claim 1 of the auxiliary request was considered to extend beyond the content of the application as filed (cf. Article 123(2) EPC).

II. Claims 1, 6 and 12 of the **main request** (claims 1 to 16) read as follows:

"1. An antibody reactive with the pyridinoline in peptide-linkedpyridinoline[*sic*] and not free pyridinoline which is useful in an assay to indicate bone resorption.

6. A kit for a biological sample to quantify bone resorption comprising:

an antibody reactive with the pyridinoline in peptide-linkedpyridinoline[*sic*] and not free pyridinoline which is useful in an assay to correlate bone resorption.

12. A method for determining bone resorption comprising obtaining a sample of urine, blood, saliva or other bodily fluid from a patient,

reacting the sample with an antibody reactive with the pyridinoline in peptide-linked-pyridinoline and not free pyridinoline which is useful in an assay to determine bone resorption, and

correlating the extent of the reaction with calibrators to determine the amount of bone resorption."

Dependent claim 2 was directed to an antibody produced by a hybridoma deposited with the American Type Culture Collection, Rockville, MD, designated HB 12254. Dependent claims 3 and 4 concerned two different embodiments of the antibodies of claim 1. Dependent claims 7 to 11 were directed to various embodiments of the kit of claim 6, and dependent claims 13 to 16 concerned specific embodiments of the method for determining bone resorption claimed in claim 12.

III. Claim 1 of the **auxiliary request** ("first auxiliary request") read as follows:

"1. An antibody reactive with the pyridinoline in peptide-linkedpyridinoline[*sic*] and not free pyridinoline which is useful in an assay to indicate bone resorption wherein the antibody is reactive with a non-linear epitope on a peptide-linked pyridinoline that is stable to acid hydrolysis, and is recognised by the antibody produced by a hybridoma deposited with the American Type Culture Collection, Rockville, MD, designated HB 12254." (additional features emphasised by the board)

Claims 6 and 12 included the same features introduced into claim 1. The remaining claims 2 to 5, 7 to 11 and 13 to 16 were identical to those of the main request.

IV. The reasons given by the examining division to deny sufficiency of disclosure may be summarized as follows:

The functional features defining the antibody of claim 1 could not be clearly and unambiguously related to a particular chemical structure of the antibody molecule which is responsible for the desired technical effect. While it was admitted that techniques for preparing polyclonal and monoclonal antibodies that react selectively with a particular known epitope belong to the routine practice in the pertinent technical field, the present application did not disclose the chemical nature of the epitope to which the specific monoclonal antibody 51A93 described in the application bound. A person skilled in the art provided only with the scarce information given in the application would not be able to produce polyclonal or monoclonal antibodies having the required biological functionality, without needing to carry out an excessively large amount of trial and error assays. Analogous arguments applied to the subject-matter of independent claims 6 (kit) and 12 (method). With regard to the additional features introduced in claim 1 of the auxiliary request, it was not apparent to the examining division how the skilled person provided only with some functional information regarding an unknown epitope could be able, without needing to carry out an excessively large amount of trial and error assays, to produce polyclonal and monoclonal antibodies against

said (unknown) epitope and having the biological functionality specified in claim 1.

V. With the statement of grounds of appeal, the appellant re-filed, as the basis for its main request and its (sole) auxiliary request, two sets of claims designated "Main Request" and "Auxiliary Request 1". These sets of claims were identical to those discussed by the examining division in the decision under appeal. In the event that the board did not intend to allow either claim request to grant, oral proceedings under Article 116 EPC were requested.

VI. The examining division did not rectify its decision and, pursuant to Article 109(2) EPC, remitted the appeal to the boards of appeal.

VII. In a communication pursuant to Article 110(2) EPC, the board expressed its provisional opinion on the issue of sufficiency of disclosure and raised objections under Articles 123(2) and 84 EPC in respect of the claims of the auxiliary request. The board also drew attention to several typographical errors in the claims and a major error concerning the general formula in claim 11.

VIII. In a letter of 22 November 2006, the appellant responded to the board's communication and filed amended claim requests in which the errors noted by the board had been corrected. Additional documentary evidence was also filed. The amended main request differed from the previous main request essentially in that a typographical error in claims 1 and 6 had been corrected ("peptide-linked pyridinoline" instead of "peptide-linkedpyridinoline"), the phrase "in peptide-

linked pyridinoline" in claim 12 replaced by "in a peptide-linked pyridinoline", and the erroneous general formula in claims 11 and 16 corrected. Claims 1, 6 and 12 of auxiliary request 1 were amended in the same manner. Additionally, the term "non-linear" in the phrase "a non-linear epitope" was deleted.

IX. The appellant was summoned to oral proceedings. By a letter dated 23 May 2007, the appellant informed the board of its intention not to attend the scheduled oral proceedings and requested a decision on the written file.

X. At oral proceedings, which were held on 27 July 2007, the appellant was not present.

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

(3):US patent No. 5 320 970, published on 14 June 1994;

(4): Pierce's Online Product Catalogue (www.piercenet.com), product website "Constant Boiling Hydrochloric Acid (6N)", dated 23 October 2006.

XII. The arguments put forward by the appellant in writing may be summarised as follows:

Main request

There was no legal requirement that the claim (or the patent application) contained any information relating to the structure of the claimed antibody or the

chemical nature of the epitope recognised by the antibody, provided that the patent application disclosed the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. In any event, the patent application provided sufficient information to the skilled person on those two points.

There was sufficient information in the application concerning the epitope to enable the skilled person to obtain antibodies to it. The production of monoclonal antibodies using an immunogen followed by selection of monoclonal antibodies with desired properties was standard practice at the relevant date. Monoclonal antibodies could readily be made using peptide-linked pyridinoline as immunogen, and the application taught how to distinguish antibodies which bind to the same epitope as recognised by 51A93 from other antibodies, such as 1H11. Also document (3), which was cited in the application, described methods for producing monoclonal antibodies as well as for screening clones for reactive monoclonal antibodies. The antigen (crosslinked or conjugated pyridinoline) could also be used to screen for antibodies by methods of antigen-driven selection for the production of antibodies which were well known to the person skilled in the art.

The skilled person was able to select specifically antibodies reactive with the pyridinoline in peptide-linked pyridinoline without undue experimentation. The application taught, for example, that pre-treatment of the samples by acid hydrolysis (as disclosed on page 9, lines 6 to 16) almost completely abolished the reactivity of 1H11, but not that of 51A93; and so an

immunoassay using urine samples pre-treated by acid hydrolysis fractionated on a Biogel P-10 column would indeed discriminate between antibodies that specifically bound to the pyridinoline in peptide-linked pyridinoline (such as 51A93) and antibodies that recognised a peptide (acid-labile) epitope (as was the case for 1H11). As shown in document (4), acid hydrolysis was a routine technique used in standard methods of protein analysis to break up peptide bonds for the purpose of amino acid content determination.

The level of skill of the skilled person at the relevant date was high, as it was standard procedure to determine, using recombinant DNA techniques, the structure of the VH and VL domains, including complementarity determining regions of an antibody produced by a hybridoma and to create chimeric antibodies of the same specificity as the parent antibody.

Auxiliary request 1

In claim 1 of the auxiliary request, the claimed antibody was characterised as being one which was cross-reactive with the antibody 51A93 (produced by HB 12254), and such antibodies could readily be selected using peptide-linked pyridinoline as antigen.

XIII. The appellant requested in writing that the decision under appeal be set aside and that a patent be granted on the basis of the main request or auxiliary request 1 filed on 22 November 2006.

Reasons for the Decision

Rule 88 EPC

1. The corrections introduced into the claims fulfil the requirements of Rule 88 EPC. It is evident to a person skilled in the art that the general formula in claims 11 and 16 as filed represents a benzene derivative, rather than a pyridine derivative as specified in the claims. Since the compound represented by the formula is defined as a pyridinoline analog, it is immediately evident also that the intended compound should have a pyridine ring. Further amendments introduced into the claims concerned only typographical errors, the correction of which was obvious.

Main request - Sufficiency of disclosure

2. Claim 1 is directed to a (monoclonal or polyclonal) antibody that reacts with the pyridinoline in peptide-linked pyridinoline, but not with free pyridinoline, which antibody is useful in an assay to indicate bone resorption.

3. The essential issue to be decided in the present case is whether the application fulfils the requirements of Article 83 EPC, ie whether, having regard to the guidance provided by the application supplemented by the common general knowledge at the time this guidance was made available to the public, a person skilled in the art would be able to carry out the invention **in the whole range claimed**, without the burden of an undue amount of experimentation or the application of inventive skills.

4. The examination as to the sufficiency of the disclosure in a patent application depends on the correlation of the facts of the case to certain general parameters, *inter alia*, the amount of reliable technical details disclosed in the application, the character of the technical field and the average amount of effort necessary to put into practice a certain written disclosure in that technical field (see decisions T 158/91 of 30 July 1991, point 2.3 of the reasons; and T 639/95 of 21 January 1998).

5. The present application relates to the technical field of antibodies for use in an immunoassay for diagnosis of osteoporosis, a disease condition that involves bone resorption. In the section headed "*Background of the invention*", the application provides an overview of the immunoassays for determining bone resorption commercially available at the filing date (see passage starting on page 4, line 23 and ending on page 6, line 2 of the application). The antibodies used in these immunoassays recognise degradation products of the organic matrix of the bone present in body fluids, in particular a linear peptide derived from collagen, collagen peptides linked through pyridinoline, or free pyridinoline or deoxypyridinoline.

6. It is stated in the application that one of the objects of the invention is to provide a specific antibody for use in a diagnostic assay for osteoporosis using bodily fluids from postmenopausal women which correlates with bone loss (cf. page 6, lines 5 to 7). In fact, one specific monoclonal antibody, monoclonal antibody 51A93 (also referred to as "Serex A93") is disclosed as an

example of the claimed antibodies. This monoclonal antibody, which is produced by a hybridoma deposited with the American Type Culture Collection (ATCC) under accession number HB 12254, is described in the application as being immunoreactive with peptide-linked pyridinoline generally, and not restricted to specific collagen peptides and therefore suitable for the quantitation of (any) cross-linked peptides which are indicative of bone loss (cf. page 6, lines 11 to 14).

7. The epitope recognised by monoclonal antibody 51A93 is said to be stable to acid hydrolysis and, therefore, not a linear peptide. It is further indicated in the application that pyridinoline is recognized only when bound or conjugated to a peptide, but not in its free form found in urine. Recognition of peptide-linked pyridinoline by monoclonal antibody 51A93 is said not to be dependent on conformation of a linear peptide but on a stable structure (cf. page 6, lines 16 to 27, and page 7, last paragraph).

8. The application discloses also the results of four different studies aiming at the characterization of the epitope bound by monoclonal antibody 51A93. In these studies, the immunoreactivity of antibody 51A93 is compared with the immunoreactivity of three different antibodies used in assays commercially available at the filing date, and in particular with that of the monoclonal antibody 1H11 described in document (3) (cf. page 4, line 30 of the application). As a conclusion, it is indicated in the application that monoclonal antibody 51A93 differed from the other antibodies tested in that it recognised pyridinoline when bound or conjugated, but not in its free form.

9. However, the application neither discloses any technical details on how the specific monoclonal antibody 51A93 was prepared nor provides any guidance whatsoever concerning the preparation of **further** antibodies as defined in claim 1. Thus, the question arises whether the availability of a hybridoma producing one specific monoclonal antibody (51A93) together with a general description of the epitope recognised by this antibody puts the skilled person in the position to obtain further (monoclonal) antibodies with the same specificity.

10. Similar questions have arisen in various cases decided by the boards of appeal of the EPO, and different boards have given different answers, depending on the circumstances of each case (cf. decisions T 510/94 of 21 April 1998 and T 513/94 of 23 April 1998 cited by the appellant, and decisions T 349/91 of 10 March 1993 and T 716/01 of 10 November 2004). In this respect, it must be stressed that, according to the well-established jurisprudence of the Boards of Appeal of the EPO, the question of sufficiency of disclosure is a question of fact which has to be answered on the basis of the available evidence **in each individual case** (see, *inter alia*, decision T 409/91, OJ EPO 1994, 653).

11. Having regard to the circumstances of the present case, the board considers that, whereas the fact that the method used to prepare monoclonal antibody 51A93 has not been disclosed in the application is not necessarily prejudicial in the context of assessing sufficiency of disclosure in respect of this specific antibody - as the hybridoma which produces this

antibody was deposited with a recognised depositary institution not later than the date of filing of the application (cf. Rule 28(1) EPC) - the absence of any directions or a suitable protocol for the preparation of further antibodies as defined in claim 1 raises serious doubts whether the requirements of Article 83 EPC, ie a disclosure of the invention which is sufficiently clear and **complete** for it to be carried out by a person skilled in the art, are fulfilled in respect of all antibodies encompassed by claim 1.

12. The appellant alleged that the structural information provided by the monoclonal antibody 51A93 produced by the deposited hybridoma ATCC HB 12254 was sufficient to prepare other antibodies falling within the scope of claim 1. The board, however, observes that claim 1 is not restricted to chimeric monoclonal antibodies sharing the complementarity determining region (CDR) of the antibody produced by the deposited hybridoma, but encompasses also monoclonal antibodies which, even though having the same specificity, are not derived from antibody 51A93.

13. In the present case, it is a verifiable fact which has not been disputed by the appellant that the application provides no guidance with respect to an antigen suitable for raising antibodies with the desired specificity and/or for screening antibody-producing clones or antibody libraries. The sole disclosure in the application in this respect is found on page 7, lines 26 to 30, where it is stated that "*other antibodies reactive with the same or similar epitopes* [as monoclonal antibody 51A93] *can be produced using known immunization conditions*".

14. Like the examining division, the board acknowledges that techniques for the production and screening of hybridomas secreting a monoclonal antibody with specific features were available in the art. However, all these techniques relied on the availability of a suitable antigen which allowed the skilled person to produce and/or select monoclonal antibodies of the desired specificity, with some perseverance and a reasonable amount of trial and error.

15. The board is not convinced that the skilled person would consider the antigen used in document (3) cited on page 4, line 30 of the application, to be a antigen suitable for the preparation of antibodies as claimed. Document (3) is cited in the application within the discussion of the prior art and only in relation to the properties of monoclonal antibody 1H11, rather than in the context of the disclosure of an antigen suitable for raising antibodies as defined in claim 1. Moreover, it is clearly indicated in the application that, while monoclonal antibody 1H11 recognises the same analytes as the monoclonal antibody 51A93 described in the present application, the two antibodies bind to very different epitopes (cf page 9, lines 12 to 14). Monoclonal antibody 1H11 recognises specific linear sequences occurring at cross-linking sites of the peptide (cf. page 4, lines 31 and 32 of the application), whereas antibody 51A93 purportedly recognises pyridinoline.

16. In view of the fact that the application does not disclose any specific antigen for preparing and/or selecting further antibodies as claimed, the board

considers that a skilled person seeking to prepare further antibodies as claimed would have to embark on a research program with the sole guidance of a *desideratum*, namely that the antibodies must react **specifically** with the pyridinoline in peptide-linked pyridinoline, but without any teaching in the application as how to achieve the desired specificity.

17. A skilled person in the field of antibodies would be aware of the fact that, if peptide-linked pyridinoline is used as antigen, as suggested by the appellant, not only antibodies which recognise specifically the pyridinoline molecule linked to a peptide chain, but also antibodies which recognise different (linear or conformational) epitopes embodied in the structure of the peptide chain are elicited. This is confirmed by document (3). The monoclonal antibody 1H11 described in this document was obtained using as antigen a specific pyridinoline-linked peptide isolated from urine. The epitope recognised by 1H11 is nevertheless located in one of the peptide chains linked to pyridinoline. Thus, the skilled person, seeking to obtain a monoclonal antibody specifically reactive with the pyridinoline, not having any guidance as how to achieve this specificity, could only rely on pure chance.

18. The appellant argued that simple tests as described in the section "*Characterization of Epitope Bound by 51A93*" on pages 8 to 10 of the application could be used for the screening of antibodies of the desired specificity. In particular, the appellant referred to the sections entitled "*Reactivity with Fractionated Urine Fractions*" (page 8, lines 21ff) and "*Reactivity with Peptide containing Fractions*" (page 9, lines 6ff).

19. The board is unable to find in the passages of the description indicated by the appellant a clear and **complete** teaching of a screening process which would lead necessarily and directly, with a reasonable amount of trial and error, towards the specific selection of antibodies as claimed.

20. The passage of the application under the heading "*Reactivity with Fractionated Urine Fractions*" describes a study in which postmenopausal and preadolescent urine samples were fractionated on a Biogel P-10 column, and pools of fractions were contacted with different antibodies, including the monoclonal antibody 51A93. As is apparent from Figures 1A and 1B and 2A and 2B of the application, both monoclonal antibody 51A93 and antibody 1H11 reacted with the same fractions, even though the two antibodies recognise different epitopes. Hence, an immunoassay using urine samples fractionated on a Biogel P-10 column as described in the application would not allow the **specific** selection of antibodies which react with the same epitope as monoclonal antibody 51A93.

21. The passage under the heading "*Reactivity with Peptide containing Fractions*" concerns a second study in which immunoreactivity of the antibodies 51A93 and 1H11 with urine pools before and after being subjected to acid hydrolysis was tested. Figures 3A to 3D and Tables 1 and 2 of the application show that, in urine pools subjected to acid hydrolysis, the reactivity with antibody 1H11 was strongly reduced whereas the

reactivity with the monoclonal antibody 51A93 was reduced only by half or even increased.

22. However, no technical details concerning the conditions employed for acid hydrolysis of the urine pools are provided in the application. These conditions are insofar critical as, in order for the desired antibodies - which according to claim 1 are not reactive with free pyridinoline - to react with the acid-treated urine pools, the pyridinoline must still be linked to a peptide. Moreover, under the same conditions, epitopes on the peptide which are recognised by antibodies other than the desired antibodies must be destroyed. The application is however silent on how much peptide must remain linked to pyridinoline for recognition by the desired antibodies, without running into the risk of isolating antibodies that bind to the peptide but not to pyridinoline. This lack of disclosure forces the skilled person to embark on further experimentation which goes beyond the routine experiments required typically - ie when sufficient guidance is provided in the application - for the identification of monoclonal antibodies of a desired specificity.

23. In support of its argument that, at the relevant date, acid hydrolysis of peptides was a routine technique, the appellant submitted document (4). In this document, acid hydrolysis conditions used in standard methods of protein analysis are described. It is, however, noted that, under the conditions described in this document, **total** protein hydrolysis is achieved (cf. fourth paragraph in document (4)). Thus, the routine methods described in document (4) are certainly not suitable

for a partial hydrolysis of peptide-linked pyridinoline as necessary for avoiding hydrolysis of the epitope recognized by the desired antibodies.

24. In its statement of grounds of appeal, the appellant alleged that, at the relevant date, phage antibody libraries were available and the methods required for screening of such libraries constituted common general knowledge. However, the board notes also that the selection of desired monoclonal antibodies among antibodies with various specificities in a phage antibody library requires a suitable antigen and a reliable screening process. However, neither a specific antigen nor a screening process are disclosed in the application in a clear and complete manner, either generally or in connection with the preparation of the specific monoclonal antibody 51A93.

25. After appraising the technical details and guidance provided by the application and the evidence submitted by the appellant, the board concludes that the disclosure in the present application is insufficient with respect to both the antigen required to raise further antibodies as claimed, and the screening process for the specific selection of the same. Due to the lack of technical details in the application, the skilled person seeking to produce further antibodies as claimed would have to carry out additional experimentation which goes beyond the average amount of effort necessary in the field of monoclonal antibodies, without any guidance from the application. The board considers that this additional experimentation amounts to an undue burden. Thus, the invention, to the extent that it relates to antibodies as claimed in claim 1

other than the specific monoclonal antibody 51A93, does not fulfil the requirements of Article 83 EPC.

Auxiliary request 1

26. In auxiliary request 1, the claimed antibodies are further defined as being reactive with an epitope on a peptide-linked pyridinoline that is stable to acid hydrolysis, this epitope being recognised by the antibody produced by the deposited hybridoma HB 12254.

27. In the board's view, there is a discrepancy between the features "*reactive with the pyridinoline in peptide-linked pyridinoline*" and "*reactive with an epitope on a peptide-linked pyridinoline that is stable to acid hydrolysis*" defining the antibodies of claim 1, insofar as the latter feature suggests that the epitope recognised by the antibodies is not limited to pyridinoline but includes further (non-defined) elements of the peptide. This lack of clarity as to the actual nature of the epitope adds to the uncertainties arising from the absence of any disclosure in the application with respect to both the antigen required for immunization and/or for selection of the desired antibodies, and a suitable screening protocol (cf. paragraphs 13 to 24 *supra*). Furthermore, in view of the lack of specific details in the application with respect to a suitable antigen, the board fails to see how the skilled person could carry out an assay for testing cross-reactivity with the monoclonal antibody 51A93 in order to select antibodies of the same specificity, without having to embark in further painstaking experimentation.

28. Thus, essentially for the same reasons as given above in connection with claim 1 of the main request, the board concludes that the invention as claimed in claim 1 of auxiliary request 1 does not fulfil the requirement of Article 83 EPC.

Article 113(1) EPC

29. The reasons given by the board in the present decision were apparent from the communication under Article 110(2) EPC, and the appellant was given the opportunity to file observations in writing and, later, invited to oral proceedings under Article 116 EPC. Nevertheless, the appellant chose not to attend oral proceedings. The provisions of Article 113(1) EPC are complied with (see also Article 11(3) RPBA).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani