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D E C I S I O N
of 30 November 1994

Case Number: T 0128/92 - 3.3.4

Application Number: 83101035.0

Publication Number: 0091539

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:
Gene coding for interleukin-2 polypeptide, recombinant DNA carrying said gene, cell lines possessing the recombinant DNA, and method for producing interleukin-2 using said cells

Patentee:
AJINOMOTO CO., et al

Opponent:
HOECHST AKTIENGESELLSCHAFT
Cetus Oncology Corporation

Headword:
-

Relevant legal provisions:
EPC Art. 123(2) and (3), 83, 84, 56

Keyword:
"Availability to the public of a complex biochemical product (no); and of cell line (yes)"
"Inventive step (yes)"

Decisions cited:
T 0301/87, T 0226/85, T 0409/91

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0128/92 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 30 November 1994

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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 26 November 1991 concerning maintenance of European patent No. 0 091 539 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: R. E. Gramaglia
S. C. Perryman

Summary of Facts and Submissions

I. The appeal is against the Interlocutory Decision of the Opposition Division of the European Patent Office dated 19 September 1991, posted on 26 November 1991, concerning maintenance of European patent 0 091 539 in amended form. This patent claims priorities of 31.03.82 (P1), of 18.05.82 (P2), of 15.12.82 (P3), of 24.12.82 (P4), of 27.12.82 (P5) and of 29.12.82 (P6).

The decision was based on claims 1 to 25 amended during the opposition proceedings.

Claim 1 (showing in bold the amendment) read as follows:

"A DNA ~~sequence~~ **fragment** comprising a DNA sequence coding for a polypeptide having human interleukin-2 activity, said polypeptide being selected from the following group:

(a) a polypeptide having the following amino acid sequence:

Met-Tyr-Arg-Met-Gln-Leu-Leu-Ser-Cys-Ile-Ala-Leu-Ser-Leu

Ala-Leu-Val-Thr-Asn-Ser-Ala-Pro-Thr-Ser-Ser-Ser-Thr-Lys-

Lys-Thr-Gln-Leu-Gln-Leu-Glu-His-Leu-Leu-Leu-Asp-Leu-Gln-

Met-Ile-Leu-Asn-Gly-Ile-Asn-Asn-Tyr-Lys-Asn-Pro-Lys-Leu-

Thr-Arg-Met-Leu-Thr-Phe-Lys-Phe-Tyr-Met-Pro-Lys-Lys-Ala-

Thr-Glu-Leu-Lys-His-Leu-Gln-Cys-Leu-Glu-Glu-Glu-Leu-Lys-

Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu-Ala-Gln-Ser-Lys-Asn-Phe-

His-Leu-Arg-Pro-Arg-Asp-Leu-Ile-Ser-Asn-Ile-Asn-Val-Ile-

Val-Leu-Glu-Leu-Lys-Gly-Ser-Glu-Thr-Thr-Phe-Met-Cys-Glu-

Tyr-Ala-Asp-Glu-Thr-Ala-Thr-Ile-Val-Glu-Phe-Leu-Asn-Arg-

Trp-Ile-Thr-Phe-Cys-Gln-Ser-Ile-Ile-Ser-Thr-Leu-Thr

(b) a polypetide [*sic*] which in respect to (a) is deficient in one or more aminoacids;

(c) a polypeptide in which in respect to (a) one or more aminoacids are replaced

(d) a fusion polypeptide comprising a polypeptide according to (a), (b) or (c) in which the additively connected aminoacids do not interfere with the biological interleukin-2 activity or may easily be eliminated,

(e) a polypeptide which is an allelic derivative of a polypeptide according to (a).

The Opposition Division considered that the objections under Articles 100(a), (b) and (c) EPC raised by the Opponents had not been made out, and came to the conclusion that the patent as amended during the proceedings met the requirements of the EPC.

II. An appeal was filed by the Appellant (Opponent II). The following documents, cited by the Parties are referred to in the present decision:

(2) Gillis *et al.*, J. Exp. Med., Vol. 152, pages 1709 to 1719 (1980)

(19) Gillis *et al.*, Curr. Top. Microbiol. Immunol., Vol. 100, pages 211 to 219 (1982)(reported during a workshop in Basle on 27 to 29 January 1982)

(23) Hinuma *et al.*, Biochem. Biophys. Res. Commun., Vol. 109(2), pages 363 to 369 (30.11.82)

(27) EP-A-0 032 134

(28) Taniguchi *et al.*, Proc. Japan. Acad., Vol. 55 Ser B, pages 464 to 469 (1979)

(29) Taniguchi *et al.*, PNAS USA, Vol. 77, pages 4003 to 4006 (1980)

(30) Taniguchi *et al.*, Nature, Vol. 302, pages 305 to 310 (1983)

(34) Harpold *et al.*, Nucl. Acids Res., Vol. 5(6), pages 2039 to 2053 (1978)

(35) Robb *et al.*, Fed. Proc., Vol. 4, 480, Abs. No. 1226 (1982)

(36) Robb *et al.*, PNAS USA, Vol. 81, pages 6486 to 6490 (1984)

(37) Livak *et al.*, J.Cell Biol., Vol. 97, 420a, Abs. No. 1586 (1983)

(38) Clark *et al.*, PNAS USA, Vol. 81, pages 2543 to 2547 (1984)

(39) Cheroutre *et al.*, Gene Expression, pages 437 to 444 (1983)

(42) Robb *et al.*, PNAS USA Vol. 80, pages 5990 to 5994 (1983)

(43) EP-A-0 118 977

(45) Stadler *et al.*, J. of Immunol., Vol. 128, pages 1620 to 1624 (1982)

(46) Gillis *et al.*, J. of Immunol., Vol. 126, pages 1978 to 1984 (1981)

(47) Caplan *et al.*, J. of Immunol., Vol. 126, pages 1351 to 1354 (1981)

(48) Declaration of Prof. K. Smith

(49) Copeland, Thymic Hormones and Lymphokines, Ed Goldstein, Plenum Pub. Corp., pages 181 to 189 (1984)

(51) Smith *et al.*, J. Immunol., Vol 131, pages 1808 to 1815 (1983)

(52) Ju *et al.*, J. Biol. Chem., Vol 262, pages 5723 to 5731 (1987)

III. Oral proceedings were held on 30 November 1994, during which the Respondent filed five auxiliary requests. The other Party (Opponent 1) was not represented at the oral proceedings, and had made no submissions in the appeal proceedings.

IV. The Appellant essentially brought forward the following arguments:

(a) Article 123(2) EPC (Amendments)

The substitution of the term "sequence" by the term "fragment" in the opposition proceedings added subject-matter to the content of the application as originally filed insofar as the term "fragment" was interpreted as "a part taken outside the whole" and "genetic material outside its natural context". Thus this term should have been interpreted in the light of the patent disclosure as meaning "a portion of a larger material produced by cleavage or nuclease activity" rather than in the broader meaning attributed by the opposition division.

(b) Articles 83 and 84 EPC (Sufficiency of disclosure and support of the claims).

The claims embraced variants which could not be worked because the patent disclosure did not offer any guidance in establishing in advance those DNA sequences which encoded active interleukin-2 (hereafter: IL-2), having regard to the possibility that mutations and deletions could be fatal to IL-2 activity. A later publication (52), Ju *et al.* indeed showed that replacement of certain Cys residues by Ser, Ala or Leu destroyed the activity. The skilled person was thus faced with the undue burden of testing each of the 20^{133} IL-2 analogs in order to find those with IL-2 activity. In decisions T 226/85 (OJ EPO 1988, 336) and T 409/91 (OJ EPO 1994, 653), however, the Board held that to fulfil the requirements of Article 83 EPC the specification must contain sufficient information to allow a skilled person to carry out the invention within the whole area claimed.

The functional limitation in claim 1 read back to the wording "DNA sequence" " rather than to "DNA fragment" so some of the claimed DNA fragments were non-functional because the claim did not exclude overall fragments which themselves were utterly inactive and incapable of expressing or providing a polypeptide with the required activity.

It was also doubtful whether a main claim covering more than 20^{133} embodiments satisfied the requirements of Article 84 EPC that a claim should be supported by the description, which Article 84 EPC was relevant in view of the amendment effected at the opposition stage.

(c) Article 54 EPC (Novelty)

The novelty issue was raised only in conjunction with the objection under Article 123(2) EPC (see Section IIIa *supra*) that insofar as the term "fragment" was given the broader meaning attributed by the opposition division of part of the whole human genome taken outside its natural context. Then the mRNA disclosed by Hinuma document (23) once reverse-transcribed into DNA would fall under the scope of the claims since it would satisfy the above definition.

(d) Article 56 EPC (Inventive step)

Two lines of argument were submitted:

Firstly the Respondent's approach for picking up the DNA coding for IL-2 was not inventive: anybody could have done so with a reasonable expectation of success by starting from the enriched IL-2 mRNA sources disclosed by documents (23) or (19) and applying the techniques disclosed by documents (27), (28) or (29). The

combination of document (23) with document (27) was the most relied on. An Appellant's expert, Prof. K. Smith confirmed in his declaration (document (48)) and at the oral proceedings that the tools, namely the Jurkat cell line producing 100 times more IL-2 message than normal T-cells, a sensitive assay for IL-2 and the reagents were available for cloning the IL-2 gene in a routine manner eg., by use of a technique paralleling the earlier procedure used for the interferon-β gene disclosed by documents (28) and (29).

It was disputed that the selection of appropriate hybridization conditions was most critical for arriving at the invention. A tabulated comparison of the techniques (see pages 35 to 40 of the appeal file) showed that the procedures followed in the patent in suit were substantially the same as in document (27). The fact that the hybridization conditions used in the patent and in the publication of the invention (document (30)) diverged and that other teams (see documents (39) and (43)) obtained the DNA coding for IL-2 following other routes, did not support the criticality of the hybridization conditions. Therefore the technique according to the patent in suit was not essential in order to arrive at human IL-2 cDNA. As to the preparation of a high IL-2 producer Jurkat 111 cell as the first critical step for arriving at the present invention, it was argued that the IL-2 mRNA abundancy in the starting Jurkat 111 cells was comparable to that of interferon-α mRNA in the induced leukocytes of document (27) and that the Jurkat 111 cell actually used by Dr Taniguchi (document (30)) produced less than 5 times rather than 40 times that reported for the parent cell line. Moreover, mRNA abundancy was not important

since, in order to screen for IFN-β with a mRNA abundancy as low as 0.1% the Taniguchi team had no hesitation in adopting the Harpold technique (see document (34)) good for 50-60% relevant mRNA abundancy.

Secondly sequenceable IL-2 was available before the recognized priority date of the patent. Thus not only could there be no invention in the provision of a sequence, but there was also another way to make available the DNA coding for IL-2 by sequencing the protein directly, then design oligonucleotides probes to screen a cDNA library. Document (35) showed that a group of researchers had obtained IL-2 amino acid sequence information before the priority date of the patent in suit. The post published documents (42) and (36) confirmed this. As shown by the post published document (37), this amino acid sequence information was used to design oligonucleotide probes for cloning the gene coding for IL-2. Further, document (38) demonstrated that another group had sufficient amino acid sequence knowledge to prepare probes for screening an IL-2 cDNA library. Prof. K. Smith stated in his declaration (document (48)), that the tools and reagents for arriving at sequenceable IL-2 and determining the amino acid sequence thereof and/or a DNA sequence were available before the priority date because only routine methods were necessary for doing this. He himself had no difficulty in selecting the monoclonal antibodies. He had used these to obtain partially purified IL-2, which he handed over to Dr Oroszlan who purified this on HPLC, and returned the separated fractions back to Prof. K. Smith. One of these fractions was identified as showing particularly high IL-2 activity and was returned to Dr Oroszlan who carried out the amino acid sequencing on it between September 1982 and March 1983, as confirmed by document (49). Prof. K. Smith indicated that the

substances had been handed over to Dr Oroszlan with no obligation of confidentiality, and the latter at some date did supply some to another researcher. This made this IL-2 publicly available.

Prior art monoclonal antibodies against IL-2, such as those disclosed by documents (45) and (46) were also suited to be used in immunoaffinity purification of IL-2 to a sequenceable purity.

V. The Respondent essentially counterargued as follows on novelty and inventive step:

The citability of document (23) and the availability to the public pursuant to Rule 28 EPC of the Jurkat FHCRC cell of document (19) were contested.

As to cloning the gene coding for IL-2 the first difficulty that had to be overcome was the low frequency of the mRNA coding for IL-2. The important step for arriving at the present invention was the provision of a mutated Jurkat 111 cell which had a higher productivity of IL-2 than the parent Jurkat FHCRC cell. It was only due to this improved IL-2-producing cell that the selection of clones could be made. Thus when starting from IL-2 mRNA produced by peripheral blood leukocytes (PBL's), spleen cells or normal Jurkat cells it would have needed more than routine work to detect the positives. Documents (27),(28) and (29) were concerned with isolating DNA sequences coding for human interferon-α or human interferon-β from sources having mRNA abundance of ca. 0.1% while in the present case mRNA abundance was 1/20,000.

The second difficulty was that there was no general method available for detecting and isolating cDNA containing clones derived from mRNA present in less than 1 part in 200.

As to the present process for isolating the DNA coding for IL-2, it differed in essential features from the known processes and there was no possibility of the skilled person knowing prior to the priority date of the patent which method and which process step would lead to cloning the gene coding for IL-2 and what were the precise conditions which would result in a successful cloning procedure. The technique disclosed by the patent in suit involved a filter hybridization step which was critical for the recovery of extremely low amounts of IL-2 mRNA.

Moreover, the Appellant had not made credible that the combination of any of documents (23) and (19) with one of documents (27), (28) or (29) would allow a full length cDNA coding for IL-2 to be picked up: another team (see (documents (39) and (43)) used a different route to that of the patent and did not obtain a full length cDNA coding for IL-2.

No sequenceable IL-2 and thus no partial or complete IL-2 amino acid sequence which could have been used as a means for providing the DNA sequence was available before the recognized priority date of the present patent. Gillis in his declaration before the USPTO (see pages 155 to 160 of the opposition file) said that all the prior art IL-2 preparations believed to be homogeneous were in fact highly impure. As to document (35) it merely stated that amino acid sequence of immunopurified IL-2 would have been a task for the future and this was confirmed by the fact that the later

document (42) reported only a partial sequence as late as October 1983. None of the prepublished documents cited by the Appellant disclosed any IL-2 amino acid sequence nor demonstrated that this had been made available to the public before the priority date of the patent. It may have been true that Livak *et al.* (document (37)) were in possession of a partial amino acid sequence of IL-2, but this was not a prepublished sequence.

With a view to the monoclonal antibodies being used for obtaining sequenceable IL-2 by immunoaffinity chromatography, the prior art ones did not work and moreover neither they nor the ones made by Prof. K. Smith were available to the public. From Prof. K. Smith's declaration it appeared that an ELISA for selecting the positive hybridomas, an RP-HPLC step and a high IL-2-producer subclone 6.8 of the Jurkat cell line were fundamental for arriving at sequenceable IL-2. Yet none of these expedients were taught nor was the subclone available to the skilled person before the priority date of the contested patent.

VI. The Appellant requested that the decision under appeal be set aside and that the European patent No. 0 091 539 be revoked.

The Respondent requested as main request that the appeal be dismissed and that the patent be maintained, and as auxiliary requests that the decision under appeal be set aside and that the patent be maintained on the basis of the first, second, third, fourth or fifth auxiliary request respectively submitted during the oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

Amendments - Articles 123 (2) and (3) EPC

2. In the context of the description and claims as a whole, the Board considers that the phrase in Claim 1 "A DNA fragment comprising a DNA sequence for a polypeptide having human interleukin-2 activity" means both that the DNA sequence codes for a polypeptide having human IL-2 activity, and that it is present in a physical embodiment that is an isolated portion of a DNA chain in a form that is processable to produce such a polypeptide, a typical example being a plasmid including the sequence, with suitable start and stop sequences. This is what the skilled reader would have understood from the originally filed description, and the wording in this case raises no problems under Article 123(2) EPC. In this case, the queries raised by the Appellant on the language used in the claim, seem to the Board not to relate to matters which would cause the practical skilled person in the art to have doubts about the scope of the claim.

3. In the Appellant's view, the reverse-transcribed IL-2 mRNA of document (23) (see Point 16 *infra*), would fall within the claim. The Board does not agree because firstly a mixture of many different mRNAs is used, all of which would have been reverse transcribed so that a fragment coding for IL-2 was not **isolated**, and secondly, at least the transcription from mRNA to cDNA was necessary, which means that the document does not directly disclose a "DNA fragment".

4. Nor does the Board interpret the claims as covering lengthy DNA chains which cannot be made to code for IL-2 activity without considerable further processing to produce a suitable fragment which does so code. Raw DNA material comprising somewhere the gene coding for IL-2 can by no means be equated to the isolated gene coding for IL-2 (see Point 16 *infra*).

5. The substitution of "fragment" for the first mention of "sequence" in Claim 1 as granted cannot be considered to be a broadening of its scope. In the context of the meaning the Board attributes to Claim 1 as granted having regard to the whole description and claims, the scope of the claim has not been changed by this substitution. The requirement of Article 123(3) EPC is thus met.

Article 84 EPC - clarity and support

6. On the interpretation of the claim given above, the Board finds it sufficiently clear and supported by the description for Article 84 EPC to be complied with. The large number of embodiments that it may cover does not in itself mean that an objection under Article 84 EPC arises.

Article 83 EPC - Sufficiency of disclosure

7. The Respondent submitted and the Appellant conceded during the oral proceedings before the Board, that the skilled person would at the priority date have been in a position with routine methods to make fragments with sequences coding for polypeptide sequences differing from the one listed in the first part of the claim in the manner stated in one of features (a), (b), (c), (d) or (e), and would have been able to test these for IL-2

activity, and select those which had such activity. This would lead to an indefinite number of fragments, other than the one specifically described, which could be used to make a polypeptide having IL-2 activity. There is no evidence that the skilled person could not find, without undue burden, many embodiments. The Board cannot, in these circumstances, see any grounds for saying that the invention is not disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

8. By providing the information in the patent, the Respondent has enabled the skilled person to make not only the polypeptide with the sequence recited in the claim, but to find many others that will have IL-2 activity. The protection granted should correspond to this disclosure. The fact that others, starting from the information in the patent might by research identify a DNA fragment encoding for a polypeptide with improved IL-2 activity infringing the present patent, is not a ground for refusing Claim 1. Such an improvement may deserve a patent of its own, but the possibility of such an improvement is no ground for limiting the present claim. Thus, even if the Appellant is correct in stating that the claim potentially covers more than 20^{133} embodiments, this is irrelevant.

9. In spite of the considerable amount of theoretically possible variation of the amino acid sequence in the present case, there is still likely to be a structural similarity between all the variants covered by the present claims. This view is confirmed when considering document (52), page 5727, Figure 3, disclosing the preparation of 50 IL-2 variants, wherein it can be seen that all the variants share a substantial number of amino acids, and to assume that a protein chemist would

expect that IL-2-analogs devoid of amino acid homology to IL-2 in the substantial part of the protein being the active centre (eg., in the instance all the 133 amino acids of IL-2 were replaced by Gly) would lead to an active protein, appears to be not reasonable. The situation here, where the claimed products are limited to those having a certain physical relation to one another, and a testable narrowly defined activity, must be distinguished from a situations where either the structure or the activity is not defined in a disputed claim, so that it can be said that some substances which it would be desirable to make fall within the claim, but the description gives no guidance as to how they can be made (cf. decision T 301/87 (OJ EPO 1990, 335)).

10. The finding of working variants is feasible in the light of the teachings of document (52) (see Point 9 *supra*). The Appellant's argument that the invention cannot be reproduced because the claims embrace variants which cannot be worked since the patent disclosure does not offer any guidance in establishing in advance those DNA sequences which encode active IL-2, having regard to the possibility that mutations, deletions and also the presence of non-coding regions could be fatal to IL-2 activity, does not hold, as such variants are excluded by the terms of the claim.

Prior art available to the public

11. It is accepted by all parties and the Board agrees that claim 1 is entitled to priority date of 24 December 1982 of the fourth priority document (P4). Accordingly document (23) published 30 November 1982 is part of the prior art.

12. Prof. K. Smith stated in his declaration and during the oral proceedings that he had given samples of IL-2 to Dr Oroszlan, and that this was done without any obligation of confidentiality being imposed (see Section IVc *supra*), which amounted to making this IL-2 available to the public. The documentary evidence submitted was the postpublished document (51), the report on the research that Prof. K. Smith and his colleagues were conducting. There was no documentary evidence predating the priority date. In view of the time that has elapsed it is not surprising that no further precise evidence was available, but this makes it impossible for the Board to be sure of what was handed over before the priority date. Prof. K. Smith cannot be considered as the notional skilled man in the art. He was a distinguished professor who had for many years been at the forefront of research trying to identify IL-2. He and his laboratory team specialized in monoclonal antibodies, but had, as he frankly admitted, little knowledge of DNA cloning. They also relied on other laboratories to carry out for them certain purification and sequencing techniques. The primary purpose of handing substance to Dr Oroszlan seems to have been to sequence it. On this evidence the Board cannot find that IL-2 as such was made available to the public. The fact that Prof. K. Smith was not able to sequence the IL-2 material himself and thus had to send it to another expert, speaks against it being public knowledge to sequence the IL-2 material. The public had no access to the sequence which was necessary for the cloning which was finished by Dr Oroszlan only on March 1983, ie., after the valid priority date. The fact that the Appellants themselves

only became aware of this supply to Dr Oroszlan shortly before the oral proceedings, as a result of contacts with Prof. K. Smith, also speaks against IL-2 being treated as having become publicly available as a result of the transfers between Prof. K. Smith and Dr Oroszlan.

13. Whether something has been made available to the public is a question of fact to be decided in each individual case. For a complex biochemical to be made available to the public, the minimum that would seem to be required for publication is a notice to those in the field that samples of the biochemical can be obtained on request, and clear evidence of exactly what the biochemical was. These requirements are not fulfilled here. The Board thus accepts neither the IL-2 nor the monoclonal antibody as something that can be treated as made available to the public within the meaning of Article 54(2) EPC.

14. Following the Appellant's line of argument on this, the Respondent argued that despite the statement in the patent (page 6, lines 21 to 22) that the Jurkat FHRC cells disclosed by document (19) were available, these were not prior art because they were not deposited with a recognised depositary institution from which anybody could obtain them.

15. However, on the evidence, the Jurkat cells were known to be available on request from three different institutions. The Respondent itself acknowledged in the patent (*loc. cit.*) that cells were obtainable from these institutions, and itself so obtained the cells on which it carried out further research. The fact that there were three different sources from which the cells could be obtained, is a strong indicator for public availability. Even though there is no evidence that any

of these institutions were under any duty to maintain these cell lines and make them available to the public, as it would have been required in case of a deposit of the cell line according to Rule 28 EPC, the Board treats the acknowledgement in the patent, uncontradicted by any other evidence, as sufficient proof that the cell line and thus the enriched IL-2 mRNA source of document (19) were publicly available.

Novelty

16. The novelty issue was raised by the Appellant only in conjunction with the objection according to Articles 123(2) and (3) EPC (see Section IVa *supra*), insofar as the term "fragment" was given the broad meaning of being part of the whole human genome taken outside its natural context. Then the mRNA disclosed by document (23) once reverse-transcribed into cDNA would fall under the scope of the claims since it would satisfy the above definition.

17. The Board cannot follow the Appellant's reasoning set out above. Firstly, document (23) does not disclose any DNA but only mentions the cloning of IL-2 cDNA as a task for the future. Secondly, even if it were conceded for the sake of hypothesis that document (23) disclosed implicitely because being complementary to the mRNA, said reverse-transcribed IL-2 cDNA, which would still be a mixture of various cDNAs and fragments thereof being reverse transcribed from a fraction below 18 S of isolated, induced mRNA, the cDNA coding for IL-2 would still need to be identified and isolated from this mixture, or, and this is the same, from a cDNA library prepared therefrom, since a fundamental step for identifying the cDNA of interest will of necessity be the preparation of a cDNA library. The question which

now arises is whether this would be possible or not. This situation corresponds to that already dealt with in decision T 301/87 (*loc. cit.*, see Reasons, point 5), where there has already been discussed the question of whether in 1980, the unknown presence of a particular polynucleotide in a Lawn gene bank could be regarded as state of the art for the purpose of Article 54(1) EPC. On the facts then, it was found that this was not the case in the absence of a known probe, or any other means, enabling the polynucleotide to be identified. Apart from the relevant point of time here being some two years later, the relevant facts are technically similar in the two cases, and general knowledge in the art concerning the preparation and screening of gene banks did not significantly change in this period. Accordingly as in this case too, no probe was known for identifying the relevant gene, the Board finds that the cDNA coding for IL-2 was not part of the prior art merely because the cDNA would have been present among the reverse-transcribed mRNA of document (23).

Inventive step

Closest prior art

18. Human IL-2, also called T-cell growth factor (TCGF) was known to exhibit a spectrum of activities including induction of cytotoxic T-cell reactivity. These identified activities of IL-2 indicated that this lymphokine could be useful in the treatment, *inter alia*, of immunological disorders including neoplastic diseases. A method for producing human IL-2 from a mitogen-stimulated human T cell leukaemia cell line by cell culture methods was known from documents (2) and (23). The latter document already describes IL-2 mRNA isolation, which would be a first step to the cloning and expression of IL-2. This document, however, provides

no teaching about how to remove the blockage of short supply of IL-2 in order to make the recombinant DNA technique route *in toto* feasible. Thus, in the Board's view, document (2) is the most appropriate starting point for a problem/solution analysis.

Problem to be solved

19. As said above, there existed the problem that all known techniques resulted in very low concentrations of human IL-2 and moreover required complex purification procedures to separate IL-2 from other immunologically active proteins produced by the T cells and from the present toxic lectins. There was still uncertainty as to whether Il-2 was a single chemical entity or a broad group of molecules having similar biologic functions. A great many attempts had been made to obtain sequenceable IL-2. None had been successful. Thus it was desirable to provide IL-2 in a quantity and quality which would have put the skilled person in a position to identify unambiguously the protein and to provide a sufficient lectin-free quantity thereof for medical purposes. The Board is satisfied that the above problem has been solved by the present invention which provides the information and means necessary for identifying and cloning of DNA fragments coding for IL-2 and for expression of IL-2.

20. When examining whether the provision of IL-2 free from toxic lectins in quantities sufficient for medical application constitutes an inventive contribution to the art, the first question is whether the skilled person would have considered the use of recombinant DNA techniques to lead to human IL-2 with a reasonable expectation of success. In order to do this, the full length cDNA coding for IL-2 had to be picked up from a

cDNA library with a method that did not involve oligonucleotide probes, as no IL-2 amino acid sequence information was available. Thus, documents (27), (28) and (29) disclosing methods for identifying cDNAs would have been taken into account by the skilled team looking for techniques to be used when, as in the present case, no amino acid sequence was available. Otherwise, the method of choice is the use of oligonucleotide probes for screening gene banks, designed according to a known amino acid sequence. Documents (23) and (19) disclose the raw material for selecting the clones, i.e., the enriched IL-2 mRNA, and thus represent the starting point for dealing with IL-2 mRNA.

mRNA frequency

21. The Respondent has placed much emphasis on the selection of a 40 times higher IL-2-producer Jurkat 111 cell as being a *conditio sine qua non* for arriving at the claimed invention whereas the Appellant denied this. Much has been submitted on a dispute about the IL-2 mRNA frequency in the Jurkat 111 cell *versus* interferon-α mRNA frequency in the induced leukocytes of document (27). The Appellant maintained that the frequency of the relevant mRNA compared to total mRNA in the Patent in suit and in document (27) were substantially the same and thus the technique used in document (27), once applied to the enriched mRNA sources of document (23) or (19) would have obviously led to the cloning and expression of the IL-2 gene. These figures were arrived at by dividing the total amount of mRNA in the pooled fractions containing activity by the total message (patent in suit: 165µg/2.4mg = 0.069 ~ 1/15; document (27): 80µg/1630µg = 0.049 ~ 1/20) and were in

good agreement with another calculation method based on the ratio of the number of clones comprising a cDNA capable of expressing the activity upon insertion in a reading frame/number of clones investigated (patent in suit: 1/2,000; document (27): 4/5,000).

22. The Respondent's figures, namely IL-2 mRNA frequency in the Jurkat 111 cell = 1/20,000; interferon-α mRNA frequency in the induced leukocyte of document (27) = ~1/1,000, diverged substantially from the above and were obtained by dividing the number of strong signals in the screening process with a ^{32}P-cDNA probe divided by the total number of clones investigated, taking also into account the 10-fold mRNA enrichment achieved by sucrose density gradient fractionation (1/2,000 ➡ 1/20,000; 95/5,000 ➡ 2/1000). These figures were respectively supported by document (30), page 308, under the heading "Discussion" and by a declaration made in relation to the cloning and expression of another protein, the t-PA, by Dr Stark before the US Patent Office, which declaration comprised a list of cDNA cloning papers involving mRNA abundance of ca. 0.1%. Human interferon-α and also interferon-β were among the list.

23. As regards the Appellant's approach based on the fraction of pooled relevant mRNA/total mRNA, this ratio would reflect mRNA frequency in the cell only if the pooled relevant mRNA resulted in a 100% transcription to (exclusively) IL-2 cDNA. The Board observes that this is normally not the case and document (27) (see page 19, lines 5 to 9) confirms this. With a view to the Appellant's calculation based on the ratio of clones comprising an insert capable of expressing the active protein/total number of clones, the Board cannot adhere either to the above calculation method. This is because a portion of the full length mRNA molecules produced in

the cell undergoes truncation during the reverse transcription, isolation and purification processes. As a consequence the clones to be screened, although they truthfully mirror the actual number of mRNA molecules present in the starting cell, comprise both full length cDNAs and shorter sequences, all of which give positive signals. But most of these positive clones will **not** yield any biologically active molecule upon insertion of the cDNA in a suitable vector and transformation of a suitable host organism because successful expression does not only require that the cDNA should be full length but also the presence of start and stop codons at the correct places, the first ones ensuring a correct reading frame and the second ones a correct termination in the translation event, be it that the regulating elements are those of the isolated gene or otherwise introduced. Since thus the calculation methods are based on ratios of active message/total message or number of "active" clones/total of the clones that do not necessarily reflect the actual number of mRNA molecules in the starting cell, the Appellant's line of argument according to which the relevant mRNA frequency in the patent in suit and in document (27) are comparable, seems to be an artificial *ex post facto* view. The Board has thus to conclude that the dispute must be resolved in favour of the Respondent, whose approach to calculating mRNA frequency (see Point 22 *supra*) finds as mentioned above, support in document (30) and in Dr Stark's Declaration.

Comparison between the IL-2-productivities of the parent Jurkat cell and the mutated Jurkat 111 cell.

24. In order to counter the Respondent's argument that the selection of the Jurkat 111 cell was a critical step for arriving at the invention, the Appellant also argued

that the IL-2-productivity of the Jurkat 111 cell actually used by Dr Taniguchi (document (30)) *versus* that of the parent Jurkat cell line was less than 5 times higher rather than the 40 times higher stated in the patent in suit (see Example 1(1)). The above conclusion was reached by comparing the figure 2,000 U/ml mentioned in the legend to Figure 1 of document (30) with the figure 462 U/ml referred to in Table V of document (2) (see page 1716). However, for a proper comparison to be made between the IL-2-productivities of the parent Jurkat cell *versus* that of the activated Jurkat 111 cell, the comparison should be effected in the same situation for both cells, ie, same cell concentration, same medium, same mitogen(s) in equal concentration(s), same temperature and same stimulation time: yet the Board observes that the value 462 U/ml relates to cells stimulated for 24 h with 10 ng/ml PMA and 1% PHA while the figure 2,000 U/ml relates to cells stimulated for 6 h with 25µg/ml concanavalin A (see document (30), page 305, l.h. column, under the heading "Hybrid plasmids containing IL-2 cDNA"). Therefore this comparison is not suitable to show that the figure 40 times referred to in Example 1(1) of the patent in suit is not reliable.

The technique for isolating the DNA coding for IL-2

25. The meaningful comparison to be made between the technique used in the patent in suit and those used in documents (27), (28) and (29) is to be done on the steps involving the screening. Analysis of the techniques used in the patent in suit and the above documents leads to the conclusion that the overall cloning strategy comprises a primary screening whose aim is to identify a clone comprising a cDNA insert capable of hybridizing with the relevant mRNA (poly(A)-mRNA), and a secondary

screening consisting of using the so-identified cDNA insert as a probe to screen rapidly other transformed clones. This strategy is summarized in document (27) (see page 31, second paragraph), wherein it is also emphasized that it cannot be taken for granted that the primary screening will of necessity yield a cDNA fragment hybridizing with the relevant mRNA, let alone the complete cDNA sequence looked for, but once said cDNA insert coding for a portion of the relevant protein has been isolated, it may at least be used as a probe to screen rapidly other transformed clones. And in fact, none of the primary screenings under consideration yields a complete cDNA. However, the incomplete insert is used as such or upon truncation as a probe to screen other colonies for the complete cDNA, as transpires from the analysis below. The above cloning strategy is also to be found in the post published document (43), cited by the Appellant for showing that the team there was successful in cloning the cDNA coding for IL-2 by following other routes.

26. In the patent in suit, the primary screening among 432 clones (see section 5-5 on page 14 of the published patent) takes place on nitrocellulose filters with 50% formamide, 0.75 M NaCl, 20mM Pipes, 5 mM EDTA, 0.2% SDS at 37°C for 18h, after the first amplified, then purified DNA had been cleaved with the restriction enzyme Hind III. The DNA of only one colony (p3-16) gave a positive signal. p3-16 turned out to comprise an incomplete cDNA of 650 bp. The secondary screening (see page 15, lines 49-59) was carried out by using the above nick translated p3-16 cDNA as a probe to screen another, separately prepared cDNA library (2,000 colonies) by the *in situ* hybridization method of Grunstein-Hogness. Clone

pIL2-50A containing the full length (in the sense that it comprises not only the complete sequence coding for the mature protein but also that coding for the signal peptide) IL-2-cDNA of 850 bp was obtained.

27. In document (27), the primary screening (see page 35, first paragraph and pages 40 to 41, "Step B") is a DNA-RNA hybridization in solution with the conditions of Davidson *et al*. (80% formamide, 0.4 M NaCl, 0.01M Pipes, 5 mM EDTA at 56°C for 4h) that yields clone "Hif-4C" comprising a cDNA insert of 320 bp (see page 46, line 2 and bottom of page 48). The secondary screening (see page 49, second paragraph) occurs with a ^{32}P-labelled Hif-4C Pst fragment used as a probe for screening on filter the λ-III colonies in 4 x SET, 0.02% (w/v) Ficoll®, 0.02% PVP, 0.02% w/v BSA, 0.5% SDS at 68°C for 16h to yield *inter alia* clone λ-III-2H with the largest insert of 900 bp (see page 50, line 7). The same probe is used to screen 5,000 colonies with the result that 95 clones gave a very strong signal (see page 51, first paragraph).

28. In connection with documents (28) and (29), these should be viewed *in toto* since the former relates to the primary screening eventually yielding the TpIF319 insert (see document (28), page 468, lines 2 to 4) capable of hybridizing with the mRNA coding for interferon-β, while the latter bears upon the secondary screening. The said primary screening comprise two steps: first an *in situ* colony hybridization effected on 3600 clones made according to Grunstein-Hogness with two probes A and B yields 4 clones which hybridize with probe A but fail to hybridize or hybridize much less with probe B. There is

then a second screening based on a RNA-DNA hybridization in solution made according to Casey-Davidson which renders possible the isolation of the incomplete (see document (29), page 4004, rh column, lines 18 to 19) TpIF319 cDNA insert of 600 bp.

29. The secondary screening is disclosed by document (29) (see page 4004, r.h. column, under the heading "Isolation of the Recombinant Plasmid TpIF319-13"). A 400 bp Bgl/Pst fragment of TpIF319 is used as a probe for screening by *in situ* hybridization according to Grunstein-Hogness 4,000 colonies. Among the 15 positive clones, clone TpIF319-13 contains 800 bp, ie., the complete cDNA sequence coding for interferon-β.

30. In conclusion, the techniques disclosed by the patent in suit and by documents (27), (28) and (29) for arriving at the sought full length cDNA coding for the protein of interest, work only if two blockages can be overcome. First a cDNA fragment has to be picked up and there is no *a priori* assurance that the primary screening would lead to such DNA fragment. Secondly, even if the first blockage can be overcome, the DNA fragment resulting from the primary screening must be used as a probe for screening other transformed clones (secondary screening) and there is again no assurance that the complete cDNA sequence looked for will be obtained.

31. Leaving out of consideration the aspects relating to the relevant mRNA supply and the lack of amino acid sequence knowledge and in the light of Point 30 *supra* wherein it has been emphasized that all the cloning strategies under consideration need to be successful in overcoming both these two blockages, the Board finds it useful to split the inventive step question in two parts, namely the primary screening and the secondary screening. In

essence the Board has to establish (i) whether starting from the known IL-2 mRNA enriched sources of documents (23) or (19) and applying known techniques (documents (27), (28) and (29) or combination thereof) would with a reasonable expectation of success result in a cDNA fragment capable of hybridization with the mRNA coding for IL-2, and (ii) whether the screening of a cDNA library with this fragment would in the same way lead to the full length cDNA coding for IL-2 with a reasonable expectation of success.

Secondary screening

32. Even if, by way of hypothesis, one conceded that the skilled person could overcome the blockage represented by the primary screening, there would remain the second blockage dwelling in the secondary screening, whose aim is identification of the **full length** cDNA coding for IL-2. Regardless of whether the primary screening is possible or not, the Board views this second blockage as a serious one. This is because many of the cDNAs are incomplete copies of the IL-2 mRNA (see document (27), page 21, lines 6 to 19 and document (43), page 17, lines 8-11). Truncation is also due to half-way termination of the cDNA synthesis by reverse transcriptase and to the isolation and purification processes. Therefore, only a fraction of the clones giving a positive response in the first screening process would comprise the complete cDNA insert coding for IL-2. For a given library, there is a relationship between the probability of picking up a full length cDNA and the relevant mRNA abundancy: the former increases with the latter. If the relevant mRNA abundancy is very low, it cannot be excluded that a clone comprising the complete cDNA insert would not be present at all among the library being screened. In fact, the authors of

document (27) (see page 32, line 9) do not exclude the possibility that an "IFN-α-related DNA sequence" might not be present at all among the 512 clones under screening. This statement, made in connection with the primary screening which does not distinguish between full length cDNAs and truncated forms thereof, is even more pertinent if account is also taken of the fact that what is sought in the secondary screening, the full length cDNAs, represents only a fraction of all the cDNAs present. Therefore, the abundance of the relevant mRNA one starts with seems to be even more critical for the success of the secondary screening than for that of the primary screening. This view is supported by the following evidence.

33. The authors of documents (27) and (28) did not encounter any difficulty in overcoming the second blockage because, as already emphasized in Point 23 *supra*, these documents were concerned with the isolation of the complete cDNA sequence coding for human interferon-α or human interferon-β from sources having mRNA abundance of ca. 0.1% (1/1,000). However, the endeavours described in postpublished documents (39) and (43) to isolate the full length IL-2 cDNA went wrong. Although these researchers were successful in isolating a 5'-truncated IL-2 cDNA fragment termed hIL2-0, they met with a failure when trying to use a 450 bp hIL2-0 fragment for screening a cDNA library comprising 2,350 clones (see document (39), page 442, lines 9-13 and document (43), page 35, lines 10 to 22) since they merely picked up a cDNA fragment of 230 bp, even shorter than the probe itself and could not find any other way of escape from this cul-de-sac situation than giving up the cDNA library approach and turning to a genomic library. The Board observes that the secondary screening disclosed by

postpublished document (43) relates to the use of human splenocytes having an abundance of mRNA coding for IL-2 lower by two orders of magnitude than that of the Jurkat 111 used in making the invention of the patent in suit.

34. The question, the Board has now to answer on the basis of the above shown technical situation, is whether the skilled person could have reasonably expected to succeed in isolating a **full length** cDNA by starting from the enriched Il-2 mRNA supplies available in the prior art and applying the known secondary screening procedures disclosed by documents (27) or (29). In answering this question the Board has to focus on the relevant mRNA frequencies and to disregard the screening method which is in all cases (even in document (43)) an *in situ* technique according to Grunstein-Hogness, as well as the measures undertaken by the present inventors for increasing the number of full length cDNA such as the addition of an inhibitor of nucleases (ribonucleosides-vanadyl complex), as such measures are the same in document (29).

35. The skilled person could have started from the enriched IL-2 mRNA source disclosed by document (23) or from the normal Jurkat cell disclosed by document (19), which normal Jurkat cells the Board considers to have been available to the public (see Points 14 and 15 *supra*). Although the normal Jurkat cells exhibit an IL-2 mRNA frequency 40 times lower than that of the Jurkat 111 cell used in the patent in suit (see Point 24 *supra*), they represented the best IL-2 mRNA source available at that time and Prof. K. Smith confirms this in his declaration (see document (48)). As regards starting from the IL-2 mRNA of document (23) and applying the known secondary screening techniques, the Board's answer to the above question is no, since the IL-2 mRNA

abundance of peripheral blood leukocytes of document (23) is less than that of the splenocytes of post published document (43) (see document (43), bottom of page 11). Since the authors of document (43) did not succeed in isolating the full length cDNA coding for IL-2 starting from splenocytes, it should be expected that departing from a lower mRNA abundance, no full length cDNA would *a fortiori* be identified.

36. With a view to the second possibility, namely starting from a IL-2 mRNA isolated form normal Jurkat cells and applying the known secondary screening procedures, it should be noted that even the Jurkat 111 cell of the present invention exhibits a very low IL-2 mRNA abundance of 1/20,000 (see Point 24 *supra*) and this is confirmed by the fact that of the 2,000 clones screened only one clone comprising the full length cDNA insert could be picked up (see document (30), page 308). If, following the Appellant's argument, the secondary screening were effected on a cDNA library made from IL-2 mRNA of the normal Jurkat cell with an abundance 40 times lower, ie, 1/800,000, the probability of identifying a full length IL-2 cDNA clone would become at most 40 times lower. Thus, in the most favourable instance, it would be 40 times more difficult to detect and isolate the unique clone comprising a full length insert which the present inventors, departing from a Jurkat 111 cell, were able to pick up from among 2,000 clones. In the worst instance, the said full length clone would not be present at all.

37. The Appellant did not provide convincing experimental or theoretical evidence in support of the above argument that a skilled person would have arrived at the full length cDNA coding for IL-2 by screening a cDNA library made from IL-2 mRNA of normal Jurkat cells.

38. Already on the basis of the preceding arguments, the Board would thus feel that the above question would have to be decided in favour of the Respondent. But the Board is given a possibility to verify what happens when a cDNA library made from normal Jurkat cells is screened, and this is provided by document (37) cited by the Appellant in a different context. This abstract shows that it was **not** possible to identify any full length cDNA coding for IL-2 upon screening said cDNA library made from normal Jurkat cells.

39. There was thus at least one blockage represented by the secondary screening which could not routinely have been overcome by the skilled person and this supports the Respondent's line of argument that the selection of the relatively high IL-2-producer mutant cell Jurkat 111 was a *conditio sine qua non* for arriving at the full length cDNA coding for IL-2. Therefore the Board has to conclude that, had a skilled person departed from any of the richest IL-2 mRNA sources available before the valid priority date of the patent in suit and applied the known screening techniques, he could not reasonably have expected to succeed in identifying the full length cDNA coding for IL-2, and would not ordinarily have been successful if he tried it.

40. Thus, the starting step of providing a cell line of enhanced production of IL-2 already contributes importantly to an inventive step and the Board observes that already the decision of the Respondent to produce a further mutant of the already mutated parent Jurkat cell line was not trivial. When producing mutants in view of one desired feature, one has always to take into consideration that processes of mutation were at the time of priority of the patent in suit, not target directed, at least those using the method carried out

according to the specification by irradiation with X-rays till 10,000 Roentgen (see specification, page 12, lines 20 to 23). This means that the mutation event causes random changes in the genome and among these, undesired ones. It is not self evident that a Jurkat cell line with enhanced IL-2 productivity could be obtained while at the same time maintaining the other desired properties of the cell line. Solely the growth rate seemed to be somewhat reduced (*ibidem*, page 12, lines 32 and 33).

Amino acid sequence availability

41. It has to be examined whether the skilled person, being discouraged by the above discussed route, would have focused with more expectation of success on an approach based on another recombinant DNA-technique, namely that of designing an oligonucleotide probe after the amino acid sequence of IL-2 and using the probe for screening a library. As already emphasized in Point 20 *supra*, this technique is to be preferred when amino acid sequence information is available. This is mainly because using an (albeit degenerate) oligonucleotide probe for screening a library is easier than using the total mRNA as a probe, as it is done for the primary screenings in documents (27) and (28) dealing with techniques to be applied when the amino acid sequence of the relevant protein is not known.

42. The Appellant's reasoning was that said IL-2 amino acid sequence knowledge was available before the recognized priority date of the patent in suit. The prior art monoclonal antibodies disclosed by document (45) and

(46) were suited for immunopurification to a sequenceable degree of IL-2 and the skilled person was moreover in a position to select by routine techniques anti-IL-2 monoclonal antibodies adequate to the same scope.

43. While agreeing that obtaining a suitable monoclonal antibody to be solid-phased onto the immunoaffinity column was one of the critical steps for arriving at sequenceable IL-2, the Board, however, is of the opinion that the way to said monoclonal antibody was not obviously derivable from the prior art. A monoclonal antibody leading to sequenceable IL-2 should not only be able to remove a substantial portion of IL-2 activity from the culture supernatant after it has been coupled to a solid support, but the bound IL-2 should be eluted easily without loss of activity. Even if these conditions are fulfilled, it cannot be taken for granted that IL-2 activity eluted from the immunoaffinity column will be sequenceable. This is mainly because IL-2 eluted from the immunoaffinity column may be contaminated by extraneous proteins due to the lack of specificity of the monoclonal antibody or to other factors, such as the impossibility of performing an efficient washing of the column to remove the contaminants.

44. That the monoclonal antibodies disclosed by the prior art document (45) and (46) do not satisfy one or more of the above mentioned conditions for leading to sequenceable IL-2, is shown by the following evidence. Although in document (45) (see page 1622, Table III) 8 monoclonal antibodies capable of binding IL-2 were selected, the bound IL-2 could not be eluted with commonly used eluants such as buffers with low pH and chaotropic agents. It was only under extremely harsh conditions (1% SDS, 79°C) that elution occurred. This,

however, resulted in an undesired denaturation of both the antibody and the antigen. The denaturated IL-2 had to be re-naturated by a further method (see document (47)). Document (46) (see page 1983, r.h. column, second full paragraph) shows that although the anti-IL-2 monoclonal antibody 4E12B2D10 was able to bind IL-2 activity, it was not possible to elute the bound activity because of the damage to the IL-2 molecule upon elution attempts. The Appellant's expert himself Prof. K. Smith states (see document (51), page 1814, r.h. column, second paragraph) that the attempts to immunopurify IL-2 by means of the monoclonal antibodies of documents (45) and (46) were unsuccessful. The Board has thus to conclude that the monoclonal antibodies of prepublished documents (45) and (46) could not yield, once coupled to a solid matrix, sequenceable IL-2.

45. The likelihood of obtaining a suitable monoclonal antibody is discussed in the following on the basis of the teachings of the two postpublished documents (42) and (51), which assist in evaluating whether the prepublished mention of a suitable monoclonal antibody in document (35) provided the skilled person with a reasonable starting point.

46. With a view to selecting better monoclonal antibodies to be used in IL-2 immunopurification, Prof. K. Smith's team had to devise a more sensitive assay (ELISA) for screening the hybridomas which proved fundamental (see document (51), last paragraph of page 1808) for arriving at monoclonal antibody DMS-3, the only one suited for immunoabsorbent purposes among three candidates (*loc. cit.* p.1812, r.h. column, first paragraph). However, while monoclonal antibody DMS-3 performed well in binding and releasing IL-2, the Board finds doubtful that the eluate from the DMS-3 column was directly

sequenceable. The Board indeed observes that Fig. 10 of document (51) (see page 1813) relating to the RP-HPLC of the DMS-3 immunoaffinity column eluate exhibit between 0 and 20 min a substantial protein peak measured at 215 nm not corresponding to IL-2. This appears to be in line with Point 5 of Prof. K. Smith's declaration (48), according to which Dr Oroszlan found that only peak n° 13 turned out to be IL-2 upon HPLC. Unlike the eluate from the 1H11-1A5 column (see document (42), Table 1, page 5991) which **was** directly sequenceable, the DMS-3 eluate was thus possibly not sufficiently pure for direct sequencing.

47. In view of the above and in particular since Prof. K. Smith's monoclonal antibodies were not available to the public (see Point 15 *supra*), it must be concluded that, for the person skilled in the art to arrive at sequenceable IL-2 by following the pathway taken by Prof. K. Smith *et al.*, it was fundamental to have the knowledge that a more sensitive ELISA assay for screening the positive hybridomas needed to be devised and also that IL-2 was a hydrophobic protein and thus it was still possible to resolve by RP-HPLC the immunoaffinity column eluate. But this information became available only with the publication of document (51) in October 1993, ie., after the valid priority date of the patent, and is missing in document (35).

48. It would seem to the Board that only monoclonal antibody 1H11-1A5 disclosed by document (42) fulfilled all the requirements mentioned above to yield an eluate of such a high purity that it could be sequenceable as such. But again, arriving at this "particularly high-affinity antibody" (*loc. cit.*, last line of page 5990) was also not within the realm of the skilled person and the

demonstration of this could lie with the fact that Prof. K. Smith's team, in spite of all the expedients used to select better monoclonal antibodies, did not succeed in selecting a monoclonal antibody which performed like the 1H11-1A5 one.

49. In the light of this factual situation, the Board has to disagree with the Appellant's argument that document (35) would have put anybody in possession of the amino acid sequence of IL-2 in order to prepare the oligonucleotide probe being used for isolating the gene coding for IL-2. The document is a conference paper relating to the 66th Annual Meeting of the FASEB held in New Orleans on 13 to 15 April 1982. Bearing in mind that postpublished documents (42) and (51) showed that there were remarkable difficulties in obtaining a suitable monoclonal antibody against IL-2, and that there was no detailed information about the process for obtaining the monoclonal antibody, let alone the provision of a hybridoma as living entity secreting the monoclonal antibody to those attending the conference, the Board cannot believe that this oral disclosure would have enabled those attending the conference to arrive at sequenceable IL-2.

50. The Appellant, moreover, did not render it plausible that IL-2 amino acid sequence information was released to the public before the valid priority date of the patent in dispute. Under these circumstances, the Board has to conclude that the skilled person could not have arrived at the claimed subject-matter by the second route departing from sequenceable IL-2 or the amino acid sequence thereof.

51. In view of the above, the claims of the main request are found to satisfy the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairwoman:

L. McGarry U. M. Kinkeldey