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D E C I S I O N
of 16 July 1998

Case Number: T 0656/94 - 3.3.4

Application Number: 86905530.1

Publication Number: 0237545

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Title of invention:
Production of pluripotent granulocyte colony-stimulating factor

Patentee:
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Opponent:
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Chugai Seiyaku Kabushiki Kaisha
Boehringer Mannheim GmbH Patentabteilung

Headword:
Colony-stimulating factor/KIRIN-AMGEN

Relevant legal provisions:
EPC Art. 123(2), (3), 54, 56, 83, 84

Keyword:
"Amendments extending originally filed disclosure or scope of granted claims (no)"
"Clarity (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
T 0292/85, T 0301/87, T 0409/91, T 0435/91, T 0296/87

Catchword:
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Case Number: T 0656/94 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 16 July 1998

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Decision under appeal: Decision of the Opposition Division of the European Patent Office posted 25 July 1994 rejecting the opposition filed against European patent No. 0 237 545 pursuant to Article 102(2) EPC.

Composition of the Board:

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

Summary of Facts and Submissions

I. European Patent No. 0 237 545 (application No. 86 905 530.1), claiming priority of 23 August 1985 (US 768 959) and 3 March 1986 (US 835548), was granted on the basis of 37 claims. The patent relates to the production of human pluripotent granulocyte colony-stimulating factor (hereinafter: hpG-CSF; a list of all relevant acronyms used throughout this decision is to be found in Annex I).

II. Notices of opposition were filed by Opponents I to V all requesting the revocation of the European patent on the grounds of Articles 100(a), (b) and (c) EPC. Opponents III and IV withdrew their oppositions. During the procedure before the Opposition Division about one hundred documents, declarations and experimental test reports were presented by the Parties. A list of the documents cited in the present decision is to be found in Annex III. By a decision posted on 25 July 1993 the Opposition Division held that the patent as granted fulfilled the requirements of the EPC and rejected the oppositions according to Article 102(2) EPC.

III. The Appellant (Opponent V) filed an appeal against the decision of the Opposition Division with the payment of the fee. The Respondent (Patentee) filed counterarguments and auxiliary requests No. 1 to 6.

IV. The Appellant was not represented at the oral proceedings held on 15 July 1998, during which the Respondent filed a new main request in replacement of any preceding request. The claims of this request differed from the granted claims (see Annex II) in that

granted claims 3 and 11 were omitted and in that claims 1 and 33 of the new main request corresponded to claims 1 and 35 as granted with the amendments shown in bold letters:

"1. **An isolated** polypeptide **consisting only of part or all of the amino acid sequence 1-174 set forth in Table VII** which:

(a) has one or more of the biological properties typical of naturally-occurring human pluripotent granulocyte colony-stimulating factor (hpG-CSF) of the sequence set forth in Table VII,

(b) is a non-naturally occurring polypeptide; and

(c) is the product of procaryotic or eucaryotic expression of an exogenous DNA sequence."

"33. **An isolated** polypeptide having the hematopoietic biological properties of naturally occurring human pluripotent granulocyte colony-stimulating factor, said polypeptide having an amino acid sequence selected from the polypeptide sequence forth in Table VII, or any allelic variants, derivatives, deletion analogs, substitution analogs, or addition analogs thereof, and characterized by being non-naturally occurring and by being the product of procaryotic or eucaryotic expression of an exogenous DNA sequence."

Otherwise the remaining claims thereof corresponded to renumbered granted claims 2, 4 to 10 and 12 to 37, with the dependencies amended as necessary.

V. The submissions and evidence provided by the Appellant can be summarized as follows:

Amendments (Article 123(2) EPC)

- The expression "non-naturally occurring polypeptide" in the claims constituted an inadmissible extension under Article 123(2) EPC. This wording was neither explicitly nor implicitly disclosed in the application as filed. Furthermore, since no "naturally occurring" hpG-CSF was known at the priority date of the patent in suit, this expression could not be a distinguishing feature for the claimed product and could not be employed as a disclaimer of prior art hpG-CSF isolates.

Sufficiency of disclosure (Article 83 EPC)

- The patent failed to fulfil the requirements of sufficiency of disclosure set out in Article 83 EPC because it did not show any reliably workable method of producing polypeptides having one or more biological properties of hpG-CSF. The fact that Example 10 of the patent in suit showed that only one of the five polypeptides prepared according to Example 8 exhibited biological activity, rendered the present situation more similar to those dealt with in decisions T 409/91 (OJ EPO 1994, 653) and T 435/91 (OJ EPO, 1995, 188), where sufficiency of disclosure had been denied, than to the ones dealt with in decisions T 292/85 (OJ EPO, 1989, 275) and T 301/87 (OJ EPO, 1990, 335), where sufficiency of disclosure had been accepted.

- Claim 6 and dependent claims were unworkable since an unacceptable effort was required for the skilled person to identify DNA sequences encoding polypeptides with hpG-CSF biological properties, among the huge number of possible DNA sequences.

- As to the cDNA coding for hG-CSF of claim 12 and referred to in claim 2, Table VII lacked the 5'-terminal region and thus the patent did not disclose a cDNA encoding hpG-CSF since expression of this cDNA of Table VII was only possible by introduction of an artificial leader sequence or by mutagenesis of the N-terminal amino acid of mature hpG-CSF.

- Insofar as claim 34 covered a non-glycosylated polypeptide having residues 1 to 174 of Table VII (i.e. $Thr^{+1}...Pro^{+174}$), the patent did not teach how to obtain such a polypeptide in a procaryotic host since expression in this cell would have implied a N-terminal Met and the absence of sugars.

Novelty (Article 54 EPC)

- The polypeptide of claims 1 and 33 lacked novelty on the grounds that the characterizing features (b) "non-naturally occurring", and (c) "is the product of eucaryotic or procaryotic expression of an exogenous DNA sequence", were incapable of distinguishing the claimed polypeptides from the products of the prior art represented by documents (P30), (P32) and (P40) in terms of glycosylation and/or amino acid sequence. The G-CSF disclosed by documents (P30) and (P32) had also been subjected to neuraminidase treatment, thus yielding a modified product.

- As for the Respondent's argument that "natural" hpG-CSF from human CHU-1/CHU-2 or 5637 cancer cells differed from recombinant hpG-CSF in that the former was a mixture of polypeptides having 174 and 177 amino acids produced by alternative splicing, there was no experimental evidence that a hpG-CSF form with 177 amino acids existed.

- The claimed recombinant hpG-CSF lacked novelty in view of the radiolabeled mouse G-CSF of documents (P24), (P25) and (P26), of recombinant mouse IL-3 of reference (K45), and of recombinant mouse GM-CSF of document (P7) and of recombinant human GM-CSF of document (P41).

- Insofar as claim 34 covered the glycosylated polypeptide having the amino acid sequence 1-174 set forth in Table VII, the claim was disclosed in an enabling manner only in the second priority document. Therefore, document (P32) was a conflicting document according to Article 54(3) EPC and was novelty destroying for the claim since it disclosed the preparation of recombinant Thr^{+1}-G-CSF.

Inventive step (Article 56 EPC)

- There was a reasonable expectation of success in isolating the cDNA encoding hpG-CSF at the priority date of the patent in suit. Document (P40) disclosed the process for isolating highly purified hpG-CSF from supernatants of the publicly available 5637 cell. The skilled person was in a position to accurately sequence the protein. There existed a direct correlation between the amount of material subjected to amino acid sequencing and the length of unambiguous amino acid information obtained. The inventor of the patent in suit, Dr

Souza, constructed in the patent a problem that did not exist. Had he used a larger amount of the hpG-CSF purified according to document (P40), this would have resulted in an unambiguous N-terminal amino acid sequence for at least 30 amino acids. ICI Exhibit 2 and document (P45) showed this. The amino acid sequence information thus obtained would have allowed application of the known cDNA library screening procedures. The screening strategies which could have successfully been used were:

- Fully degenerate oligonucleotide probes, as disclosed in, e.g., in document (P7) for the isolation of the cDNA encoding GM-CSF.

- The long probe approach (guessmer) of Anderson and Kingston (document (P48)).

- The inosine substitution approach of documents (P35) and (P28).

Three test reports were submitted in support of the above proposition. These were the Appellant's test report dated 21 February 1992, the ICI Exhibit 1 dated 19 February 1992 and Exhibit (K30). Later document (P32) showed the successful application of the inosine substitution approach.

- rhpG-CSF did not exhibit surprising advantageous properties in comparison with G-CSF of document (P40).

VI. The submissions and evidence provided by the Respondent can be summarized as follows.

Sufficiency of disclosure (Article 83 EPC)

- The patent disclosure could be practised by the skilled person without undue effort and expense.

Novelty (Article 54 EPC)

- The Appellant had not argued lack of novelty in his opposition, and so was not entitled to argue the point on appeal.

- "Natural" G-CSF from human CHU-1/CHU-2 or 5637 cancer cells differed from recombinant hpG-CSF in that the former was glycosylated differently and that it was a mixture of polypeptides having 174 and 177 amino acids produced by alternative splicing. The terms "isolated" and/or "only" in the claims to proteins of the new main request ensured that these claims covered a single polypeptide, not the mixtures of said polypeptides of the prior art.

Inventive step (Article 56 EPC)

- There existed no reasonable expectation of success in isolating the DNA encoding hpG-CSF at the priority date of the patent in suit. Example 1 (see Tables I and II) of the patent showed that three amino acid sequencing runs performed on the isolates of document (P40) failed to provide unambiguous amino acid sequence information. Further, the amino acid sequence of Table I and II could not be used for designing a fully degenerate oligonucleotide probe because they comprised a

great many amino acids with 6-fold and 4-fold codon degeneracy. Additional amino acid sequence information devoid of ambiguity and having a sufficient length was therefore required for applying a successful screening strategy. But even after the inventor, Dr Souza, succeeded in obtaining the less ambiguous and sufficiently long amino acid sequence recited in Table IV of the patent in suit, there were still difficulties to be overcome because the additional amino acids identified were found to be encoded by highly degenerate codons and this prevented the use of the highly degenerate probe approach. He had to turn to a highly unreliable and not yet explored technique of the inosine substitution technique.

VII. The Appellant (Opponent V) requested that the decision under appeal be set aside and that the European patent No. 0 237 545 be revoked.

The Respondent (Patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the new main request submitted at the oral proceedings on 15 July 1998.

Reasons for the Decision

Article 123(2) and (3) EPC

1. The Appellant argued that the wording "non-naturally occurring" in the granted claims had no basis in the application as filed. In the Board's view, although the expression "non-naturally occurring" in claims 1, 29, 33 and 34 of the new main request is to be found nowhere expressis verbis, there is an implicit basis in the application as filed for this wording. In the

original description (see paragraph bridging pages 2 and 3), it is stated that human pluripotent colony-stimulating factor has been identified in the culture medium of human bladder carcinoma cell line 5637 and this is again mentioned in Example I (page 10, lines 6 to 10). This factor is then later defined as "natural" in the application as filed (see eg page 25, line 10). In this context, "natural" means that no technical intervention of man, such as DNA manipulation, is required in order that this protein comes into existence, but is produced by a natural cell, although a cancer cell. The production of recombinant hpG-CSF according to the patent in suit, however, requires intervention of man and thus it involves the use of DNA engineered cells which are not to be found as such in nature even under pathological situations. Hence the claimed molecules are implicitly "non-naturally occurring" and no infringement of Article 123(2) EPC arises. Whether or not the term "non-naturally occurring" has any distinguishing power vis-à-vis "naturally occurring" hpG-CSF, is not an issue to be treated under Article 123(2) EPC.

2. The term "isolated" in claims 1 and 33 of the new main request finds a basis on page 6, line 33 of the application as filed.

The term "only" in claim 1 of the new main request means that the claimed rhpG-CSF must be a "single species" (see point 11 infra). This term, already present in granted claim 36, finds also support in the application as filed (see eg, page 7, lines 3 to 9), where it is stated that the claimed polypeptides can be the product of expression of exogenous cDNA sequences, a condition for obtaining the "single species" (see point 10 infra).

Further, since the terms "non-naturally occurring", "isolated" and "only" are restrictive in nature, no infringement of Article 123(3) EPC takes place.

Article 84 EPC

3. Claims 1, 33 and 34 of the new main request now include at least one of the terms "isolated" and "only", which the Board regards as clear in their technical meaning and furthermore as true distinguishing features vis-à-vis the hpG-CSFs of the prior art on the grounds explained in detail in points 10 and 11 infra. Insofar as these claims include at least one true distinguishing feature, it does not matter whether or not the remaining features "non-naturally occurring" and "is the product of eucaryotic or procaryotic expression of an exogenous DNA sequence" in claims 1 and 33 of the new main request are true distinguishing features.

In conclusion, the claims satisfy the requirements of Article 84 EPC.

Article 83 EPC

4. According to the Appellant, an unacceptable effort was required for the skilled person to identify, among the huge number of claimed DNA sequences, those DNA sequences encoding polypeptides with hpG-CSF biological properties, having regard that Example 10 of the patent in suit showed that only one of the five polypeptides prepared according to Example 8 exhibited biological activity (see paragraph IV supra). In the Board's view, however, this objection is beside the point when discussing Article 83 EPC requirements, since the patent teaches how to prepare hpG-CSF analogs and how to check the biological activity thereof by using the ^{3}H

thymidine uptake and the CFU-GM and WEHI-3B D* assays (see pages 35 and 36, Examples 10.2 to 10.4 of the patent in suit). If a given analog does not fulfil the biological activity requirement recited in the claims, it has to be discarded. In spite of the considerable amount of theoretically possible variation of the DNA and hence amino acid sequence in the present case, there is still likely to be a structural similarity among all the variants covered by the present claims. The situation here, where the claimed DNAs are limited to those having a certain structural relation to one another, and encoding proteins with a testable narrowly defined activity, must be distinguished from situations where either the structure or the activity or both is/are not defined in a disputed claim, as e.g. in the cases dealt with in the decisions cited by the Appellant, namely decisions T 435/91 (supra, see point 2.2.1) and T 409/91 (supra, see point 3.5: "... it was not contested by the appellant that no information was given to perform the claimed invention successfully without the structurally defined class of additives...").

5. The Appellant maintained that the cDNA coding for hpG-CSF of claim 12 and referred to in claim 2 and disclosed by Table VII of the patent in suit lacked the 5'-terminal region and thus the patent was not enabling for this cDNA encoding hpG-CSF since it could not be expressed as such. In the Board's view, for the purpose of enablement of a cDNA encoding hpG-CSF, the patent in suit has to disclose all the details necessary for a skilled person to arrive at a cDNA coding for hpG-CSF. These technical instructions are to be found on page 8, line 30 to page 14 line 25 of the patent in suit, where reference is made to a number of publications relating to the preparation of cDNA and according to which the cDNA as claimed can be produced by reverse transcription of mRNA from 5637 cells, it can be

isolated by means of probes designed in the light of Table VII of the patent in suit and identified by comparing it to the DNA sequence encoding mature hpG-CSF disclosed by Table VII. Regarding expressibility, the skilled person could have used a construct comprising a DNA encoding a leader sequence from another protein (eg, yeast α-factor: see Exhibit (8), published 31 October 1984) which was processed off by the yeast host cell to yield the mature protein. This technique had already been applied for expression of mature erythropoietin (see Exhibit (7), published 20 June 1985, page 83, lines 5 to 21). Thus, it was known before the earliest priority date of the patent in suit (August 1985), a time where genetic engineering was already advanced to such a high degree as to render this technique a routine one.

6. It was also argued by the Appellant that a non-glycosylated polypeptide ($Thr^{+1}...Pro^{+174}$) according to claim 34 could not be arrived at. The Board disagrees with this proposition because the claim in question does not require that the protein be non-glycosylated. The skilled person was in a position to obtain the glycosylated polypeptide ($Thr^{+1}...Pro^{+174}$) by expression in an eucaryotic cell of a DNA encoding a leader sequence from another protein in combination with the DNA sequence of Table VII and (see point 5 supra).

For the above reasons, the arguments provided by the Appellant are not convincing and the claims are found to fulfil the requirements of Article 83, EPC.

Novelty

7. The question of novelty was dealt with in the decision under appeal, and it had been raised by some Opponents. Opposition proceedings are to be treated as a single proceedings, so that once, as in this case, the ground

of lack of novelty was before the Opposition Division, this ground can be raised on appeal even by an Opponent who had not objected on the basis of novelty in his own opposition.

The prior art

8. A method for producing hpG-CSF from the conditioned medium of a bladder carcinoma 5637 cell line is known from document (P40). This document already describes the isolation and partial characterization of hpG-CSF, however, without disclosing any amino acid sequence thereof. Also representing prior art, but only according to Article 54(3) EPC, are European patent applications (P30) and (P32) disclosing natural hpG-CSF from CHU-1 or CHU-2 cells. Unlike document (P32), neither document (P40) nor document (P30) discloses any DNA sequences encoding hpG-CSF.

Novelty of claims 6 to 23, 28, 30 and 31

9. These claims are directed to DNAs encoding hpG-CSF, plasmids or viral vectors including these DNAs, hosts transfected with these plasmids or viral vectors and processes for the production of hpG-CSF by using these DNAs. In fact, the novelty of these claims has never been questioned by the Appellant. No prior art document, including documents (P30) and (P40), discloses any DNA encoding hpG-CSF, exception made for document (P32). This document is based on seven priority documents PD1 to PD7. PD1 (see document (P33)), dated 8 February 1985, is the only priority document filed before 23 August 1985, i.e., the earliest priority date of the patent in suit. However, document PD1 does not disclose any DNA sequences encoding hpG-CSF, while said DNA sequences are to be found in the earliest priority document on which the patent in suit is based. Consequently, document (P32)

is not prior art according to Article 54(3) EPC insofar as claims 6 to 23, 28, 30 and 31 relating to DNAs encoding hpG-CSF are concerned. In view of these findings, the Board has to conclude that the DNA sequence as claimed in claim 6 is novel. Claim 6 therefore fulfils the requirements of Article 54 EPC. Since the host cells, the plasmids or viral vectors including these DNAs, the hosts transfected with these plasmids or viral vectors and the processes for the production of hpG-CSF by using these DNAs of claims 7 to 18 and 20 to 23 all rely on the DNA of claim 6 found by the Board to be novel, these claims also satisfy the requirements of Article 54 EPC. This conclusion also applies to the DNAs encoding the muteins of hpG-CSF of claim 19 ([Ala^1]hpG-CSF) and claim 28 (hpG-CSF having one or more Cys replaced with Ala or Ser) and to claim 30 directed to plasmids or viral vectors including the DNA of claim 28, and to claim 31 covering the hosts transfected with the plasmids or viral vectors of claim 30.

In view of these findings, the novelty of the subject-matter of these claims is acknowledged.

Novelty of claims 1 to 5, 24 to 27, 29 and 32 to 35

10. These claims relate to the polypeptides and to the first and further medical application thereof. The novelty of these claims has been challenged by the Appellant. A first ground of challenge was that the features "non-naturally occurring", and "is the product of eucaryotic or procaryotic expression of an exogenous DNA sequence", were incapable of distinguishing the claimed polypeptides from the products of the prior art represented by documents (P30), (P32) and (P40) in terms of glycosylation and/or amino acid sequence.

11. In the Board's judgement, the "natural" hpG-CSF from human CHU-1/CHU-2 cells of document (P30) and (P32) or from 5637 cancer cells according to document (P40) differs from the claimed rhpG-CSFs in that "natural" hpG-CSF is a mixture of polypeptides having 174 and 177 amino acids in a molar ratio of about 80:20 produced by alternative splicing (i.e. it is "multiple species"), while rhpG-CSF according to the patent in suit is "single species", i.e. it comprises only the 174 amino acid polypeptide. The Board has come to this conclusion in the light of a series of post-published documents showing that bladder carcinoma cell line 5637 of document (P40) and CHU-1/CHU-2 cells of document (P30) express two different mRNA's encoding hpG-CSF with 174 and 177 amino acids, respectively, the latter having a Val-Ser-Glu insertion after amino acid position 35 (see Exhibit (15), paragraph bridging pages 177 and 178; Exhibit (16), page 113, right hand column, lines 1 to 21); document (P21), page 577, left hand column, third full paragraph and Exhibit (25), Figure 2.10 on page 25).

12. The Appellant maintains that there is no experimental evidence that a hpG-CSF form with 177 amino acids exists by relying, inter alia, on the sentence on page 113, right hand column, lines 21 to 24 of Exhibit (16): "However, it is not yet known whether two different G-CSF molecules are actually expressed in the primary cellular sources of G-CSF". In the Board's view, however, this sentence means that it was not yet known whether in the **human body** , i.e. in its (then not yet known) primary source, hpG-CSF was expressed as two species. This statement thus does not relate to 5637 or CHU-1/CHU-2 cells, which are "pathological" cells and are not the primary source. The latter has turned out after the priority date of the patent in suit to be peripheral blood monocytes (see document (K33), page 1179, right hand column, last full paragraph).

Furthermore, as to the question whether or not there are two different hpG-CSF molecules in 5637 or in CHU-1/CHU-2 cells, there is no evidence on file that a "selective blocker" existed in these cells that inhibited translation of the mRNA encoding hpG-CSF of 177 amino acids **but not** translation of the mRNA encoding hpG-CSF of 174 amino acids.

In conclusion, the Appellant's arguments that the 177 amino acid long form of hpG-CSF does not exist in the hpG-CSF compositions of the prior art are not convincing.

13. This means that the claims now comprise **at least one** technical feature appropriate for distinguishing the claimed hpG-CSF consisting of one molecular species only from "natural" hpG-CSFs of the prior art comprising two molecular species. This feature is represented by the terms "**isolated**" and "**only**" in claim 1, the term "**isolated**" in claim 33 and the term "**only**" in claim 34 of the new main request. Owing to this feature, these claims cover a single polypeptide having part or all of the amino acid sequence 1-174 set forth in Table VII of the patent in suit, not the mixtures of polypeptides of the prior art comprising 80% of the 174 amino acid long species and 20% of the 177 amino acid long species produced by alternative splicing. Since the prior art documents disclosed neither that there was a mixture of proteins of different lengths, nor the presence of the 177 amino acid long species of hpG-CSF, and thus no need for a process of isolating a single component had been realized to exist, the rationale emerging from decision T 296/87 (OJ EPO, 1990, 195, see items 6.1 to 6.3) also applies to the present situation. Thus the mixtures of polypeptides of the prior art comprising 80% of the 174 amino acid long species and 20% of the 177 amino acid long species produced by alternative splicing do not

destroy the novelty of the isolated 174 amino acid long hpG-CSF. In view of this, the Board need not evaluate whether or not the features questioned by the Appellant "non-naturally occurring" and "is the product of eucaryotic or procaryotic expression of an exogenous DNA sequence" further distinguish the claimed subject matter from that of the prior art.

14. It is the Appellant's view that the claimed recombinant hpG-CSF lacks novelty in view of the radiolabeled mouse G-CSF of references (P24), (P25) and (P26). However, there is no evidence before the Board that the protein disclosed in these documents has part or all of the amino acid sequence recited in the claims of the patent in suit.

15. As regards recombinant mouse GM-CSF of document (P7), recombinant human GM-CSF of document (P41) and recombinant murine IL-3, there is no homology at all between these proteins and hpG-CSF. For instance, there is no homology between hpG-CSF and GM-CSF (see Exhibit 17, page 2187, right hand column). The person skilled in the art would not consider meaningful the fact that two proteins might exhibit a few amino acids (2 or 3) homology over more than hundred amino acids.

16. It was argued by the Appellant that the polypeptide of claim 34 covering the glycosylated polypeptide having the amino acid sequence 1-174 set forth in Table VII ($Thr^{+1}...Pro^{+174}$) was enabled only in the second priority document US 835548. Therefore, conflicting document (P32) (a document according to Article 54(3) EPC in the Appellant's view) was novelty destroying for the claim since it disclosed the preparation of recombinant hpG-CSF having the amino acid sequence $Thr^{+1}...Pro^{+174}$.

However, as already emphasized under point 9 of the reasons supra, document (P32) is based on seven

priority documents PD1 to PD7. PD1 (see document (P33)), dated 8 February 1985, is the only priority document filed before 23 August 1985, i.e., the earliest priority date of the patent in suit. However, document PD1 does not disclose any DNA sequences encoding hpG-CSF and hence also no recombinant hpG-CSF, while both the DNA sequences and the expression thereof in host cells are to be found in the earliest priority document on which the patent in suit is based. Consequently, document (P32) is not prior art according to Article 54(3) EPC.

17. Finally, when looking at the prior art documents, both by themselves and in the light of later publications, the Board is left in serious doubt as to the alleged identity of the products of the prior art with the claimed products (see points 12 and 14 supra). It is up to the Appellant to convince the Board of the above identity. But the Appellant has not availed himself of the opportunity to dispel this doubt at the oral proceedings or otherwise. In this situation, the novelty of the polypeptide of claim 1 has to be acknowledged. On this basis, novelty can also be acknowledged for claim 34 comprising the same expression "consisting only of the amino acid sequence 1-174 set forth in Table VII". Claims 2 to 5, are directly or indirectly dependent on claim 1 so that there is no need to consider the presence of novelty separately from that of claim 1. Claims 24 to 27 are directed to a pharmaceutical composition or to the second/further medicinal use of the hpG-CSF of claim 1. No evidence is before the Board that the minimal quantities of hpG-CSF produced by cancer cell 5637 were available in a form suitable as a pharmaceutical. These claims thus also involve novelty. On the same basis can be acknowledged the novelty of claims 32 and 33, directed to muteins or allelic variants of hpG-CSF, and

of claim 35, covering pharmaceutical composition comprising them. Thus, the novelty (Article 54 EPC) of claims 1 to 5, 24 to 27, 29 and 32 to 35 has to be acknowledged.

Inventive step

Closest prior art

18. Human pluripotent granulocyte colony stimulating factor (hpG-CSF), a human hematopoietic growth factor, was known to exhibit a spectrum of activities including granulocyte colony-stimulating activity. This identified activity of hpG-CSF indicated that this growth factor could be useful in the treatment, inter alia, of granulocytopenia in patients receiving cytotoxic chemotherapy. A method for producing hpG-CSF from the conditioned medium of a bladder carcinoma 5637 cell was known from document (P40). This document already describes the isolation and partial characterization of hpG-CSF, however, without disclosing any amino acid sequence thereof. There is a link of continuity between document (P40) and the patent in suit because the latter also starts from hpG-CSF produced by cells of a bladder carcinoma cell line 5637 (see page 6, line 49) that is further purified and subjected to amino acid sequence analysis in order to design oligonucleotide probes useful to isolate the DNA and to render possible the production of hpG-CSF via the recombinant technology route according to the patent in suit. Thus, in the Board's view, document (P40) is the appropriate starting point for a problem/solution analysis.

Inventive step of claims 6 to 23, 28, 30 and 31

Problem to be solved

19. These claims are directed to DNAs encoding hpG-CSF, plasmids or viral vectors including these DNAs, hosts transfected with these plasmids or viral vectors and processes for the production of hpG-CSF by using these DNAs. There existed the problem that the technique for producing hpG-CSF from the conditioned medium of bladder carcinoma 5637 cells known from document (P40) resulted in very low concentrations of hpG-CSF. There existed also restrictions against the commercial use of Human Tumor Bank cells such as the human bladder carcinoma cell line 5637 (see patent in suit, page 4, lines 16 to 17). In view of this, the process described in document (P40) had to be considered to be unsuitable for the manufacture of hpG-CSF in quantities sufficient for use in therapy. Thus, it was desirable to provide hpG-CSF in a quantity sufficient for clinical investigation and medical purposes via the recombinant technology route. In conclusion, the technical objective problem can be stated, for the claims to the DNA sequences, as being making available the means or the tools to enable the manufacture of hpG-CSF in quantities sufficient to meet the demand for hpG-CSF for extended clinical studies and for therapeutical applications.

The solution

20. The Board is satisfied that the above problem has been solved by the present patent which provides the information and means necessary for identifying and cloning of DNA fragments coding for hpG-CSF and for expression of hpG-CSF.

21. The question thus arises whether the skilled person would have considered the use of recombinant DNA techniques for producing hpG-CSF with a reasonable expectation of success. One way to do this, was picking up a cDNA coding for hpG-CSF from a cDNA library. The method of choice was the use of oligonucleotide probes for screening gene banks, designed according to the amino acid sequence of the protein.

22. An essential element of the Appellant's objection of lack of inventive step is based on the assumption that unambiguous N-terminal sequence for at least 30 amino acids could have been obtained by the skilled person, provided **more** protein purified according to document (P40) had been subjected to amino acid sequencing. ICI Exhibit 2 and Dr Lottspeich's declaration (P45) were relied upon.

23. Upon reviewing ICI Exhibit 2, however, the Board observes that the procedure used therein for purification of hpG-CSF substantially diverges from the purification protocol disclosed by document (P40). This is because of the introduction of RP-HPLC steps with C4 or C18 columns not disclosed in document (P40) and of the use of rhpG-CSF, i.e., a product not available to the authors of document (P40), for tracking hpG-CSF through the purification procedure. Moreover, the amino acid sequencing results obtained (see Table 2 on page 8) are affected by errors and uncertainties. Thus, this experimental report does not support the above Appellant's proposition.

24. Dr Lottspeich's test report (see document (P45)) purports to demonstrate that before the earliest priority date of the patent in suit, the skilled person would have obtained reliable amino acid sequence information by sequencing **larger** quantities of the material from document (P40). He calculated in his

experimental report that if the inventor of the patent in suit, Dr Souza, had sequenced 50 pmol instead of the 25 pmol of the material of run 2 (see the patent in suit page 6, line 24) prepared according to the protocol disclosed in document (P40), he would have easily obtained up to about 30 unambiguous amino acid long sequence information instead of the highly ambiguous 22 amino acid long sequence of Table II of the patent in suit. However, Dr Lottspeich miscalculated the 5 µg subjected to amino acid sequencing in run 2 (patent in suit, loc. cit.) as corresponding to 25 pmol hpG-CSF. In fact, these 5 µg correspond to more than 250 pmol (for a protein having a m.w. of about 18,000 as hpG-CSF, 18,000 g = 18 x 10^9 µg = 1 mol = 10^{12} pmol, thus 0.9 µg = 50 pmol, hence 5 µg > 250 pmol). Thus, Dr Souza actually used much more material in his sequencing attempt than Dr Lottspeich calculated. Hence Dr Lottspeich's argument that Dr Souza had sequenced too low amounts of the material of document (P40) is also not convincing.

25. The Board must conclude that the skilled person could not have obtained unambiguous N-terminal sequence for at least 30 amino acids even if he/she had sequenced **more** protein purified according to document (P40). Nevertheless, the Parties agree that although no amino acid sequence information was available before the earliest priority date of the patent in suit, i.e. in 1985, when advanced techniques for amino acid sequencing of very small quantities of proteins had already become available, it was within the normal reach of the skilled person departing from the material purified according to the disclosure of document (P40) to obtain the amino acid sequence information of Tables I and II of the patent in suit. This is because on the one hand Example 1 of the patent in suit states that the amino acid sequence of Table I and II have been obtained "by literature methods" applied to a

sample of hpG-CSF "isolated according to Welte et al., Proc. Natl. Acad. Sci. USA Vol. 82, pages 1526-1530 (1985)", namely document (P40)(see lines 4 to 7). On the other hand, the Appellant, when arguing that the skilled person would have picked up the DNA encoding hpG-CSF without exercise of inventive ingenuity, relied on the test report ICI Exhibit 1 (see point 29 infra) and on its own test report dated 21 February 1992 (see point 30 infra), in which the probe had been designed in the light of the amino acid sequence of Table II of the patent in suit. This is an implicit admission that Table I and II of the patent in suit are representative of what amino acid sequence information the skilled person could have obtained at the priority date of the patent in suit.

Assuming that the skilled person was actually in a position to arrive at this amino acid sequence information provided by Table I or II of the patent in suit, it remains to be established whether or not this amino acid information was sufficient for the design of oligonucleotide probes to be used with a reasonable expectation of success. The Appellant cited three test reports in an attempt to demonstrate that the skilled person would have reasonably expected to clone the DNA encoding hpG-CSF by (1) a fully degenerate set of mixed oligonucleotide probes, (2) the so-called "long probe" or "guessmer" approach or (3) the inosine substitution approach. The three test reports are the Appellant's test report dated 21 February 1992, the ICI Exhibit 1 dated 19 February 1992 and Experiment C (document (K30)).

Fully degenerate set of mixed oligonucleotide probes

26. As regards the approach based on a fully degenerate set of mixed oligonucleotide probes, the Board notes that the amino acid sequences of Table I and Table II of the patent in suit, which as stated above could have been representative of what amino acid sequence information the skilled person could have obtained at the priority date of the patent in suit, not only comprise errors and uncertainties but also contain several amino acids for which a 6-fold (leucine (L), serine (S) and arginine (R)) or a 4-fold (proline (P), alanine (A), glycine (G), valine (V) and threonine (T)) codon degeneracy exists. Therefore, the preparation of a fully degenerate mixture of oligonucleotide probes sufficiently long to identify the DNA encoding for hpG-CSF would have involved an unreasonably large number of DNAs, with the consequence of a very high background radiation due to non-specific binding. In support of this view may be cited the fact that the inventor of the patent in suit, Dr Souza, would have needed a mixture of 1536 oligonucleotide probes designed in the light of a lesser degenerate region of hpG-CSF than the N-terminal end (see post published document (P34), page 61, right hand column, line 24), had he not incorporated inosines at three locations (see point 31 infra). In conclusion, the situation here is prohibitive and by no way comparable to the one described in document (P7), where accurate N-terminal amino acid sequence information for the first 29 amino acid of the protein was available (see Figure 5) and where low degeneracy probes could be used (see page 763, right hand column, line 19: "48-fold degenerate sets").

27. The Appellant relies upon the test report (K30) when arguing that the skilled person would have easily isolated the DNA coding for hpG-CSF by using a fully degenerate mixture of oligonucleotide probes designed according to the amino acid sequence of Table I. However, the Board notes that the technique adopted in test report (K30) does not rely on the use of a true fully degenerate mixture of oligonucleotide probes, otherwise a mixture of 884,736 different DNAs would have been needed for representing all possible codons for the eleven amino acids specified (4x4x6x4x4x4x6x6x6x4x2). Rather, the number of probes has been reduced from 884,736 to 512 by taking into account the Lathe preferred codon usage tables (see document (P13)). This expedient of reducing the number of probes, however, has never been disclosed by any prior art document. Further, in the probe of test report (K30), the codon representing Ser^7 (AGC) matches the one present in hpG-CSF of Table VII of the patent in suit, in spite of the fact that Table 5 of Lathe (document (P13)) states that TCC and TCT are the preferred codons for serine in DNAs coding for human proteins. The Board is thus left with the doubt that ex post facto information might have influenced the design of the probe used in this test report. Finally, test report (K30) is a test to determine whether some members of a set of 512 oligonucleotides probe hybridize to DNA coding for hpG-CSF which has been already cloned and **amplified** by polymerase chain reaction, a procedure not available at the priority date of the patent in suit. Therefore the test (K30) is not predictive on establishing whether or not the probe can be used to screen a cDNA library and to identify a cDNA encoding hpG-CSF.

"Long probe" or "guessmer" approach

28. It was argued by the Appellant that the skilled person would have easily isolated the DNA coding for hpG-CSF by using *a* "long probe" (guessmer) designed according to the teaching of Anderson et al. (document (P48)). A list of 18 publications (document (P49)) showed the successful application of the guessmer approach. However, the long probes of the Anderson's team had been designed in the light of the known complete amino acid sequence of BPTI. Therefore, the Anderson's team was able to chose areas of least degeneracy, whereas no such regions of least degeneracy for hpG-CSF were available to the skilled person who at most could have arrived at the N-terminal sequences of Table I (five amino acids with six-fold codon degeneracy) or Table II (nine amino acids with six-fold codon degeneracy). Furthermore, the great majority of the 18 publications cited by the Appellant for illustrating the success of the "guessmer" approach are concerned with the use of multiple probes rather than a single guessmer probe. These documents are, therefore, no evidence that the skilled person would have adopted the guessmer technique in the present case, once he/she had realised that N-terminal amino acid sequence of hpG-CSF was affected by a prohibitively high codon degeneracy and that no possibility of using more than a single probe existed.

29. As regards the test report ICI Exhibit 1, which according to the Appellant shows the successful use of a guessmer for identifying the DNA encoding hpG-CSF, the Board observes that the probe has been designed in the light of the amino acid sequence of Table II. Positions 1, 8 and 10 in Table II are now known to be correct, while the assignments for these residues in Table I are now known to be incorrect. Thus the Board

is left again with the doubt that ex post facto information might have biased the design of the probe used in this test report. Further, the screening in this test had been performed on a human monocyte library quite rich in the DNA looked for, which library was not available to the skilled person before the earliest priority date of the patent in suit. Finally, the 33-mer turned out to fail in this test because of high background noise (see page 8). In view of these facts, test report ICI Exhibit 1 does not convince the Board that the skilled person would have routinely isolated the DNA coding for hpG-CSF by using a "long probe" (guessmer).

30. The results of the Appellant's test report dated 21 February 1992 are also not convincing. They purport to show that a guessmer designed according to Lathe (document (P13)) is "twice as sensitive" as the probe used by the inventor of the patent in suit for isolating the DNA coding for hpG-CSF. However, this test is a Northern blot analysis (DNA/RNA hybridization) showing that the guessmer binds to a mRNA fraction. The Board is not convinced that this test could be predictive upon establishing whether or not the probe can be used to screen a cDNA library for identifying a cDNA encoding hpG-CSF. Moreover, the position by the Board pointed out under point 29 supra that ex post facto information has biased the design of the probe, also applies here since the probe used in this test report has also been designed in the light of Table II of the patent in suit.

Inosine replacement approach

31. This approach relies on the use of deoxyoligonucleotide probes carrying deoxyinosine residues at positions corresponding to the third position of the amino acid codon which is ambiguous. It is the Appellant's view that before the earliest priority date of the patent in suit the skilled person had a reasonable expectation of success in picking up the gene coding for hpG-CSF by using the inosine replacement approach disclosed by documents (P28) and (P35). Later document (P32) showed the successful application of the inosine substitution approach.

32. The Board observes that in the procedure disclosed by post published document (P32) (see page 41, line 24 to page 42, line 20), the positive clone pHCS-1 comprising a piece of DNA encoding hpG-CSF has been identified by the use of **two** probes, namely probe (IWQ) comprising inosines and probe (A), the latter being a degenerate short oligonucleotide probe (see ibidem, Figure 1). Thus, the conclusion cannot be drawn that document (P32) shows the successful application of the inosine substitution approach. As regards this technique, in the Board's view, there was little expectation of success by a skilled person when using this technique for isolating the gene coding for hpG-CSF. This was because it was not known whether the inosine replacement technique would have worked in the specific case faced for hpG-CSF, where one needed bonding of inosine with the base guanine (see document P(28), page 2607: "Inosine in a tRNA anticodon is known to form hydrogen bonds with A, C or U"). Further, document (P28) (see end of page 2607) warned that "In order to extend the applicability of this system, **a systematic analysis** of the stability of inosine-containing hybrids as a function of the pairing partner, the interval between the inosine residues, and the total length of

the oligonucleotide **is required**." (emphasis added). Therefore it cannot be said that the inosine substitution approach was a well established and straightforward tool at the earliest priority date of the patent in suit.

33. Finally, it has also to be noted that in any case the skilled person wishing to isolate the gene coding for hpG-CSF was faced with another complication. Even if he/she were lucky enough to obtain positive clones by using one of the above discussed three techniques, confirmation of a positive clone as being the DNA coding for hpG-CSF looked for was difficult if not impossible. This was because N-terminal amino acid information (Table I or II of the patent in suit, which as stated above, could have been representative of what amino acid sequence information the skilled person could have obtained at the priority date of the patent in suit) provided no information about the DNA upstream and downstream of the probe region.

34. In view of these findings, the Board has to conclude that the DNA sequence as claimed in claim 6 does not follows from the prior art in an obvious fashion. Claim 6 therefore fulfils the requirements of Article 56 EPC. Since the host cells, the plasmids or viral vectors including these DNAs, the hosts transfected with these plasmids or viral vectors and the processes for the production of hpG-CSF by using these DNAs of claims 7 to 18 and 20 to 23 all rely on the DNA of claim 6 found by the Board to involve an inventive step, these claims also satisfy the requirements of Article 56 EPC. This conclusion also applies to the DNAs encoding the muteins of hpG-CSF of claim 19 ([Ala^1]hpG-CSF) and claim 28 (hpG-CSF having one or more Cys replaced with Ala or Ser) and to

claim 30 directed to plasmids or viral vectors including the DNA of claim 28, and to claim 31 covering the hosts transfected with the plasmids or viral vectors of claim 30.

Inventive step of claims 1 to 5, 24 to 27, 29 and 32 to 35

Problem to be solved

35. Claim 1 is directed to a polypeptide which has part or all of the primary structure consisting only of the amino acid sequence 1-174 set forth in Table VII and one or more of the biological properties typical of naturally occurring hpG-CSF. As already emphasized under point 13 supra, the feature "consisting only of the amino acid sequence 1-174 set forth in Table VII" distinguishes it from hpG-CSF obtained by the known process starting from the conditioned medium of bladder carcinoma 5637 cells known from document (P40) since the latter yielded hpG-CSF consisting of a mixture of polypeptides having 174 and 177 amino acids in a molar ratio of about 80:20 produced by alternative splicing (i.e. it was "multiple species"), while rhpG-CSF according to claim 1 of the patent in suit is "single species". In relation to the product from bladder carcinoma 5637 cells of the closest prior art document (P40), the problem to be solved by the patent in suit can be stated as the provision of an alternative form of hpG-CSF, namely the "single species form" of hpG-CSF.

The solution

36. This problem is solved according to the present invention by expression in procaryotic or eucaryotic host cells the DNA of claim 6 to produce a recombinant hpG-CSF.

37. While in theory the way to solve this problem was obviously to use a recombinant route, the discussion above concerning claim 6 makes clear that this route involved an inventive step over the prior art. As, on the evidence, the provision of the "single species form" of hpG-CSF was only possible by solving the problem of developing a recombinant route, an inventive step can here be acknowledged for the provision of the alternative product.

38. On this basis, inventive step can also be acknowledged for claim 34 comprising the same expression "consisting only of the amino acid sequence 1-174 set forth in Table VII". Claims 2 to 5, are directly or indirectly dependent on claim 1 so that there is no need to consider the presence of an inventive step separately from that of claim 1. Claims 24 to 27 are directed to a pharmaceutical composition or to the second/further medicinal use of the hpG-CSF of claim 1. To make a pharmaceutical available would once again require the problem of finding a recombinant route to be solved, as was done with the provision of the DNA of Claim 6. These claims thus also involve an inventive step. On the same basis can be acknowledged the inventive step of claims 32 and 33, directed to muteins or allelic variants of hpG-CSF, and of claim 35, covering pharmaceutical composition comprising them. Thus, the inventive step (Article 56 EPC) of claims 1 to 5, 24 to 27, 29 and 32 to 35 has to be acknowledged.

39. In conclusion, the claims of the new main request are found to satisfy the requirements of the EPC.

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 claims for the Contracting States BE, CH, DE, FR, GB, IT, LI, LU, NL and SE filed as New Main Request at the oral proceedings on 15 July 1998 and the claims as granted for the Contracting State AT, and amended page 4 of the description as filed as New Main Request at the oral proceedings on 15 July 1998 and the remaining pages of the description and drawings as granted.

The Registrar: The Chairwoman:

D. Spigarelli U. M. Kinkeldey

ANNEX I

Glossary and list of acronyms

hpG-CSF:	human pluripotent granulocyte colony-stimulating factor
GM-CSF:	granulocyte macrophage colony-stimulating factor
rhpG-CSF:	recombinant human pluripotent granulocyte colony-stimulating factor
IL-3:	interleukin-3
BPTI:	bovine pancreatic trypsin inhibitor
m.w.:	molecular weight
HPLC:	high-pressure (performance) liquid chromatography
RP-HPLC:	reversed phase-HPLC.

ANNEX II

Claims (Non-AT)

Claims 1 to 37 as granted read:

1. A polypeptide which:

(a) has part or all of the primary structure and one or more of the biological properties typical of naturally-occurring human pluripotent granulocyte colony-stimulating factor (hpG-CSF) of the sequence set forth in Table VII,

(b) is a non-naturally occurring polypeptide; and

(c) is the product of procaryotic or eucaryotic expression of an exogenous DNA sequence.

2. A polypeptide according to Claim 1 wherein the exogenous DNA sequence is a cDNA sequence.

3. A polypeptide according to Claim 1 wherein the exogenous DNA sequence is a genomic DNA sequence.

4. A polypeptide according to Claim 1 wherein the exogenous DNA sequence is carried on an autonomously replicating DNA plasmid or viral vector.

5. A polypeptide according to Claim 1 further characterized by being covalently associated with a detectable label substance.

6. A polypeptide according to Claim 5 wherein said detectable label is a radiolabel.

7. A DNA sequence which codes upon expression in a procaryotic or eucaryotic host cell for a polypeptide product having at least a part of the primary structure and one or more of the biological properties of naturally-occurring pluripotent granulocyte colony-stimulating factor, said DNA sequence being selected from among:

(a) the DNA sequences set out in Table VII or the complementary strands thereof;

(b) DNA sequences which hybridize to the DNA sequences defined in (a) or fragments thereof; and

(c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize to the DNA sequences defined in (a) or (b) and which sequences code for a polypeptide having the same amino acid sequence.

8. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to Claim 7 in a manner allowing the host cell to express said polypeptide product.

9. A host cell according to Claim 8 wherein the host is E. coli

10. A host cell according to Claim 8 wherein the host is a mammalian cell.

11. A non-naturally occurring polypeptide product of the expression of a DNA sequence of Claim 7 in a procaryotic or eucaryotic host.

12. A cDNA sequence according to Claim 7.

13. A genomic DNA sequence according to Claim 7.

14. A DNA sequence according to Claim 7 and including one or more codons preferred for expression in E. coli cells.

15. A DNA sequence according to Claim 7 and coding for expression of human pluripotent granulocyte colony-stimulating factor.

16. A DNA sequence according to Claim 15 and including one or more codons preferred for expression in yeast cells.

17. A DNA sequence according to Claims 12 or 13 coding for expression of human pluripotent granulocyte colony-stimulating factor.

18. A DNA sequence according to Claim 7 covalently associated with a detectable label substance.

19. A DNA sequence according to Claim 18 wherein the detectable label is a radiolabel.

20. A single-stranded DNA sequence according to Claim 18.

21. A DNA sequence coding for (Ala^{1})-hpG-CSF.

22. A biologically functional plasmid or viral DNA vector including a DNA sequence according to Claim 7.

23. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to Claim 22.

24. A process for the production of a polypeptide having part or all of the primary structure and one or more of the biological properties of naturally occurring pluripotent granulocyte colony-stimulating

factor, which process is characterized by culturing under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with a DNA sequence according to Claim 7 in a manner allowing the host cell to express said polypeptide, and isolating desired polypeptide products of the expression of DNA sequence.

25. A process for the production of a polypeptide having the primary structure of human pluripotent granulocyte colony-stimulating factor, which process is characterized by culturing under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with a DNA sequence set forth in Table VII in a manner allowing the host cells to express said polypeptide, and isolating desired polypeptide products of the expression of DNA sequence.

26. A pharmaceutical composition comprising an effective amount of the polypeptide according to Claim 1 and/or produced by the process of Claim 24 or 25 and a pharmaceutically acceptable diluent, adjuvant or carrier.

27. A pharmaceutical composition according to Claim 26, further characterized by being free of association with any human protein.

28. Use of a polypeptide according to Claim 1 for the manufacture of a medicament for providing hematopoietic therapy to a mammal.

29. Use of a polypeptide according to Claim 1 for the manufacture of a medicament for arresting proliferation of leukemic cells.

30. A DNA sequence coding for a polypeptide analog of hpG-CSF having one or more cystein residues deleted or replaced by alanine or serine residues.

31. A non-naturally occurring polypeptide product of the expression in a procaryotic or eucaryotic host cell of a DNA sequence according to Claim 30.

32. A biologically functional plasmid or viral DNA vector including a DNA sequence according to Claim 30.

33. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to Claim 32.

34. A polypeptide according to Claim 1 preceded by a methionine residue.

35. A polypeptide having the hematopoietic biological properties of naturally occurring pluripotent granulocyte colony-stimulating factor, said polypeptide having an amino acid sequence selected from the polypeptide sequence forth in Table VII, or any allelic variants, derivatives, deletion analogs, substitution analogs, or addition analogs thereof, and characterized by being non-naturally occurring and by being the product of procaryotic or eucaryotic expression of an exogenous DNA sequence.

36. A non-naturally occurring polypeptide consisting only of the amino acid sequence 1-174 set forth in Table VII.

37. A pharmaceutical composition comprising an effective amount of the polypeptide according to Claim 35 or 36 and a pharmaceutically acceptable diluent, adjuvant or carrier.

ANNEX III

(P7) Gough et al., Nature, Vol. 309, pages 763 to 767 (1983)

(P13) Lathe, J. Mol. Biol., Vol. 183, pages 1 to 12 (1985)

(P21) Nagata et al., EMBO J., Vol. 5, pages 575 to 581 (1986)

(P24) Nicola et al., Immunology Today, Vol. 5, pages 76 to 80 (1984)

(P25) Nicola et al., J. Biol. Chem., Vol. 258, pages 9017 to 9023 (1983)

(P26) Nicola et al., Nature, Vol. 314 pages 625 to 628 (1985)

(P28) Ohtsuka et al., J. Biol. Chem., Vol. 260, pages 2605 to 2608 (1985)

(P30) EP-B-0 169 566

(P32) EP-A-0 215 126

(P33) English translation of JP 23777/85 filed 8 February 1985 (earliest priority document on which (P32) is based)

(P34) Souza et al., Science, Vol. 232, pages 61 to 65 (1986)

(P35) Takahashi et al., PNAS USA, Vol. 82, pages 1931 to 1935 (1985)

(P40) Welte et al., PNAS USA, Vol. 82, pages 1526 to 1530 (1985)

(P41) Wong et al., Science, Vol. 228, pages 810-815 (1985)

(P45) Declaration of Dr Lottspeich dated 28 December 1993 (Appellant)

(P48) Anderson et al., PNAS USA Vol. 80 6838-6842 (1983)

(P49) Annex to document (P48) comprising a list of 18 articles

(K30) Experiment C filed on 28 March 1994 by Opponent II

(K33) Komatsu et al., Jpn J. Cancer Res. Vol. 78, pages 1179 to 1181 (1987)

(K45) Fung et al., Nature, Vol. 307, pages 233 to 237 (1984)

Exhibit (7) WO-A-85/02610

Exhibit (8) EP-A-0 123 294

Exhibit (15) Zsebo et al., Immunobiol., Vol. 172, pages 175 to 184 (1986)

Exhibit (16) Nagata, Bio Assays, Vol. 10, pages 113 to 117 (1990)

Exhibit (17) Metcalf, Cancer, Vol. 65, pages 2185 to 2195 (1990)

Exhibit (25) Hamblin, Lymphokines, IRL Press, Oxford, Washington, pages 25, 26, 32, 33 (1988)

ICI Exhibit 1 Test Report annexed to Dr Graham's declaration dated 19 February 1992 submitted by Opponent I

ICI Exhibit 2 Test Report annexed to Dr Camble's declaration dated 19 February 1992 submitted by Opponent I

Appellant's Test Report is a Test Report filed by Opponent V on 21 February 1992