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Datasheet for the decision of 13 May 2016

Case Number: T 0125/12 - 3.3.02

Application Number: 99971863.8

Publication Number: 1124984

IPC: C12P21/02, C12N15/19, C07K14/505, C12N5/06, C12N15/63

Language of the proceedings: EN

Title of invention:
HOST CELLS EXPRESSING RECOMBINANT HUMAN ERYTHROPOIETIN

Patent Proprietor:
Sterrenbeld Biotechnologie North America, Inc.

Opponent:
Polymun Scientific
Immunbiologische Forschung GmbH

Headword:
Host cells/STERRENBELD

Relevant legal provisions:
EPC Art. 100(a), 100(b), 56, 83

Keyword:
Requirements of the EPC met (yes)

Decisions cited:

Catchword:

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Case Number: T 0125/12 - 3.3.02

D E C I S I O N
of Technical Board of Appeal 3.3.02
of 13 May 2016

Appellant: (Opponent)	Polymun Scientific Immunbiologische Forschung GmbH Nussdorfer Lände 11 1190 Wien (AT)
Representative:	Bogensberger, Burkhard Bogensberger Patent- und Markenbüro Fallsgasse 7 9492 Eschen (LI)
Respondent: (Patent Proprietor)	Sterrenbeld Biotechnologie North America, Inc. 1209 Orange Street Wilmington, DE 19801 (US)
Representative:	MacLean, Martin Robert Mathys & Squire LLP The Shard 32 London Bridge Street London SE1 9SG (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 10 October 2011 rejecting the opposition filed against European patent No. 1124984 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	U. Oswald
Members:	K. Giebeler
	L. Bühler

Summary of Facts and Submissions

I. European patent No. 1 124 984, based on European patent application No. 99971863.8 (published as WO 00/28066) and entitled "Host cells expressing recombinant human erythropoietin", was granted with ten claims.

II. The claims as granted read:

"1. A host cell comprising first and second vectors, wherein said first vector comprises:
(i) a nucleotide sequence encoding the erythropoietin polypeptide consisting of the amino acid sequence in SEQ ID NO: 1, wherein said nucleotide sequence does not include 5' and 3' non-coding regions of the EPO gene;
(ii) a viral promoter; and
(iii) a viral terminator;
and wherein said second vector comprises a nucleotide sequence encoding dihydrofolate reductase (DHFR).

2. The host cell deposited as DSM ACC2397.

3. The host cell of claim 1, wherein said viral promoter and viral terminator comprises an early promoter and terminator of a SV40 virus.

4. The host cell of claim 1, wherein said first vector comprises pVex 1, deposited as DSM 12776.

5. The host cell of claim 1, wherein said second vector comprises pDHFR, deposited as DSM 12777.

6. The host cell of claim 1, wherein said host cell is resistant to neomycin-derived antibiotics and methotrexate.

7. The host cell of claim 1, wherein said host cell is a mammalian cell.

8. The host cell of claim 1, wherein said host cell comprises a CHO cell.

9. A method for producing an EPO polypeptide, comprising:
(a) culturing the host cell of claim 1 in medium with methotrexate;
(b) isolating viable cells from step (a);
(c) amplifying the cells from step (b) in medium without methotrexate; and
(d) isolating said polypeptide from the cell supernatant in step (c).

10. The method of Claim 9, further comprising isolating viable cells (b) that produce said polypeptide at a concentration of 50 mg of EPO per litre of medium per day."

III. Notice of opposition was filed against the granted patent, on the grounds of lack of inventive step (Article 100(a) EPC) and insufficiency of disclosure (Article 100(b) EPC).

IV. The opposition division decided to reject the opposition.

V. The opponent (hereafter: appellant) lodged an appeal against the opposition division's decision.

VI. The patent proprietor (hereafter: respondent) filed counter-arguments to the appeal.

VII. On 30 November 2015, the board issued a communication as an annex to the summons to oral proceedings, expressing its preliminary opinion.

VIII. With letter of 13 April 2016, the respondent filed an auxiliary request.

IX. Oral proceedings before the board were held on 13 May 2016.

X. The following documents are mentioned in this decision:

D2: Yanagi et al., J. Ferment. Bioeng. (1989) 68:257
D3: US 5,618,698
E1: Powell et al., P.N.A.S. (1986) 83:6465
F1/E11: US 5,688,679
F2: Park et al., Molecular Biology (2001) 35:413

XI. The appellant's arguments, insofar as they are relevant for the present decision, can be summarised as follows:

Sufficiency of disclosure - Article 100(b) EPC

The claimed invention was not sufficiently disclosed and not commensurate with the inventors' contribution to the art. Whereas it could be accepted that the deposited host cell of claim 2 was patentable and sufficiently disclosed, claim 1 was too broad because it encompassed host cells with an unlimited number of variations with respect to the cells, vectors, promoters and terminators to be used, and the skilled person was left in doubt as to whether all of these conceivable variations would qualify for carrying out the claimed invention. There was only one single example in the patent in suit, namely Example 7, which described a cell according to claim 1. Although

performing the claimed invention for a host cell system other than the host cell line disclosed in Example 7 was a routine task in principle, it nevertheless amounted to a tremendous amount of work and effort, and thus undue burden, to find such a high Epo-producing cell line. Moreover, the post-published document F2 referred to by the respondent reported only very low levels of Epo. Already during the examination proceedings, the examining division had expressed serious doubts as to the essential features required to achieve the high production levels of Epo reported in the application as filed, and these doubts had never been overcome by the respondent. It was thus not necessary for the appellant to prove that the disclosure of the patent in suit was not enabling over the full scope of the claims.

Inventive step - Article 56 EPC

The claimed subject-matter did not involve an inventive step. There was no evidence in the patent in suit for a causative correlation between the removal of the untranslated regions (UTRs) and the surprisingly high Epo product yield reported in Example 7 of the patent in suit. Moreover, the post-published document F2 did not provide such evidence either, because the product yields reported therein were two orders of magnitude lower than in the patent in suit. Therefore, additional factors not stated in the claims had to be responsible for the high product yields reported in Example 7.

The host cells of claim 1 differed from those of documents F1/E11, E1 or D3 merely in the removal of the UTRs. This feature was however well known from the prior art, which described the use of Epo cDNA, i.e. Epo gene constructs without UTRs present. Furthermore,

there was no general prejudice in the art against the removal of UTRs from the Epo gene sequence in order to reach high protein expression levels. Consequently, the requirements of Article 56 EPC were not fulfilled.

XII. The respondent's arguments, insofar as they are relevant for the present decision, can be summarised as follows:

Sufficiency of disclosure - Article 100(b) EPC

The requirement for a sufficient disclosure was fulfilled because the skilled person was enabled to perform the claimed invention by cutting off the UTRs from the Epo-encoding nucleotide sequence. This structural change could easily be put into effect without any undue burden. Moreover, the selection of suitable cells was conventional and well known from the prior art, as confirmed by documents F2/E11, E1 and D2. The expression levels described in Example 7 of the patent in suit were comparable to those in the prior art, and there was no evidence on file showing that such levels would not be achieved with cells other than those used in said example.

Inventive step - Article 56 EPC

The claimed subject-matter involved an inventive step over the closest prior art represented by document F1/E11. The problem to be solved was the provision of a simplified alternative for high-yield Epo production, as correctly formulated in the opposition division's decision. The solution to the problem as provided by claim 1 was inventive because the prior art consistently taught that the 5' and 3' UTRs were essential for Epo gene expression. Moreover,

document F2 confirmed that the deletion of the 5' and 3' non-coding regions of the Epo gene led to improved expression levels.

XIII. The final requests of the parties were:

The appellant requested that the decision under appeal be set aside and that the patent be revoked.

The respondent requested that the appeal be dismissed, or, alternatively, that the patent be maintained in amended form on the basis of the auxiliary request filed with letter of 13 April 2016.

Reasons for the Decision

1. The appeal is admissible.

Main request

Sufficiency of disclosure - Article 100(b) EPC

2. Claim 1 relates to a host cell comprising
- a first vector which comprises (i) a nucleotide sequence encoding the erythropoietin (hereafter: Epo) polypeptide consisting of the amino acid sequence in SEQ ID NO: 1, wherein said nucleotide sequence does not include 5' and 3' non-coding regions of the Epo gene; (ii) a viral promoter; and (iii) a viral terminator; and
- a second vector which comprises a nucleotide sequence encoding dihydrofolate reductase (DHFR).

Claim 9 relates to a method for producing an Epo polypeptide, comprising *inter alia* a step of culturing

the host cell of claim 1. Claim 10 is dependent on claim 9 and requires a step of isolating viable cells that produce the Epo polypeptide at a concentration of 50 mg per litre of medium per day.

3. The examples of the patent in suit report the cloning of the Epo coding sequence into the expression vector "pVex1" (Example 5) and the subsequent co-transfection of DHFR-deficient CHO cells with the vector thus obtained ("pVex-EPO") and a vector including a DHFR coding sequence ("pDHFR"), followed by a selection procedure using geneticin (G418) and methotrexate, which ultimately resulted in the selection of a clone of recombinant host cells producing 50 mg Epo per litre of culture medium per day (Examples 6 and 7). This host cell was deposited under the accession number DSM ACC2397.

4. The appellant has submitted that the host cell of claim 1 was not sufficiently disclosed, because it would represent an undue burden for the skilled person to find a high-producer cell line of Epo other than the deposited cell line of Example 7 of the patent in suit.

5. Neither independent claim 1 relating to a host cell nor independent claim 9 relating to a method for producing an Epo polypeptide requires that the host cell referred to produces Epo at any particular (high) concentration. Therefore, the board considers that there are no reasons to doubt that the invention claimed in claims 1 and 9 can be carried out by a person skilled in the art.

6. The method of dependent claim 10, however, comprises a step of isolating viable cells that produce Epo polypeptide at a concentration of 50 mg of Epo per

litre of medium per day. Hence the question arises as to whether or not, on the basis of the disclosure of the patent in suit and/or common general knowledge, the skilled person would be able, without undue burden, to perform the step of isolating viable cells that produce Epo at the specified concentration.

6.1 Whereas Example 6 of the patent in suit reports that a CHO (Chinese Hamster Ovary) cell line deficient in the DHFR gene was used for the co-transfection and selection procedure, page 6, lines 20-21 additionally refers to COS, BHK, Namalwa and HeLa cells as host cells which are especially preferred. The general part of the description furthermore refers to a number of preferred vectors, promoters and terminators other than those used in the specific constructs of the examples, see paragraphs [0045]-[0058] on pages 5 and 6.

6.2 The board further notes that at the priority date of the patent in suit, host cells which produce Epo at concentrations in the same order of magnitude as the concentration referred to in claim 10 had been described, see documents F1/E11 and E1. Both documents report the transformation of BHK cells with appropriate vectors and subsequent selection of high Epo-producing cells using methotrexate, resulting in cells which produce 80 mg of Epo per litre of culture medium in 24 hours (see document F1/E11, Examples 4 and 5; document E1, page 6467, column 2, paragraph 1). Additionally, the principle of using vectors comprising a DHFR gene and selection with methotrexate in order to achieve high-level expression of Epo was mentioned in documents D2 (see page 260, column 1, last four lines) and D3 (see column 26, lines 38-56).

6.3 The board acknowledges that at the priority date, isolating host cells which produce Epo at concentrations as high as 50 mg per litre of medium per day, other than those host cells that were already available, required time and effort. However, the necessary screening and selection procedures were in principle well known and had been successfully applied, not only in the patent in suit but also in the prior art (see documents F1/E11 and E1). It is furthermore undisputed between the parties that the skilled person would be able to remove the 5' and 3' non-coding regions of the Epo gene on the basis of his/her common general knowledge.

In this situation, the board is convinced that the patent in suit provides a technical teaching which placed those skilled in the art in a position to reproduce the claimed invention, possibly in a time-consuming and cumbersome way, but without undue burden.

7. As concerns the appellant's submission that claim 1 did not state the essential features of the invention, the board observes that in cases like the present one, where there are no serious doubts, substantiated by verifiable facts, that the claimed invention is reproducible, the question as to whether or not a claim states the essential features of the invention is entirely a matter of Article 84 EPC which does not constitute a ground for opposition (Article 100 EPC).

8. For these reasons, the board considers that the information provided in the patent in suit is sufficiently clear and complete to enable a skilled person to carry out the claimed invention.

Consequently, there are no reasons for the board to conclude that the disclosure of the claimed invention is not sufficient within the meaning of Article 100(b) EPC.

Inventive step - Article 56 EPC

9. The closest prior art is represented by document F1/E11, a US patent document which relates to the expression of high levels of biologically active human Epo from stably transfected cells. An ApaI restriction fragment of the human Epo gene, which included untranslated sequences both at its 5' and 3' ends (see column 3, lines 62-64 and column 4, lines 2-9), was inserted into a plasmid expression vector comprising an adenoviral promoter and terminator (column 4, lines 18-44). To establish stable cell lines producing high levels of Epo, BHK cells were co-transfected with the resulting plasmid and a second plasmid containing a cDNA for dihydrofolate reductase (column 5, lines 52-67). Cells obtained were subsequently challenged with very high levels of methotrexate (column 6, lines 15-24). Selected cell lines secreted up to 7000 units Epo per millilitre into the supernatant, corresponding to amounts of up to 80 µm/ml (column 6, Table 2; column 7, line 64 to column 8, line 4).

10. The host cell of claim 1 differs from those described in document F1/E11 solely in that the Epo-encoding nucleotide sequence does not include 5' and 3' non-coding regions.

11. Starting from document F1/E11 as the closest prior art, the technical problem to be solved is the provision of an alternative host cell suitable for Epo production.

11.1 The respondent submitted that the post-published document F2 showed that the deletion of the 5' and 3' non-coding sequences of the EPO gene resulted in increased expression levels.

However, it is not plausible from the application as filed underlying the patent in suit that the deletion of the 5' and 3' non-coding sequences of the Epo gene results in increased expression levels. Therefore, the improvement shown in document F2 may not be taken into account when formulating the technical problem (cf. point 11 above).

12. As a solution to the problem posed, claim 1 proposes a host cell comprising a vector comprising an Epo-encoding nucleotide sequence which does not include 5' and 3' non-coding regions of the Epo gene.

13. Having regard to the description of the patent in suit and in particular to its Example 7, the board is satisfied that the problem has indeed been solved.

14. It remains to be examined whether the claimed solution involves an inventive step.

15. Several prior-art documents describe host cells suitable for the production of Epo.

Document F2/E11, the closest prior art, states that the ApaI restriction fragment of the human Epo gene, which includes 5' and 3' non-coding regions, "was selected to maximize efficient transcription of erythropoietin messenger RNA and effective translation and post-translational modification of the RNA into mature biologically active erythropoietin glycoprotein" (column 2, lines 20-14). The document further indicates

that "at the 5' end of the erythropoietin gene it was important to remove interfering sequences but retain enhancing sequences" (column 2, lines 25-27) and that "some 3' untranslated sequences were retained to optimize putative regulating sequences" (column 2, lines 29-31). Column 4, lines 2-9 again stresses that the 5' and 3' untranslated sequences were included in order to retain putative regulatory sequences and processing signals. The board considers that based on the teaching of this document, the skilled person faced with the problem posed would not remove the 5' and 3' non-coding regions, and thus would not arrive at the subject-matter of claim 1.

Document D2 compares the expression efficiency of Epo cDNA and Epo genomic DNA in otherwise identical expression plasmids. The lower translational efficiency for the plasmid containing the Epo cDNA is assumed to be related to the replacement of the immediately upstream region of the ATG initiation codon of the cDNA by a *Bgl*II recognition sequence leading to the loss of an essential purine residue at position -3. The authors conclude that they "can expect a higher expression level by using a cDNA sequence with the natural 5' non-coding region" (page 259, column 2, lines 4-7). In view of this disclosure, a skilled person faced with the problem posed would thus retain at least the 5' non-coding region of the Epo gene, and would thus not arrive at the subject-matter of claim 1.

Additionally, document D3 discloses Epo DNA clones including 5' and 3' untranslated regions (see Figures 5 and 6) and refers to "5' and 3' DNA sequences which may be significant to promoter/operator functions of the human gene operon" (column 21, lines 18-21).

To sum up, said prior-art documents teach that 5' and/or 3' non-coding regions of the Epo gene either were intentionally retained when aiming to produce the Epo polypeptide or should be included in order to achieve higher Epo expression levels. The board is convinced that in this situation a skilled person would not have solved the problem posed by providing the claimed host cells, which comprise an Epo-encoding nucleotide sequence that does not include 5' and 3' non-coding regions of the Epo gene.

16. The appellant submitted that the skilled person would have been well aware of using Epo gene constructs without 5' and 3' non-coding regions, because the prior art disclosed the use of Epo cDNA constructs.

The board cannot follow this line of argument and agrees with the respondent's submission that cDNA, by virtue of being produced by reverse transcription of mRNA, includes 5' and 3' non-coding regions.

Therefore, the prior-art disclosure of Epo production using cDNA constructs cannot render the claimed host cell obvious.

17. In view of these considerations, the board comes to the conclusion that the host cell of claim 1 involves an inventive step (Article 56 EPC).

The same applies to the host cells of dependent claims 2 to 8 and to the methods of claims 9 and 10, which involve the culturing of the host cell of claim 1.

18. It follows that the main request fulfils the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

N. Maslin U. Oswald

Decision electronically authenticated