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D E C I S I O N
of 25 March 2003

Case Number: T 0505/00 - 3.3.8

Application Number: 91913019.5

Publication Number: 0540573

IPC: G01N 33/573

Language of the proceedings: EN

Title of invention:
Assay of free and complexed prostate-specific antigen (PSA)

Patentee:
LILJA, Hans, et al.

Opponent:
Centocor, Inc.
CIS Bio International
Bio Merieux S.A.
Diasorin S.r.l.
Diagnostics Products Corporation
Dade Behring Marburg GmbH

Headword:
Anti-free PSA antibody/LILJA

Relevant legal provisions:
EPC Art. 123(2), 54, 56, 83

Keyword:
"Added subject-matter - no"
"Novelty over alleged prior use - yes"
"Inventive step - yes"
"Sufficiency of disclosure - yes"

Decisions cited:
T 0019/90, T 0097/94, T 0194/94, T 0848/94

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0505/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 25 March 2003

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Decision under appeal:

Interlocutory decision of the Opposition Division of the European Patent Office posted 31 March 2000 concerning maintenance of European patent No. 0 540 573 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of Facts and Submissions

I. European patent No. 0 540 573 with the title "Assay of free and complexed prostate-specific antigen (PSA)" was granted with 16 claims for all Designated Contracting States except ES and GR and 18 claims for the Designated Contracting States ES and GR, based on European patent application No. 91 913 019.5.

II. Nine notices of opposition were filed requesting the revocation of the patent under Article 100(a) to (c) EPC. Opponents 5 and 7 withdrew their oppositions before the decision under appeal, and so are not parties to the appeal. The other party (Opponent 1) withdrew its opposition during the appeal proceedings and so is only a party for formal purposes.

III. By the interlocutory decision dated 31 March 2000, the Opposition Division maintained the patent in amended form on the basis of the third auxiliary request then on file.

IV. Appellants I (Patentees) as well as Appellants II, III and IV (Opponents 3, 4 and 8, respectively) filed an appeal against this decision.

V. The Appellants filed further submissions in response to their respective statements of grounds of appeal.

VI. The Board sent a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal, indicating its preliminary non-binding opinion.

VII. Appellants I and IV answered the Board's communication. Appellants I submitted a new main and four auxiliary requests.

VIII. At oral proceedings which took place on 24 and 25 March 2003, Appellants III and IV withdrew their appeals and became Respondents IV and V, respectively. Oral proceedings were not attended by Respondents I and III who had accordingly informed the Board. Appellants I replaced all of the requests on file by one main request.

Claims 1, 3, 5, 8, 9 and 11 of the main request for all Designated Contracting States except ES and GR read as follows:

"1. An immunoassay for quantitatively determining the amount of free prostate-specific antigen (free PSA) in a sample, **characterized in** that the sample is exposed to a monoclonal antibody which binds free PSA, but does not bind PSA in complex with α_1-antichymotrypsin and that the amount of free PSA bound by the antibody is detected."

"3. An immunoassay for quantitatively determining the amount of prostate-specific antigen complexed with α_1-antichymotrypsin (complexed PSA) in a sample, characterized in that the sample is exposed to both an antibody which binds complexed PSA and an antibody which binds α_1-antichymotrypsin and that the amount of complexed PSA bound by both antibodies is detected."

"5. An immunoassay for determining the ratio between the free non-complexed prostate-specific antigen (free PSA) and the total amount of PSA (total PSA), characterized in that

(a) the amount of free PSA is measured in accordance with claim 1,

(b) the amount of total PSA is measured, and

(c) the ratio between the amount of free PSA and the amount of total PSA is determined."

"8. A purified PSA-α_1-antichymotrypsin complex (PSA-ACT)."

"9. A monoclonal antibody capable of binding free PSA, but not capable of binding PSA complexed with α_1-antichymotrypsin."

"11. A method for differentiating between benign prostatic hyperplasia and prostate cancer by determining the ratio between free PSA and total PSA, between free PSA and PSA complexed with α_1-antichymotrypsin, or between PSA complexed with α_1-antichymotrypsin and total PSA in a patient's serum, wherein the free PSA is determined in accordance with claim 1, and wherein PSA complexed with α_1-antichymotrypsin is determined in accordance with claim 3."

Claim 2 related to a further feature of the immunoassay of claim 1. Claim 4 related to an immunoassay for determining the ratio between free PSA and PSA complexed with α_1-antichymotrypsin (PSA-ACT). Claims 6 and 7 related to further features of the immunoassays of claims 1 to 5. Claim 10 related to a cell line producing the monoclonal antibody of claim 9 and claim 12 related to a method for screening prostate cancer having the same characteristics as the method of claim 11.

Claims 1 to 12 for the Designated Contracting States ES and GR were identical to claims 1 to 12 for the other Designated Contracting States. Claims 13 and 14 read as follows:

"13. A process for preparing an antibody capable of binding free PSA, but not capable of binding PSA complexed with α_1-antichymotrypsin, the process comprising the steps of:

(a) immunizing mice with PSA,

(b) preparing lymphoid cells of the spleen from the mice,

(c) mixing and fusing these cells with plasmacytoma cells to form a hybridoma cell line capable of producing anti-PSA antibodies,

(d) cultivating the hybridoma cell line to produce anti-PSA antibodies, and

(e) screening for anti-PSA specific antibodies that do not concomitantly bind to PSA in complex with α_1-antichymotrypsin."

"14. A process for preparing a purified PSA-ACT complex comprising mixing purified PSA and purified ACT to form the PSA-ACT complex".

IX. The following documents are mentioned in the present decision:

(1): Certified English translation of Japanese laid-open patent application No. 62-46263 of 28 February 1987;

(24): Akiyama, K. et al., FEBS Letters, Vol. 225, No. 1, 2, pages 168 to 172, 1987;

(25): Schaller, J. et al., Eur.J.Biochem., Vol. 170, pages 111 to 120, 1987;

(26a): English translation of the information accompanying the "Chugaï kit" to measure γ-seminoprotein in serum, March 1986;

(48): Demura, T. et al., Cancer, Vol. 77, No. 6, pages 1137 to 1143, 1996;

(50): Product sheet "Cappel" relating to Goat IGG fraction to human alpha-1-antitrypsin, Catalog No. 55030, ICN pharmaceuticals Inc;

(83): Pettersson et al., Clin.Chem., Vol. 41, No. 10, pages 1480 to 1488, 1995.

X. The arguments by Appellants II (Opponents 3) in writing and during oral proceedings insofar as they are relevant to the present decision may be summarized as follows:

Article 123(2) EPC

Claims 1 and 9

The application as filed disclosed a monoclonal antibody (mAb) which **preferentially** recognized free PSA and **poorly** recognized (PSA-ACT) on page 7, first par. and Table 1, assay B, 2nd column but it did not disclose a mAb specific for free PSA ie which would **only** bind to free PSA, let alone a method for quantitatively measuring free PSA using such an antibody. For this reason, the requirements of Article 123(2) EPC were not fulfilled.

Article 83 EPC

in relation to the subject-matter of claims 1, 5, 9 and 10

- The patent in suit did not disclose a mAb according to claim 9 which was necessary to carry out the immunoassay of claim 1: indeed, the examplified mAb

allegedly specific for free PSA, ie 5A10, was, in fact, cross-reactive with PSA-ACT (Table 1, Figures 2 and 3). For the same reason, the cell-line producing 5A10 could not be considered as being a cell line in accordance with claim 10.

Even if it was accepted that 5A10 answered the definition of an anti-free PSA Mab according to claim 9, it would remain that in the absence of any deposit, it would be undue burden for the skilled person to reproduce it, taking into account that no information was available on how many monoclonal antibodies had been tested in order to find 5A10, and on the epitope which the mAb should recognize.

Even if it was accepted that 5A10 was reproducible, it would remain that the description failed to provide an enabling disclosure of the immunoassay of claim 1 because of the cross-reactivity with PSA-ACT to be expected from 5A10-like mAbs which in the case of 5A10 was of about 6% (Table 1). Such a level of cross-reactivity would distort the results obtained when testing patients' sera in such a manner as to render the immunoassay meaningless.

- In addition, the description failed to give any information on how to quantify total PSA (claim 5).

in relation to the subject-matter of claim 8 as well as of claims 13 and 14 for Contracting States ES and GR

The patent in suit did not teach any methods for obtaining a **purified** PSA-ACT complex, let alone the purified complex per se. Visualizing this complex on gels as the product resulting from the mixing of purified ACT and purified PSA was an analytical

detection rather than a purification method. It was not even sure from Figure 1 that PSA-ACT had been separated from free ACT.

In the absence of purified PSA-ACT, it was impossible to carry out the method of claim 13 (ES/GR) which made use of that molecule for screening anti-free PSA mAbs.

in relation to the subject-matter of claims 11 and 12

- The patent in suit showed that the ratios free PSA/total PSA and complexed PSA/total PSA were not suitable tools for diagnosing prostate cancer:

(i)- Figures 4 and 5 were diagrams respectively representing the amounts of free or complexed PSA present in the sera of 65 individual cancer patients, as a function of the amount of total PSA. On page 4, lines 26 to 28, mathematical formulas were derived from these data which were representative of the relationship between the amount of free or complexed PSA and the amount of total PSA. When these formulas were used to calculate the ratios free PSA/total PSA and complexed PSA/total PSA, one found that for **increasing** quantities of total PSA, **both** these ratios **decreased**. This inconsistency led to the conclusion that the ratios had no meaning and, consequently, were not suited for any kind of diagnosis.

(ii)- The same conclusion was reached by considering the data shown in Table 2. This table disclosed specific average ratios of free PSA/total PSA and complexed PSA/total PSA allegedly characteristic of populations of patients suffering from benign prostate hyperplasia or from prostate cancer at different stages of the disease. It could readily be seen that for some

of these populations, the sum of the two ratios was superior to 1 by as much as 30%, which made no technical sense.

(iii)- Figures 6, 7 and 8 showed the amounts of free, complexed or total PSA found in different fractions resulting from gel filtration of the serum of three individual patients. The relationship between these amounts varied very widely from patient to patient and the amount of complexed PSA was sometimes higher than that of total PSA. As for Figure 10, it disclosed ratios of complexed PSA to total PSA in the serum of individual cancer patients which overlapped with those which according to page 4, lines 43 and 44 were found in the sera of healthy males. All these results cast doubts on the significance of **average ratios** for distinguishing patients with prostate cancer from other patients or from healthy individuals.

- No mention was ever made of the ratio free PSA/PSA-ACT as a tool for differentiating between benign prostate hyperplasia and prostate cancer, or for screening prostate cancer.

For all these reasons, the requirements of Article 83 EPC were not fulfilled in relation to the subject-matter of claims 11 and 12.

Article 54 EPC

Claims 9 (product) and 1 (method)

- As early as 1986, the firm Chugaï sold an immunoassay kit for the detection of prostate cancer described in document (26a), which kit was detrimental to the novelty of claims 9 and 1. Admittedly, document (26a) did not disclose that the mAb contained in the Chugaï

assay kit was capable of binding free PSA but was not capable of binding PSA-ACT. Yet, post-published evidence such as document(48), pages 1137 to 1139, showed that it was indeed the case.

- Various test reports filed at the opposition stage showed that the Chugaï mAb was specific for free PSA. These tests were carried out using kits manufactured after 1992, ie after the date when the according to document (48), the Chugaï company modified the method for free PSA determination. Yet, also according to document (48), test values obtained with the old and new versions of the Chugaï kit could be directly correlated. This allowed the conclusion that the specificity of the mAb had not been changed in the course of time and that, therefore, the test reports constituted adequate evidence that the Chugaï mAb and immunoassay fell within the scope of claims 9 and 1, thus destroying novelty.

- Starting from the Chugaï mAb, it would have been possible before the filing date of the patent to clone the corresponding DNA without undue burden and, thus, to reproduce said mAb and determine its proprieties.

Article 56 EPC

Claims 9 and 1

Document (1) was the closest prior art for the subject-matter of claims 9 and 1. It disclosed radioimmunoassays for measuring the total amount of γ-seminoprotein (γ-Sm) present in blood serum, as a means to detect prostate cancer. It also taught that γ-Sm existed in the serum in free and complexed forms and provided an immunological method for measuring only complexed γ-Sm, also as a means to detect prostate cancer.

Knowing from document (1) that the γ-Sm complexed form or total γ-Sm could be used as markers for detecting prostate cancer by immunoassays, and from document (25) that γ-Sm was the same molecule as PSA, the skilled person would have found it obvious to isolate a mAb specific for the alternative form of γ-Sm ie for free PSA for carrying out a further immunoassay with the same purpose.

Isolating this antibody could be done in a routine manner taking into account that document (1) taught how to separate complexed PSA from free PSA.

- In document (1), γ-Sm (PSA) was said to be present in serum as a complex with antitrypsin (AT). At the priority date, the skilled person knew that PSA was a serine protease "expressing trypsin- and chymotrypsin-like specificities" (document (24), page 171, document (25), page 115). This knowledge made it obvious that PSA would bind to antichymotrypsin, ie that the complex defined in document (1) as γ-Sm-α-antitrypsin (PSA-AT) was in fact a complex between γ-Sm and α-anti**chymo**trypsin (PSA-ACT). This, in turn implied that it was obvious to isolate the mAb of claim 9 which recognized free PSA but did not recognize PSA-ACT, as a means to be used in the detection of prostate cancer.

- As document (1) rendered obvious the antibody of claim 9, it also rendered obvious the immunoassay of claim 1 which made use of this antibody in the same manner and for the same purpose as taught in said document.

Claim 3

The difference between the subject-matter of claim 3 and the teachings of document (1) was only that the

antibody used to determine the amount of complexed PSA in a sample was specific of PSA-ACT rather than of PSA-AT. As already explained in relation to claim 9, it was obvious at the priority date and on the basis of documents (24) or (25) that PSA-ACT was the complex present in blood serum which was worth measuring. Thus, the use of a mAb against PSA-ACT as part of an immunoassay for the quantitation of complexed PSA did not require an inventive step.

Claim 8

The closest prior art was document (1) which provided a method to separate free PSA from PSA-ACT (even if then identified as PSA-AT). For the skilled person to purify the PSA-ACT complex starting from PSA-ACT as isolated in document (1) would only require standard routine techniques. Thus, purified PSA-ACT per se did not fulfill the requirements of Article 56 EPC.

Claims 11 and 12

The subject-matter of these claims was directed to methods for identifying prostate cancer which made use of the ratios free PSA/complexed PSA, free PSA/total PSA or complexed PSA/total PSA. This way of expressing the results was argued by the patent Proprietors to add a second dimension to the diagnosis of prostate cancer in that it put emphasis on the quality of the PSA present in blood serum rather than on its quantification. This aim, however, could equally be achieved by defining ranges of total PSA concentrations and identifying within each of these ranges the concentrations of free or complexed PSA indicative of cancer. Thus, the subject-matter of claims 11 or 12 amounted to a way of expressing obvious results which was not suited by itself for imparting inventive step to said results.

XI. The arguments by Appellants I (Patentees) in writing and during oral proceedings insofar as they are relevant to the present decision may be summarized as follows:

Article 123(2)

Claims 1 and 9

The application as filed, page 6, lines 7 to 20 disclosed the mAb, 5A10, which identified PSA blotted to PVDF-membranes but did not identify PSA-ACT. On page 9, lines 3 to 18, this antibody was used in a quantitative method for distinguishing free PSA from complexed PSA (Table 1). This disclosure constituted a valid basis for acknowledging that the subject-matter of claims 1 and 9 fulfilled the requirements of Article 123(2) EPC.

Article 83 EPC; sufficiency of disclosure

in relation to the subject-matter of claims 1, 5, 9 and 10

- The patent in suit gave the information necessary to isolate the cell line of claim 10 in the passage bridging page 3, line 29 to page 4, line 20, it also disclosed a mAb according to claim 9, capable of binding free PSA but not capable of binding PSA-ACT: 5A10. That 5A10 did not show any cross-reactivity with PSA-ACT could be seen in Figure 1 D. The background level of PSA-ACT-anti-free PSA mAb binding observed in Figure 3 and Table 1 was due to residual free PSA in the PSA-ACT preparation and that observed in Figure 2 was an artefact due to the way the experiment had been conducted, ie under denaturing conditions.

Post-published document (83) showed that using the methods of the patent in suit, it was possible to isolate further anti-free PSA mAbs according to claim 9.

Appellants II had merely expressed assumptions that mAbs such as 5A10 would not give reliable results in an immunoassay for quantitatively determining the amount of free PSA in a sample (claim 1) but they had not discharged their burden of proof that it was indeed so.

- Methods for measuring total PSA were part of the common general knowledge at the priority date as could be inferred from document (1) which referred to such methods. Thus, the skilled person would have been able to reproduce the subject-matter of claim 5 without undue burden on the basis of the common general knowledge and of the teachings of the patent in suit.

in relation to the subject-matter of claim 8 as well as claim 13 and claim 14 for Contracting States ES and GR.

The patent in suit provided a method for obtaining purified PSA-ACT, according to which the complex made from purified PSA and purified ACT was separated from the individual constituents on agarose gels (page 6, lines 14 to 15, Figure 1). No evidence was provided by Appellants II that PSA-ACT was contaminated by free ACT on the gel. It would be well within the skilled person's ability to retrieve said complex from the ~~gel~~ using techniques well-known in the art for this purpose.

Since the method led to the preparation of purified PSA-ACT, it was possible to carry out the method of claim 13 (ES/GR).

in relation to the subject-matter of claims 11 and 12

- Table 2 showed that there were clear differences between the ratios of free or complexed PSA to total PSA, depending on whether the tested samples originated from sera of cancer patients or of patients suffering from hyperplasia.

- The arguments presented by Appellants II on the basis of mathematical formulas allegedly representing the relationship between free or complexed PSA and total PSA in cancer patients were the result of a misconception of the data. By simply looking at the Figures 4 and 5, it was immediately apparent that complexed PSA constituted about 89% of the amount of total PSA present in the sera of these patients (Figure 4) whereas free PSA contributed about 10% (Figure 5). Thus, the cumulative amounts of free and complexed PSA did correspond to the total amount of PSA within experimental variations. Consequently, the above mentioned ratios were not without technical meaning and the fact that they were different in cancer patients and other individuals could be used as a mean for diagnosis.

- This conclusion was not affected by the fact that in Table 2, the sum of the ratios of free and complexed PSA over total PSA was superior to 1 for some of the tested groups. This artefact was simply due to the way the assay for the PSA-ACT complex was calibrated, in particular to the fact that the PSA-ACT preparation would not be expected to be 100% pure.

- As for the doubt raised by Appellants II on the meaning to be given to the ratios established from populations of patients, taking into account that the discrepancies sometimes observed when analysing the sera of individual patients or taking into account that the ratios established for cancer patients sometimes overlapped with those established for healthy

individuals, it amounted to no more than an allegation which, in accordance with the case law of the Boards of Appeal, was not sufficient for concluding that the requirements of Article 83 EPC were not fulfilled.

- The ratio free PSA/complexed PSA could be used as a tool for diagnosis in exactly the same way as the other ratios.

The requirements of Article 83 EPC were fulfilled in relation to the subject-matter of claims 11 and 12.

Article 54 EPC

Claims 1 and 9

- The novelty of the subject-matter of claim 9 had been challenged on the basis that the Chugaï immunoassay kit which was on the market before the priority date must have contained an anti-free PSA mAb. Yet, document (26a), ie the information accompanying the kit did not specify the nature of the mAb. The Chugaï company never gave any further information in this respect.

- The kit as available at the priority date was changed over the year as shown in document (48) and one could not be sure that the later Chugaï kit contained the same mAb as the earlier. Thus, the experiments presented by the opponents during the opposition procedure which were all carried out on the later Chugaï kit were not suitable to show which antibody was originally contained within the earlier kit.

- For these reasons, the prior sale of the Chugaï immunoassay kit, even taking into account the written evidence on file did not destroy the novelty of the subject-matter of claims 9 and 1.

Article 56 EPC

Claims 9 and 1

- Document (1) was the closest prior art as it taught determining the amount of complexed PSA by immunoassay in order to diagnose prostate cancer.

Starting from document (1), the problem to be solved could be defined as providing further means which were useful for differentiating between patients suffering from prostate cancer and those suffering from benign hyperplasia.

Document (1) taught away from the solution provided by claim 9 as it directed the skilled person's attention to complexed PSA rather than to free PSA. And besides, document (1) did not teach to measure PSA complexed with anti**chym**otrypsin but PSA complexed with trypsin. Thus, even if one was to assume that document (1) suggested the measurement of free PSA, the skilled person could only deduce from the document that the relevant mAb would be the one which did not bind antitrypsin.

- Appellants II' argument that the skilled person would take the teachings of document (1) as referring to PSA complexed with antichymotrypsin rather than with trypsin was not founded. On page 6 of this document, it was mentioned that the antitrypsin mAb necessary for the quantitation of complexed PSA was that described in document (50) (page 4) ie it had no cross-reactivity with any other serum proteins. Besides, it was not yet known at the priority date whether PSA had "trypsin- or chymotrypsin- like specificities" (document (24), page 171). Finally, even if the skilled person was to think on the basis of document (25) (page 115) that PSA

had chymotrypsin-like specificity, it did not necessarily mean that it would be recognized by and, thus, form a complex with antichymotrypsin, ie that an mAb specific for free PSA should have the property of distinguishing between free PSA and PSA complexed with antichymotrypsin.

For these reasons, the subject-matter of claims 9 and 1 was inventive.

Claims 8 and 3

The closest prior art, document (1), did not mention that PSA would bind to antichymotrypsin but only that it bound to antitrypsin. Furthermore, the fact that PSA was known as having trypsin-and chymotrypsin-like specificities (document (24), page 171) did not necessarily imply that antichymotrypsin would bind PSA. Thus, it could not have been expected that the major complexed form of PSA in serum would be PSA-ACT. Accordingly, the subject-matter of claims 8 and 3 was inventive.

Claims 11 and 12

Document (1) disclosed the quantitative measurements of PSA-AT and total PSA as diagnostic means. The sole fact of measuring free PSA as that PSA which was not bound to ACT, and of measuring complexed PSA as that PSA which was bound to ACT was already inventive for the above mentioned reasons. Making use of the ratios between the different forms of PSA as diagnostic tools added the further dimension to the diagnosis that the quality of the PSA present in the serum was taken into account.

XII. Appellants I requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request filed during oral proceedings on 24 March 2003.

Appellants II requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Article 123(2)(3) EPC

Claims 1 and 9

1. The only request at issue (in both versions) differs from the granted claim request in that the claimed antibody has been qualified as "monoclonal" and granted claims 2, 4, 5 and 13 have been deleted with consequent renumbering and review of dependencies.

2. The passage bridging page 2, line 30 to page 3, line 2 and the passage page 6, lines 16 to 20 of the application as filed respectively disclose: "**Free PSA** and the PSA complex are according to the nature of the invention **measured** by a **non-competitive immunoassay** employing at least two different monoclonal antibodies." and "PSA blotted to PVDF-membranes from the agarose gels was identified by all three monoclonal antibodies whereas the PSA complexed to α_1-antichymotrypsin was identified by the 2E9 and the 2H11 antibodies but **not by the 5A10 antibody**" (emphasis added by the Board). Thus, there is a formal basis in the application as filed for a mAb capable of recognizing free PSA and not capable of recognizing PSA-ACT as well as for its use in an immunoassay for measuring free PSA.

3. Granted claims 1 and 12 which correspond to claims 1 and 9 at issue related to polyclonal as well as monoclonal antibodies. In contrast, claims 1 and 9 now only relate to monoclonal antibodies. Thus, the scope of the claims has been restricted.

4. The requirements of Article 123(2)(3) EPC are fulfilled.

Article 83 EPC

5. Appellants II argued that the requirement of sufficiency of disclosure was not fulfilled in relation to the subject-matter of claims 1, 5, 8 to 12 as well as in relation to claims 13 and 14 for the Contracting States ES and GR. As the features objected to recur in these claims, they will be considered in turn.

Sufficiency of disclosure in relation to the purified PSA-ACT complex (representing the invention (claim 8 as well as claims 13 and 14 ES/GR) or directly or indirectly necessary for carrying it out (all other claims)).

6. In accordance with the case law of the Boards of Appeal, an objection for lack of sufficient disclosure can only be considered relevant if there are serious doubts substantiated by verifiable facts that the invention cannot be reproduced on the basis of the teachings provided by the patent in suit (T 19/90, OJ EPO 1990, 476).

7. The patent in suit teaches how to produce PSA-ACT starting from purified PSA and purified ACT in the passage bridging pages 3 and 4 and it shows that the complex may be separated from free PSA on agarose gel (page 3, lines 56 and 57, Figure 1). Appellants II argued that observing the complex on an analytical gel does not amount to providing a method for preparing it

and that one could not be sure by looking at Figure 1 that PSA-ACT had been separated from free ACT. Yet, they failed to show that it is not possible to elute PSA-ACT from the agarose gel in purified form by methods generally known in the art for retrieving proteins from gels and did not provide any experimental evidence to show that ACT would migrate so near to PSA-ACT that separating them would be impossible.

8. In accordance with the case law and in absence of any evidence to the contrary, the Board concludes that the purified PSA-ACT complex is made available in a reproducible manner by the teachings of the patent in suit. Sufficiency of disclosure is, thus, acknowledged in relation to the subject-matter of claim 8 as well as of claims 13 and 14 (ES/GR).

Sufficiency of disclosure in relation to a mAb capable of binding free PSA but not capable of binding PSA-ACT (claims 9 and 10)

9. The patent in suit (page 3, lines 29 to 51) teaches how to obtain mAbs against PSA and also (passage bridging page 3, line 55 to page 4, line 15) how to select from a population of anti-PSA mAbs those which are capable of binding free PSA but not PSA-ACT. The Board was not provided with any evidence that these teachings could not be reproduced taking into account the finding in point 8 above that PSA-ACT is available for the screening of the relevant mAb. In particular, Appellants II' assumptions that too high a number of mAbs would have to be tested and that the relevant epitope would have to be known before one could obtain an Ab such as claimed is not substantiated by any data.

10. Appellants II also argued that the mAb isolated by the above mentioned methods (5A10) as representative of the mAbs of claim 9 did not in fact possess the required

properties since it showed 6% cross-reactivity with PSA-ACT when free PSA and PSA-ACT were present at the same high concentration in the respective test samples (Table 1, assay B, column 2 and Figures 2 and 3). However, the experiment which leads to this result makes use of a preparation of PSA-ACT which was simply obtained by mixing PSA and ACT without further purification steps and, as such, contains some residual free PSA (Figure 1). Under these experimental conditions, it is expected that 5A10 (which recognizes free PSA) will be seen as binding to the preparation of PSA-ACT to the extent that it binds to the residual free PSA within it. Accordingly, the apparent cross-reactivity reported in Table 1 and Figure 3 cannot be taken as a proof that 5A10 is indeed cross-reactive with PSA-ACT. As for the binding observed in Figure 2 when the experiment is carried out under denaturing conditions, ie when PSA and PSA-ACT have lost their tertiary conformations, it is not suitable to draw any conclusion as to the binding properties of 5A10 to the natural molecules.

11. Document (83) (to be taken as an expert document) provides a study of anti-PSA mAbs which involves using a PSA-ACT preparation which went through a purification step after PSA and ACT were reacted together (page 1481). Under such experimental conditions, 5A10 shows less than 0.2% cross-reactivity with PSA-ACT and it is said to recognize an epitope only accessible on the free PSA molecule (Table 1 and Figure 1). This constitutes additional evidence that it is possible to obtain an anti-free PSA mAb such as claimed by using the method described in the patent in suit.

12. Taking into account the findings in points 9 to 11 above, sufficiency of disclosure is acknowledged in relation to the subject-matter of claims 9 and 10.

Sufficiency of disclosure in relation to an immunoassay for quantitatively measuring the amount of free PSA in a sample (claim 1)

13. The argument was also presented that the immunoassay for quantifying the amount of free PSA in a sample making use of a mAb such as 5A10 would lead to false results since the mAb would bind to PSA-ACT to a non negligible extent under conditions where PSA-ACT could be expected to be present in a much higher concentration than free PSA (ie in serum). This argument, however, only holds under the assumption that the anti-free PSA mAb indeed binds to PSA-ACT. This assumption has not been proven. To the contrary, as above mentioned (points 10 and 11), there is evidence on file that the anti-free PSA mAb according to the patent in suit does not bind to PSA-ACT but to the residual free PSA present in the PSA-ACT preparation ie the observed apparent cross-reactivity is only an in vitro artefact.

14. In the absence of any evidence that there is a PSA form other than free PSA **in a serum sample** which would be capable of binding 5A10-like mAbs, the argument is not found convincing.

15. Sufficiency of disclosure is acknowledged in relation to the subject-matter of claim 1.

Sufficiency of disclosure in relation to an immunoassay involving in particular the measurement of total PSA (claim 5)

16. Appellants II drew the Board's attention to the fact that the patent in suit did not mention how to measure total PSA whereas Appellants I cited document (1) as evidence that the measurement of total PSA was already a matter of common general knowledge some four years

before the filing date of the patent in suit. On page 3 of this document, it is stated: "...radioimmunoassay (RIA), EIA and the like using the anti-γ-Sm antibody may be used to measure the gross weight of the disengaged γ-Sm and the γ-Sm-α_1-AT complex. Such a method is reported in articles. Nichihinyoukaï Journal, Volume 74, pp.1320-1325. 1983..., Rinshoukensa, Volume 23, pp.1755-1758, 1984...", the term γ-Sm being an earlier denomination of PSA. On this basis, the Board concludes that the skilled person would have known at the filing date how to measure total PSA. Sufficiency of disclosure is acknowledged in relation to the subject-matter of claim 5.

Sufficiency of disclosure in relation to methods of differentiating between benign prostate hyperplasia and prostate cancer or for screening prostate cancer (claims 11 and 12).

17. Appellants II submitted a critical analysis of the data presented in Table 2, Figs. 4 to 8 and 10 of the patent in suit as an argument against the validity of using the ratios free PSA/total PSA or complexed PSA/total PSA in diagnostic methods. Thereagain, as pointed out in point 6 above, the Board must emphasize that bringing forward allegations against the credibility of data or casting doubts on their relevance does not meet the standard applied by the European Patent Office when deciding on sufficiency of disclosure, which standard is that serious doubts must be substantiated by verifiable facts (T 19/90, above). No such facts have been put forward by Appellants II who bear the onus of proof. On the contrary, it is readily apparent from studying Table 2 that the above mentioned ratios are different when they are calculated from the

measurements of free, complexed and total PSA carried out on sera of patients suffering from prostate cancer or respectively on sera of patients suffering from hyperplasia.

18. For the sake of completeness, the Board will, however, consider Appellants II' arguments. One of them is that the ratios free PSA/total PSA and complexed PSA/total PSA calculated using mathematical formulas given in the patent in suit make no technical sense (see Section X above). The Board, however, notices that the calculated values of free or complexed PSA starting from a given amount of total PSA using said formulas do not coincide with the corresponding experimental values which may simply be obtained from Figures 4 or 5 by correlating de visu any value of total PSA with the corresponding value of complexed PSA (Figure 4) or free PSA (Figure 5). The only conclusion which can therefore be drawn is that the mathematical formulas, whatever they might represent, do not provide a valid relationship between the amount of free or complexed PSA and the amount of total PSA. Accordingly, the conclusion cannot be drawn that the ratios are meaningless.

19. Appellants II also pointed out that the ratios free PSA/total PSA and complexed PSA/total PSA calculated for specific groups of patients in Table 2 do not add to 1 for some of the groups. Appellants I argued that this was only due to the experimental conditions which were used (see Section XI, above). This observed discrepancy, however, does not alter the fact that the ratios per se are distinctly different depending on the disease the patients are suffering from and, thus, can be used in diagnostic methods.

20. Finally, Appellants II expressed doubts that the average ratios obtained from groups of patients could be of significance for individual patients which could not be expected to be "average" and pointed out that ratios determined from healthy males may overlap with those characteristic of cancer. In the Board's judgment, the skilled person would be well aware that the claimed diagnostic method is only a part of a complete diagnosis and would not expect it to give an absolute answer on its own but would, nonetheless, regard it as useful information.

21. For the reason given in point 17 above, sufficiency of disclosure is acknowledged in relation to the subject-matter of claims 11 and 12 irrespective of the ratio which is used even if this ratio is that of free PSA over complexed PSA which is not exemplified in the patent in suit but is directly derivable from the other two ratios.

22. The requirement of Article 83 EPC is fulfilled in relation to the subject-matter of claims 1 to 12 (all states except ES/GR) and claims 1 to 14 (ES/GR).

Article 54 EPC

Claims 9 and 1

23. The novelty of the subject-matter of these claims was challenged on the basis of the prior sale of an immunoassay kit by the firm Chugaï. The overall information on file about this kit is as follows:

- Document (26a) which corresponds to the sheet accompanying the kit in 1986 teaches that it is suited for measuring PSA concentrations in blood serum in order to assess the prognosis and identify any recurrence of prostate cancer. The antibody which the

kit contains is described as a mAb for specifically assaying PSA (page 5). No information is given as to the PSA forms which are recognized.

- Document (48) (sentence bridging pages 1138 and 1139), to be used as an expert document, discloses the quantitation of free PSA in serum specimens with an enzyme immunoassay using the Chugaï kit reagent dating from 1993 (two years after the filing date of the patent; ref.13). On the same page, it is mentioned that "the Chugaï company modified the method for γ-seminoprotein determination in 1992" and that "when γ-seminoprotein values measured with the old version kit reagent were lower than 15ng/mL, they were well correlated with the γ-seminoprotein values measured with the new version kit". Document (48), however, does not provide any information as to whether the main reagent ie the mAb recognizing PSA is the same in the two kits.

- A number of test reports were carried out by the Opponents on the Chugaï immunoassay kit dating from 1992, which showed that this kit contained a mAb specific for free PSA.

24. The question now to be answered is whether or not this information is sufficient to lead to the conclusion that the prior sale of the Chugaï immunoassay kit is a novelty destroying prior use for the subject-matter of claims 1 and 9.

25. There are numerous decisions of the Boards of Appeal dealing with the standards to be met by a prior use for being novelty-destroying. In decision T 194/94 of 17 May 1988, it is stated (point 2,(iii)): "...mere assertions, no matter how believable they may be, are not generally sufficient". In accordance to T 97/94 of 17 July 1997 (point 5.1), there must be an unbroken

chain of evidence linking the prior use to the claimed subject-matter. In decision T 848/94 of 3 June 1997 (point 3.1.2,(iv)), it is found that evidence on materials other than the materials constituting the prior use is not sufficient to destroy novelty even if the two materials are of the same kind.

26. Here, the state of the art is silent as to a possible identity between the mAbs contained in the early and old Chugaï kits. Appellants II presumed on the basis of indirect evidence that it is indeed so but a decisive link is lacking in the chain of evidence leading to this presumption: a declaration by the manufacturers of the immunoassay that the mAb was not one of the kit's reagents which were changed in 1992. Finally, as already mentioned, the data provided by the Opponents was not obtained for the material which, they allege, constitutes the prior use.

27. For these reasons, the Board finds that the written evidence is not adequate to support the conclusion that the 1986 Chugaï immunoassay kit is novelty destroying for the subject-matter of claims 9 and 1.

28. The further argument was also presented that at the filing date of the patent, the skilled person was able to clone the DNA encoding the Chugaï mAb starting from the protein itself and, thus, could reproduce said protein and determine its functionality. No evidence was provided to support the assertion that at that date it would have been a matter of routine to clone mAb encoding DNA and that the knowledge of the resulting recombinant protein structure would necessarily help in finding its properties. The argument, thus, fails.

29. It is concluded that the prior use of an mAb such as claimed has not been demonstrated. Furthermore, there are no documents on file which disclose a mAb according to claim 9 nor any of the other claimed subject-matter. The requirements of Article 54 EPC are fulfilled.

Article 56 EPC

Claims 1, 2, 4 to 7, 9 and 10

30. The closest prior art to the subject-matter of claim 9 is document (1) which teaches that PSA (identified as γ-Sm) is present in blood serum in free form (identified as disengaged γ-Sm) and as a complex with **antitrypsin** (PSA-AT identified as the γ-Sm-α_1-AT complex). It mentions on page 3 the existence of immunoassays for quantifying the total amount of PSA in blood serum using anti-total PSA mAbs and describes on page 7 the use of a mAb against PSA-AT in an alternative immunoassay for the diagnosis of prostate cancer.

31. Starting from the closest prior art, the problem to be solved can be defined as providing further means to be used in an immunoassay for detecting prostate cancer.

32. The solution given is a mAb capable of binding free PSA but not capable of binding PSA complexed with anti**chymo**trypsin.

33. In the Board's judgment, as total PSA and complexed PSA had already been quantified by immunoassay for the purpose of diagnosing prostate cancer, the skilled person would find it obvious also to quantify the alternative form of PSA, ie free PSA, for the same purpose. Starting from document (1) which identifies PSA-AT as the complexed form of PSA, it would follow in

a straightforward manner that the relevant tool for quantifying free PSA would be a mAb which bound to PSA but not to PSA-**AT**. Yet, as just mentioned, the claimed mAb for quantifying free PSA has the properties of binding to PSA but not to PSA-**ACT**.

34. Between the publication date of document (1) and the filing date of the patent in suit, document (24) identified γ-Sm as being identical to PSA (page 168) and as being a serine protease expressing trypsin and chymotrypsin-like specificities (page 171), said chymotrypsin-like specificity being characterized as strictly restricted in terms of substrate recognition (page 172). In document (25) (page 117, left-hand column), PSA is characterized as "being able to hydrolyse synthetic substrates specific for trypsin and chymotrypsin although to a much lesser extent (about 200 times less in case of chymotrypsin)".

35. In the Board's judgment, the skilled person would not infer from these disclosures that PSA must be so alike to chymotrypsin that it will bind to antichymotrypsin to form PSA-ACT. Otherwise stated, there is no suggestion in the art that the complex to be measured in blood serum is PSA-ACT. Consequently, it is not obvious when deciding to use an anti-free PSA mAb, to select that mAb for its property of not binding to PSA-ACT.

36. For these reasons, the Board concludes that the subject-matter of claim 9 and of claims 1, 2, 4 to 7 and 10 which directly or indirectly comprises said mAb is inventive.

Claims 8 and 3

37. The closest prior art to the subject-matter of claim 8 is document (1) which, as above mentioned, identifies total PSA and complexed PSA-AT as the PSA forms worth quantifying for the diagnosis of cancer.

38. Starting from the closest prior art, the problem to be solved can be defined as identifying other forms of PSA which may be relevant for the diagnosis of cancer.

39. The solution provided is PSA complexed with antichymotrypsin.

40. In the prior art on file, no mention is made of the existence of this complex. As mentioned in point 35 above, PSA was then considered as having only very weak and very substrate-specific chymotrypsin-like activity (documents (24) and (25)) which leaves the question entirely open of whether its homology to chymotrypsin could be such that it would bind antichymotrypsin. It is, thus concluded that the skilled person did not find in the prior art any incentive for looking for the PSA-ACT complex when trying to solve the above mentioned problem. Said complex and the immunoassay using a mAb directed against it are inventive.

Claims 11 and 12

41. The closest prior art is document (1) which discloses a diagnostic test for prostate cancer whereby the PSA-AT complex is measured and found to be present at elevated levels in the serum of prostate cancer patients compared to in the serum of healthy males or patients suffering from begnign prostate hyperplasia (page 7, lines 19 to 26).

42. Starting from the said closest prior art, the problem to be solved can be defined as developing a further diagnostic test.

43. The solution provided is a test which makes use of the ratios free PSA/total PSA, PSA-ACT/total PSA or free PSA/PSA-ACT as means for distinguishing between individuals affected by either one of the diseases or healthy.

44. This approach to the diagnosis of cancer is not suggested in any document of the prior art. It requires mAbs recognizing (or unable to recognize) a form of complexed PSA which at the priority date was unidentified (PSA-ACT) (point 40, above) and it has the advantage that the quality of the PSA present in the serum is taken into account as well as the respective quantities of its different forms. For these reasons, the subject-matter of claims 11 and 12 is considered inventive.

45. The requirements of Article 56 EPC are fulfilled by the subject-matter of claims 1 to 12 as well (all states except ES/GR) as claims 1 to 14 (ES/GR).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The matter is remitted to the first instance with the order to maintain the patent on the following basis:

Claims: as submitted on 24 March 2003 during oral proceedings.

Description: as granted.

Drawings: as granted.

The Registrar:

A. Wolinski

The Chairman:

L. Galligani