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D E C I S I O N
of 2 March 1999

Case Number: T 0542/95 - 3.3.4

Application Number: 85115918.6

Publication Number: 0186833

IPC: C07K 3/18

Language of the proceedings: EN

Title of invention:

A monoclonal antibody recognizing a cytotoxin, a hybridoma cell line expressing same and a process for the preparation of a purified cytotoxin

Patentee:

YEDA RESEARCH AND DEVELOPMENT COMPANY LIMITED

Opponent:

- (01) BAYER AG, Leverkusen Konzernverwaltung RP Patente Konzern
(02) PHARMACIA S.p.A.

Headword:

TNF/YEDA

Relevant legal provisions:

EPC Art. 54, 56, 84, 87, 123(2)(3)

Keyword:

- "Priority (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:

G 0001/93, T 0184/84, T 0381/87, T 0597/92, T 0923/92,
T 0512/94

Catchword:

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Boards of Appeal

Chambres de recours

Case Number: T 0542/95 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 2 March 1999

Appellant:
(Opponent 01)

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Decision under appeal: Interlocutory decision of the Opposition Division

of the European Patent Office posted 29 May 1995
concerning maintenance of European patent
No. 0 186 833 in amended form.

Composition of the Board:

Chairman: R. E. Gramaglia
Members: F. L. Davison-Brunel
S. C. Perryman

Summary of facts and submissions

- I. European patent No. 0 186 833 with the title "A monoclonal antibody recognizing a cytotoxin, a hybridoma cell line expressing same and a process for the preparation of a purified cytotoxin" was granted with 22 claims, on the basis of European application No. 85 115 918.6 filed on 13 December 1985 and claiming the priority date of 20 December 1984 from the patent application IL 73883.

Granted claims 1 and 3 read as follows:

"1. A hybridoma cell line expressing a monoclonal antibody which specifically recognizes and binds a cytotoxin having M.W. of $17,000 \pm 500$ D as determined by polyacrylamide SDS gel electrophoresis said hybridoma being formed by fusion of murine myeloma cells with spleen cells from a mouse previously immunized with a pure or impure preparation of a human cytotoxin obtained from stimulated monocytes or monocyte-like cells, which is specifically recognized and bound by the reference monoclonal antibody produced by the hybridoma cell line CNCM I-472 deposited with the Institute Pasteur."

"3. A monoclonal antibody which specifically recognizes and binds a cytotoxin having M.W. of $17,000 \pm 500$ D as determined by polyacrylamide SDS gel electrophoresis."

Claim 2 related to the deposited hybridoma. Claims 4 and 5 related to the monoclonal antibodies (MAbs) produced by the hybridoma cell lines of claims 1 and 2, respectively. Claims 6 to 13 and 21 were directed to

processes for the preparation/isolation of the cytotoxin making use of the MAbs of claim 3 and/or claim 5. Independent claim 14 was directed to a solid phase immunoassay for the screening of hybridoma cultures producing antibodies against the cytotoxin having a M.W. of 17,000 ± 500 D. Dependent claims 15 to 18 specified further features of the assay. Claims 19 and 20 related to a process for preparing MAbs against the cytotoxin making use of the immunoassay of any of the claims 15 to 18. Claim 22 related to the use of the cytotoxin for the preparation of a medicament for treatment of virus infected cells.

- II. Two notices of opposition were filed requesting the revocation of the patent in suit under Article 100(a) EPC (lack of novelty and inventive step) and Article 100(b) EPC (insufficiency of disclosure).

- III. The opposition division maintained the patent in suit in amended form on the basis of claims 1, 2, 4 and 6 to 22 as granted, claim 3 as filed during the oral proceedings before the opposition division, namely with the words "which cytotoxin is recognized and bound by the reference monoclonal antibody produced by the hybridoma cell line CNCM I-472 deposited with the Institute Pasteur" added to the wording of claim 3 as granted and claim 5 as filed with the letter dated 14 March 1994 respectively.

- IV. The appellants (opponents 1) filed an appeal, paid the appeal fee and submitted a written statement setting out the grounds of their appeals as well a legal opinion. Opponents 2 remained party to the proceedings pursuant to Article 107 EPC.

- V. A third party sent observations under Article 115 EPC.
- VI. The respondents (patentees) submitted an answer to the grounds of appeal and to the observations filed by the third party.
- VII. A communication was sent according to Article 11(2) of the Rules of Procedure of the Boards of Appeal, setting out the board's provisional, non-binding opinion.
- VIII. A further exchange of submissions took place amongst the parties. The respondents filed a new main request and Auxiliary Requests 1A/B to 10A/B as well as three legal opinions respectively on what was the "same invention" for the purpose of claiming priority, on whether the issue of Nature of 20/27 December 1984 was available to the public, and on Israeli law on naming inventors.
- IX. Oral proceedings took place on 2 March 1999. As already announced in their letter of 8 January 1999, the other party (opponents 2) did not attend the proceedings. The respondents made the set of claims headed "Auxiliary request 2A" submitted on 2 February 1999 their main request.

Claims 1 to 3 of the new main request read as follows:

- "1. A hybridoma cell line expressing a monoclonal antibody which specifically recognises and binds a cytotoxin having M.W. of $17,000 \pm 500D$ as determined by polyacrylamide SDS gel electrophoresis, said hybridoma being formed by

fusion of murine myeloma cells with spleen cells from a mouse previously immunised with a pure or impure preparation of a human cytotoxin obtained from stimulated monocytes or monocyte-like cells, wherein said cytotoxin

- (i) exhibits a cytotoxic effect on CHI-sensitised SV-80 cells and
- (ii) is obtainable in a state of enhanced purity by adsorption of the cytotoxin from said preparation onto controlled pore glass beads (CPG) and desorption, and
- (iii) is specifically recognized and bound by the reference monoclonal antibody produced by the hybridoma cell line CNCM I-472 deposited with the Institut Pasteur."

"2. A monoclonal antibody which specifically recognises and binds a cytotoxin having M.W. of $17,000 \pm 500D$ as determined by polyacrylamide SDS gel electrophoresis, which cytotoxin

- (i) exhibits a cytotoxic effect on CHI-sensitized SV-80 cells and
- (ii) is obtainable in a state of enhanced purity by adsorption of the cytotoxin from said preparation onto controlled pore glass beads (CPG) and desorption."

"3. A monoclonal antibody according to claim 2 produced by a hybridoma cell line according to

claim 1."

Independent claims 4, 11, 16, 18 and 19 differed from the corresponding granted claims 6, 14, 19, 21 and 22 only in that the cytotoxin was further characterized by features (i) and (ii) as set out in claim 1. All other claims were the same as the corresponding granted claims.

X. The following documents on file were considered by the board:

I-3: WO 83/00930;

I-10: Aggarwal et al., The J. of Biochem. Chem.,
Vol. 259, No. 1, pages 686 to 691, 1984;

I-17: Declaration of Dr. Lawson dated 2 February 1999;

I-18: Declaration of Dr. Cerami dated 20 November
1995;

II-16: Williamson, B. et al., Proc. Natl Acad. Sci.
USA, Vol. 80, pages 5397 to 5401, 1983;

II-17: Matthews, N., Immunology, Vol. 48, pages 321 to
327, 1983;

II-19: Pennica, D. et al., Nature, Vol. 312, pages 724
to 729, 20/27 December 1984;

II-20: Gray, P. et al., Nature, Vol. 312, pages 721 to
724, 20/27 December 1984;

- II-25: Pennica, D. et al., Proc. Natl. Acad. Sci. USA, Vol. 82, pages 6060 to 6064, 1985;
- II-28: Beutler, B. and A. Cerami, The New England J. of Med., Vol. 318, No. 7, pages 379 to 385, 1987;
- II-39: Oppenheim, J. et al., Immun. Today, Vol. 7, No. 2, pages 45 to 56, 1986;
- P-9: Holtmann, H. et al., Immunobiol., vol. 177, pages 7 to 22, 1988;
- III-1: EP-A- 0 183 198;
- III-2: JP-59-246184 (application whose priority is claimed in document III-1) filed on 22 November 1984;
- III-3: JP-58-138383.
- XI. The submissions in writing and during oral proceedings by the appellants were as follows:

Claim 1:

Formal requirements

- Feature (ii): "is obtainable **in a state of enhanced purity**" was not mentioned in the application as filed.

- Feature (iii) could not serve to limit the scope of the claim if, as argued by the respondents, it was only accessory in defining the invention.

Accordingly, the claimed hybridomas were only characterised by features (i) and (ii) rather than by feature (iii) as in granted claim 1. This amounted to an enlargement of the scope of said claim to comprise hybridoma cell lines expressing a MAb specific to other cytokines than CT.

Clarity

- The expressions "cytotoxic effect" and "enhanced purity" in added features (i) and (ii) were unclear in the absence of any quantitative characterisation.

Claim 2:

Formal requirements

- The MAb was solely defined by features (i) and (ii). If feature (iii) was an essential feature of the cytotoxin, then the scope of the claim was changed compared to that of claim 3 as accepted by the opposition division. Furthermore, it was not allowable to delete said feature from the claim accepted by the opposition division. Indeed, a parallel had to be drawn with the situation dealt with in the decision G 1/93 (OJ EPO 1994, 541) whereby the Enlarged Board of Appeal determined that a limiting feature introduced in a claim in the examination procedure and objected to under Article 123(2) EPC at the opposition stage could not be deleted without offending the requirements of Article 123(3) EPC. The same reasoning applied to claims 4, 11, 16, 18 and 19.

Priority rights

- The patent in suit was not entitled to priority rights from the filing date of the priority application on the following grounds:

- the priority application was not directed to the same invention as the patent in suit since it comprised under the denomination CT, the now claimed cytotoxin **and** other cytotoxins produced by peripheral blood mononuclear cells (PBMC). Moreover, the priority application did not contain any claims to hybridoma cell lines.

- the subject-matter of claim 1 was not enabled at the priority date, since the deposited hybridoma which was essential for carrying out the invention had not yet been deposited at that date.

- the description of the priority application did not disclose the subject-matter of claim 1 in an enabling manner. The two protocols for the production of a crude preparation of CTs made use of peripheral blood mononuclear cells (PBMC) as starting material so that as many as 20 cytotoxic substances could be present in the crude preparation.
The first step in purification procedure for CT involved the use of controlled pore glass beads (CPG) to which lymphotoxin (LT) adsorbed as well as interleukin-1 (IL-1) to a lesser extent (documents (I-17) and (I-18)).
The second step involving size fractionation would

not separate the three molecules because their molecular weights were practically identical and, furthermore, all fractions with cytotoxic activity were pooled and used together for immunisation. Even if LT and IL-1 were eliminated to some extent, the remaining LT and IL-1 molecules would stimulate the mouse's immune system, as they were highly immunogenic in mice. Thus, the corresponding MAbs would be produced following immunisation and fusion. MAbs against LT or IL-1 could not be distinguished from MAbs against CT because the cytotoxic assay used to identify CT which involved the killing of CHI-treated SV-80 cells was not specific: LT and IL-1 were equally susceptible of killing these cells.

Novelty

- Document (III-1) was relevant to novelty pursuant to Article 54(3)(4) EPC. Document (III-2), the priority application upon which document (III-1) relied, disclosed the construction of a DNA encoding a physiologically active human TNF-like substance (page 4), the amino acid and corresponding DNA sequence of which were given on pages 5 to 7. This human TNF-like substance corresponded to CT. In Example 2 on page 22, it was shown how recombinant human TNF could be purified "using a column of monoclonal antibody". This MAb had to be directed against human TNF. No procedure was disclosed for its isolation but it would have been standard practice to obtain it. Document (III-2) was enabling with regard to the preparation of a MAb and therefore document

(III-1) was detrimental to the novelty of claim 2. Furthermore, since there existed a very strong probability that the monoclonal antibody of document (III-2) had been made in mice, the subject-matter of claim 1 also lacked novelty.

- Document (I-3) was also detrimental to the novelty of claims 1 and 2 as it described a mediator produced by endotoxin-stimulated macrophages which must have been of human origin. The high molecular weights ascribed to this molecule (page 26) represented aggregated forms. MAbs against the mediator were disclosed on page 6.

Inventive step

- Document (II-17) described the isolation from stimulated human monocytes of a 34,000 MW mediator said to closely resemble rabbit TNF. This mediator had to be hTNF in particular because its synthesis was stimulated by compounds known to activate the production of hTNF and the assay to measure its activity was based on the same principle as the assay used for measuring CT activity in the patent in suit.
- A preparation thereof was used to raise an antiserum in rabbits which, in turn, enabled a 40 fold purification of the mediator by immuno-adsorption and desorption.
- Starting from this prior art, the problem to be solved could be defined as raising MAbs against purified hTNF. The case law of the Boards of

Appeal (for example, T 512/94 of 22 June 1998) was clear that the isolation of a MAb to a purified known substance could not be considered inventive. Claims 1 and 3, thus, lacked inventive step. In the final part of document (II-17), mention was made of the difficulty inherent to isolating a MAb to the mediator substance. This, however, should not be considered as having an impact on the above reasoning since two years had passed between the date of publication of document (II-17) and the priority date of the patent in suit, during which much experience had been gathered in the isolation of MAbs.

- The same reasoning applied if document (III-3) was taken as closest prior art as this document disclosed the isolation of a human TNF of molecular weight 48,000 from the same cell source as used in the patent in suit, its purification on Blue Sepharose as for hTNF, an assay for its activity and the production of an antiserum used for further purification of this hTNF.

- Document (II-19) was published in the 20/27 December 1984 issue of Nature. Nature was normally sent by post to subscribers, being posted by the printers on the day before the cover date. Also, however, according to submitted evidence from the Nature Office Manager, a small number of copies were supplied to the publishers offices and could there be purchased on the afternoon of the day before the cover date. No records of such sales were kept. The evidence also showed that subscribers had received copies by post arriving

on 20 December 1984. The presumption thus was that this issue was available and distributed in the normal way, and thus would have been available to purchasers at the publishers office on 19 December 1984, and that this was accordingly the date when document (II-19) was published. Decision T 381/87 (OJ EPO 1990, 213) was relied on for the proposition that whether anyone had actually purchased a copy from the publishers or read the article was irrelevant.

- Document (II-19), of which the inventor was co-author described the cloning and expression of hTNF having a molecular weight of 17,500 Daltons as well as the corresponding antiserum. The same reasoning on inventive step as presented above with regard to document (II-17) led to the conclusion that the MAbs against hTNF were not inventive.

XII. The respondents' submissions were essentially as follows:

Claim 1:

Formal requirements:

- Feature (ii) ("obtained in a state of enhanced purity") found a basis in claim 1 as filed which recited the steps:

".. b. absorbing the cytotoxin from said preparation onto controlled pore glass means;

c. desorbing the cytotoxin in a state of enhanced purity from said controlled glass means...".

- The combination of features (i) and (ii), or feature (iii) alone both defined exactly the same subject-matter. Accordingly, claim 1 which comprised features (i) and (ii) in addition to feature (iii) was of identical scope to granted claim 1 which only comprised feature (iii).

Clarity

- The skilled person would have no difficulty in understanding what the terms "cytotoxic effect" and "enhanced purity" meant. Conditions in which to measure the cytotoxic effect were given on page 3 of the application. "Enhanced purity" was synonymous with enriched preparation (passage bridging pages 7 and 8).

Claim 2:

Formal requirements

- This claim corresponded to claim 3 as granted with the cytotoxin being additionally defined by features (i) and (ii). This addition amounted neither to adding subject-matter contrary to Article 123(2) EPC, the cytotoxin having been described as having these characteristics as originally filed, nor to an enlargement of the scope of the claim pursuant to Article 123(3) EPC. Claim 2 did not differ by deletion of feature

(iii) from granted claim 3 but only from claim 3 as accepted by the opposition division. The decision of the Enlarged Board of Appeal G 1/93 (see supra) was not relevant to such a situation.

Priority rights

- The priority application was enabling with regard to isolating MAbs against CT. The CT preparation used for immunisation did not contain LT or IL-1 because the purification procedure for CT had been devised in such a way as to eliminate these contaminants and a way had been provided to check whether the MAbs ultimately obtained were specific for CT.

LT was produced in much smaller quantities than CT under the stimulation conditions used in the patent in suit (document (II-28)). Thus, it would only be present at low level at the beginning of the purification. Furthermore, it would be separated from CT by the step involving size fractionation since it had a molecular weight of 20 to 25,000 whereas CT had a molecular weight of 17500.

IL-1 was made in a low amount from monocytes since the amount of mRNA encoding IL-1 in these cells was 0.01% to 5% (upon stimulation) of the total mRNA (document (II-39)). Further, IL-1 did not bind to the CPG column used as the first purification step. It was also not active in the CHI-treated SV-80 assay (document (P-9)).

Finally, the priority application described an assay (killing of CHI-treated SV-80 cells) to check whether the isolated MAbs were specific for

CTs.

Novelty

- Document (III-1) could only be detrimental to the novelty of claims 1 and 2 under Article 54(3)(4) EPC if its priority application (document (III-2)) disclosed in an enabling manner the preparation of a MAb against CT (mature hTNF). On page 22, document (III-2) disclosed a MAb which served to purify recombinant human TNF (rhTNF) synthesized from an expression vector named pHTNF-lacUV5-1. No information was provided on the structure of the hTNF DNA insert cloned in this vector. The insert could code for premature hTNF or even for the hTNF sequence fused to a bacterial polypeptide. In any event, there was no demonstrated link between the sequence of this DNA insert and the DNA encoding mature hTNF. In the same manner, document (III-2) was completely silent with regard to the source of the MAb and to the epitope, it recognized. Thus, it was not clear whether or not document (III-2) disclosed an antibody against the amino acid sequence corresponding to mature hTNF. Nor was document (III-2) enabling with regard to obtaining the MAb: there is only a reference to the "monoclonal antibody" on page 22, lines 2 to 5 and on page 24, lines 21 to 22 whereas document (III-1) provided a ten pages long description disclosing how to isolate this MAb (pages 10 to 20).

- Document I-3 disclosed antibodies to an unidentified mediator of mammalian origin

isolated under various forms, the molecular weights (300,000 Daltons, 70,000 Daltons) of which drastically differed from that of CT (17,500 Daltons). A human mediator was not mentioned, let alone hTNF. The document was thus not relevant to novelty.

Inventive step

- Document (II-17) disclosed an antiserum raised against a crude fraction having cytotoxic activity. This crude fraction was not shown to contain hTNF, because the assay used to measure cytotoxic activity was not specific for hTNF. Furthermore, there was doubt whether hTNF was present in the preparation since according to Figure 2, the cytotoxic effect of monocytes on A549 cells did not decrease in the presence of an antiserum raised against said preparation, whereas hTNF was cytotoxic to A549 cells (document (II-25)). Document (II-17) failed to provide the purification and screening system which would lead to a preparation containing only hTNF and, consequently to a MAb specific thereto.

- Document (III-3) disclosed the isolation from the same U-937 cells as used in the patent in suit of a factor with cytotoxic activity with a molecular weight of 48,000 on SDS PAGE (i.e. as a monomer). There were no reasons why this factor should be hTNF. Nor would the skilled person have thought of it as hTNF for the reason that it bound to Blue Sepharose since this binding property of hTNF was not known at the priority date.

- Document (II-19) was not state of the art before the priority date for the reasons that the evidence of the relevant person in the publishers being that such purchasers were rare and mainly of back copies, no records being required to be kept, thus:
 - there was no evidence that the 20/27 December 1984 issue of Nature which contained document (II-19) was actually available from the publishing house on the 19 December 1984 and,
 - there was no evidence that anybody had bought it from the publishing house on this date.

Accordingly, document (II-19) could not be taken into account for the assessment of inventive step.

XIII. The appellants requested that the decision under appeal be set aside and that the European patent No. 0 186 833 be revoked.

The respondents requested that the decision under appeal be set aside and that the patent be maintained as main request on the basis of the set of claims headed Auxiliary Request 2A submitted on 2 February 1999 or by way of auxiliary requests on the basis of the Auxiliary Requests 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 7A, 7B, 8A, 8B, 9A, 9B, 10A or 10B in that order all submitted on 2 February 1999.

Reasons for the decision

1. The appeal is admissible.

Main request

Article 123(2) EPC

2. The addition of the feature "exhibits a cytotoxic effect on CHI-sensitized SV-80 cells" (claim 1, (i)) has not been objected to under Article 123(2) EPC, and the board agrees that there is a basis for this in the description as originally filed.
3. The feature "obtainable **in a state of enhanced purity**" (claim 1, (ii)) objected to under Article 123(2) EPC has a basis in the wording of claim 1 of the application as filed. The objection, thus, fails.
4. These features now appear in other claims too, but for the same reasons give rise to no objections under Article 123(2) EPC.

Article 123(3) EPC

5. Present claim 1 to a hybridoma cell line expressing a monoclonal antibody differs from the corresponding granted claim 1 by the **additional requirement** that the cytotoxin has the characteristics of features (i) and (ii).
6. Similarly present claim 2 to a monoclonal antibody has been derived from granted claim 3 by the addition of

these same features (i) and (ii) which must be met by the cytotoxin which the claimed antibody specifically recognizes and binds. Also independent claims 4, 11, 16, 18 and 19 of the main request differ from the corresponding granted claims 6, 14, 19, 21 and 22 only in that the cytotoxin has been further characterized by features (i) and (ii) as set out in claim 1. These amendments by recitation of additional requirements must either restrict the scope of the respective claims or leave the scope unchanged: in either case the requirements of Article 123(3) EPC are met.

7. As explained in more detail below in connection with entitlement to priority, the board finds that on the evidence before it the cytotoxin having M.W. of 17,000 \pm 500 D as determined by polyacrylamide SDS gel electrophoresis which is characterized by features (i) and (ii) must be regarded as being unique and the same as the cytotoxin having M.W. of 17,000 \pm 500 D as determined by polyacrylamide SDS gel electrophoresis meeting feature (iii) recognition by the deposited monoclonal antibody. The addition of features (i) and (ii) thus does not alter the scope of claim 1 as granted. On this finding of facts by the board, which is contrary to the position argued by the appellants, feature (iii) could have been deleted without altering the scope of claim 1. However by retaining (iii) in claim 1 as a characteristic of the cytotoxin there could be no argument that claim 1 violates Article 123(3) EPC even if a different view of the facts to that of the board were taken.

8. Claim 2 is a more restricted version of claim 3 as granted. The argument by the appellants that claim 2

should not be allowable because it differs from claim 3 **as accepted by the opposition division** by deletion of feature (iii) and, thus, provides a wider scope of protection than this latter claim is not an argument under Article 123(3) EPC, which requires only a comparison with the scope of the claims as granted. The argument will be considered below in connection with amendments a respondent may make in response to the arguments of an appellant.

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

Clarity (Article 84 EPC)

10. The term "cytotoxic effect" is defined in the patent in suit on page 3, last paragraph. A way is described how to test this effect.
11. The term "enhanced purity" appeared in the application as filed in claim 1 in the passage "... absorbing the cytotoxin from said preparation onto controlled pore glass means; desorbing the cytotoxin in a state of enhanced purity from said controlled glass means...". Thus "enhanced purity" will be understood as enrichment using controlled pore glass means as described on pages 7 and 8. This is meaningful even without any quantification.
12. The meaning of the above terms, objected to as unclear, is considered by the board as clear to the skilled person, and the claims are considered to meet the requirements of Article 84 EPC.

Right to priority (Articles 87 and 88 EPC)

13. According to Article 87(1) EPC, a person who has duly filed in or for any state party to the Paris Convention for the Protection of Industrial Property an application for a patent shall enjoy for the purpose of filing a European patent application a right of priority **in respect of the same invention**. In the case law of the EPO, this has been interpreted to mean that the subject-matter of the claims of the European application must be clearly identifiable in the previous application **taken as a whole** (T 184/84 of 4 April 1986, T 597/92, OJ EPO 1996, 135).

14. For an understanding of the issues raised in this case, it is necessary to explain the following background. The monoclonal antibody produced by the hybridoma cell line CNCM I-472 deposited with the Institute Pasteur referred to in claim 1 was deposited 16 July 1985. The priority claimed was an earlier application in Israel on December 1984. The opposition division held that "...the opposed patent should be accorded priority right for the deposited cell line CNMC-472c because the Patentee complied with the requirements he was aware of at the date of filing both the priority application and of the European patent application." This view has no basis in the European Convention, whether in Rule 28 EPC or elsewhere, and not even the respondents sought to support it before the board. It is therefore not necessary to set out the detailed reasoning which led to this view by the Opposition Division, which was influenced by there then being no provision for referring to deposited materials in applications in Israel, or a refutation thereof.

15. The board follows established case law in the view that

for an invention to be entitled to priority, the priority application must have been enabling. For the priority to be claimed, the definition of the invention, as given in the claims should not depend uniquely on technical information clearly not available from the priority document, such as here the deposited hybridoma cell. (Compare decision T 923/92, human t-PA/GENENTECH (OJ EPO 1996, 564) point 7 where Figure 5 referred to in a claim showed a different sequence to that shown in Figure 5 of the priority document).

16. In the present case, the following statements are found in the priority application IL 73 883 upon which the patent in suit is based under the heading "Summary of the invention": "There is provided a purified cytotoxic protein referred to as cytotoxin (CT)...The purified CT has a molecular weight of about 17000 daltons...There is provided a technique for establishing lines of lymphocytes producing such (*monoclonal*) anti CT antibodies. Such lines are advantageously established by screening of plurality of hybridomas derived from spleenocytes of such immunized mice and the monoclonal antibodies produced by such hybridoma cell lines are used for isolating CT in essentially homogeneous purified form." (word in brackets added). Furthermore, claims 1 and 2 of the priority application respectively relate to a cytotoxic protein...of 17,500 daltons ... which is specifically recognized by the MAb CT-1" and to "A process for preparing the CT, comprising ... applying the lymphotoxins on an immunoadsorbent constructed from **a** monoclonal antibody against CT" (emphasis added). Thus, the priority application comprises CT, and any MAb (including CT-1 the MAb

produced by the deposited hybridoma) against CT, and the hybridoma secreting it, and provides a basis for the hybridoma cell line expressing a monoclonal antibody against the cytotoxin of stated molecular weight and meeting features (i) and (ii).

17. It was further argued that the priority application and the patent in suit did not disclose the same invention because the protocol described in the former led to the isolation of MAbs specific for LT or IL-1 in addition to MAbs specific for CT, which were not distinguishable from each other, whereas the protocol in the patent in suit ensured that only MAbs against CT would be obtained.

18. The protocol in the priority application comprises the following steps:
 - (a) induction of cytotoxin production by appropriate stimulation of PBMC,

 - (b) adsorption on CPG,

 - (c) size/charge fractionation by polyacrylamide gel electrophoresis or on Ultrogel ACA44,

 - (d) use of the cytotoxin preparation so obtained to isolate MAbs against CT,

 - (e) testing the ability of the cytotoxin recognized by the MAbs for their ability to kill CHI-sensitized SV-80 cells.

In step (a), PBMC synthesize LT and IL-1 as well as CT.

- Thus, this protocol will only lead to the exclusive isolation of MAbs against CT if steps (b) to (e) are instrumental in eliminating LT and IL-1 or the corresponding antibodies. This, in turn, depends on the intrinsic properties of LT and IL-1.
19. At the priority date, it was known that LT had a molecular weight of about 20,000 Daltons (document(I-10)) compared to a molecular weight of 17,500 Daltons for CT. It was also known that IL-1 only bound very poorly to CPG (document (P-9), page 9). Furthermore, post-published document (II-28) (first column) discloses that LT is made in low amounts under the stimulating conditions used in step (a). In post-published document (II-39), page 42, right hand column), the percentage of IL-1 mRNA in stimulated monocytes is evaluated as 0.1% of total mRNA which could indicate that IL-1 is made in relatively low amounts.
 20. Accordingly, it would seem that the small quantities of LT and IL-1 present in the CT immunizing preparation would be discarded from it at step (c) and step (b) respectively.
 21. It cannot be ruled out, however, that some LT or IL-1 remained in the CT preparation, which, by virtue of their immunogenicity, would result in the production of MAbs specific to them. MAbs against IL-1 will be discarded after the test assay (step (e)), as IL-1 does not kill CHI-sensitized SV-80 cells (document (P-9), Fig. 7). At the priority date, MAbs against LT could be disposed of as a matter of routine by virtue of their ability to bind LT which had already been purified to

homogeneity (document (I-10)).

22. Accordingly, at the priority date, the skilled person whilst not knowing all of the properties of LT and IL-1 would have been able to isolate MAbs against CT in the absence of any deposited hybridoma, on the basis of the instructions given in the priority application and then available general knowledge.
23. In the application as filed and the present claims the reference is to stimulated monocytes or monocyte like cells, whereas in the priority application the reference is to CT being induced in human peripheral blood mononuclear cells (PBMC). Monocytes are known to be the major constituent of PBMC, so that this difference cannot be regarded as significant, or as making the claims directed to something other than the invention disclosed in the priority document.
24. On the facts before it, the Board thus finds that the cytotoxin having M.W. of $17,000 \pm 500$ D as determined by polyacrylamide SDS gel electrophoresis which is characterized by features (i) and (ii) must be regarded as being unique and the same as the cytotoxin having M.W. of $17,000 \pm 500$ D as determined by polyacrylamide SDS gel electrophoresis meeting feature (iii) recognition by the deposited monoclonal antibody. Thus feature (iii) in claim 1 can now be regarded as mere surplus definition, not having any effect on the priority of claim 1.
25. Claim 2 can also be regarded as entitled to the priority, as features (i) and (ii) have a basis in the priority application as shown above.

26. The board thus finds that the priority application discloses in an enabling manner the same invention as the patent in suit and that the claims are entitled to the filing date of the priority application.

Novelty

27. Document (III-1) discloses on page 101 a MAb specific for mature hTNF (CT in the patent in suit). It is relevant to novelty under Article 54(3)(4) EPC providing the priority application, document(III-2), discloses said MAb.
28. Document (III-2) provides the amino-acid sequence of hTNF and of the DNA sequence encoding it as well as a method for purifying rhTNF. A general protocol to obtain a rhTNF clone is described on pages 7 to 10. From page 10 to page 12, it is disclosed that rhTNF may be expressed in a variety of forms: mature, with the presequence, as fused protein or recombined with natural or artificially synthesised DNA. There is no mention in this general part of the description of isolating MAbs against rhTNF.
29. The method for purifying rhTNF is exemplified on page 20 starting with the expression vector phTNF-lacUV5-1, the structure of which is only described by reference to the Japanese patent application 59-115497. As the latter application was not published until the 23 December 1985, the skilled person had no way to know, which of the rhTNF forms mentioned in the general part of the description was expressed from the recombinant plasmid. Starting from page 22, the

purification method is compared with another method which involves "a column of MAb". There is no information at all provided on the MAb. It should, of course, recognise an epitope present on the hTNF-like substance made from phTNF-lacUV5-1. This, however, leaves open the possibility that the MAb is directed to an epitope which is not present on mature hTNF. The situation is, thus, such that MAbs to mature hTNF are not directly and unambiguously disclosed in document (III-2). Accordingly, it is concluded that document (III-1) as regards the MAb directed against mature hTNF is not entitled to priority from document (III-2) and, therefore, it is not detrimental to the novelty of claims 1 and 2 of the patent in suit.

30. Document (I-3) was also cited in the context of assessing novelty. It describes a mediator composition obtained from mammals with cytotoxic activity and the corresponding MAbs (page 6). As an example for such mediator, a protein composition is obtained from mice which contains two proteins of 300,000 and 70,000 Daltons, respectively. There is no evidence that these proteins which are of murine origin would have their equivalent in humans, let alone would be hTNF. Therefore, document (I-3) is not relevant to novelty.
31. No other documents on file disclose hybridomas or MAbs to a cytotoxin as defined in claims 1 or 2. Novelty is acknowledged.

Inventive step

The status of document (II-19)

32. Document (II-19) which discloses the cloning and expression of hTNF in E. coli was published in the 20/27 December 1984 issue of Nature. The appellants argued that the magazine was already available to the public on 19 December 1984 (i.e. on the day before the priority date), on the basis of the information from the Operations Editor of Nature magazine that copies of Nature could be obtained from the Nature office on the afternoon of the day before the cover date. The board finds that the evidence is not sufficient to establish even that the particular 20/27 December issue of Nature was available to would-be purchasers at the publishers office on 19 December 1984. No records were kept, and the evidence that libraries had received copies from the printers by 20 December 1984 does not go to show that the publisher's office actually had copies available on 19 December. The appellants' case on pre-publication of this document thus fails in limine, and it is not necessary to decide whether for publication it would be necessary also to show that one member of the public actually so obtained a copy on 19 December 1984, which is the view the board would rather incline to, because availability at a publisher's or printer's appears to fall in a rather different category to availability at a library (as in decision T 381/87, OJ EPO 1990, 213) where the document can already be considered in the public domain.
33. It is decided that document (II-19) published in the 20/27 December 1984 issue of Nature cannot be taken into account against claims entitled to the priority date.

Other documents:

34. At the priority date, a number of documents could be found in the art which described the isolation of human proteins which were identified as hTNF. Thus, document (II-16) discloses a 70,000 MW hTNF on the basis of its reactivity in the Met-sarcoma assay. The identity of this protein was later queried as its origin made it more likely to be a lymphotoxin (see document (II-20), page 724). Furthermore, document (III-3) discloses isolating from the same cell line as used in the patent in suit, a protein capable of inhibiting the growth of L929 cells. SDS PAGE electrophoresis shows the molecular of this protein (in monomeric form) to be 48,000 Daltons (page 10, example 6). There is no reason to believe it would be the $17,500 \pm 500$ D hTNF. Finally, document (II-17) discloses a protein of molecular weight 34,000 Daltons and the raising of the corresponding antiserum. Figure 2 shows that this antiserum is not able to prevent the cytotoxic effect of a monocyte preparation on A549 cells. This indicates that the 34,000 Daltons cytotoxin does not contribute to the growth inhibition of these cells by monocytes. Doubt, thus, exists whether the 34,000 Daltons protein is hTNF, as hTNF is cytotoxic to A549 cells (document (II-25), page 6063).
35. The prior art does not disclose the purification of the same hTNF (CT) as in the patent in suit. Nor does it give any hint that a different protein from the one it describes would be the "real" hTNF, nor suggest ways of isolating a desirable protein, the making of which would lead in any obvious manner to the subject of the present patent.

36. The claims other than claims 1 and 2, have not been subject to attacks separate from those which the board finds have failed against claims 1 and 2.

37. Accordingly, the presence of inventive step can be acknowledged for the claims. The requirements of Article 56 EPC are fulfilled.

Admissibility of amended claims on appeal

38. As stated above, the claims considered comply with the requirements of the European Patent Convention. The amendments made to the claims during appeal proceedings are considered by the board as necessary and appropriate to meet objections raised by the appellants.

39. The appellants have in particular objected that claim 2, derived from claim 3 as granted, does not contain the limitation "which cytotoxin is recognized and bound by the reference monoclonal antibody produced by the hybridoma cell line CNCM I-472 deposited with the Institute Pasteur" introduced into granted claim 3 before the opposition division. However this claim was objected to on appeal. When it became clear that the board's view on entitlement to priority for the deposited cell line differed from that of the opposition division, it became legitimate for the respondents to attempt to amend claim 3 as granted in a new way to meet the objections raised.

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 set of claims headed Auxiliary Request 2A submitted on February 2, 1999, and a description to be adapted.

The Registrar:

The Chairman:

S. Hue

R. Gramaglia