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# Datasheet for the decision of 1 July 2022

**Case Number:** T 2695/18 - 3.3.08

**Application Number:** 06847508.6

**Publication Number:** 1957630

**IPC:** C12N5/02, C07K14/505

**Language of the proceedings:** EN

**Title of invention:**
Improved production of glycoproteins using manganese

**Patent Proprietor:**
Amgen Inc.

**Opponent:**
Potter Clarkson LLP

**Headword:**
Production of EPO/DPO using manganese/AMGEN

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

**Keyword:**
Main request - sufficiency of disclosure - (yes)
Main request - inventive step, try-and-see - (not applicable)

**Decisions cited:**
G 0001/03, G 0003/14, T 0985/98, T 1172/06

**Catchword:**

Europäisches Patentamt
European Patent Office
Office européen des brevets

**Beschwerdekammern**

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**Case Number: T 2695/18 - 3.3.08**

# D E C I S I O N
# of Technical Board of Appeal 3.3.08
# of 1 July 2022

| | |
|---|---|
| **Respondent:** (Patent Proprietor) | Amgen Inc.<br>One Amgen Center Drive<br>Thousand Oaks, CA 91320-1799 (US) |
| **Representative:** | André Guder<br>Uexküll & Stolberg<br>Partnerschaft von<br>Patent- und Rechtsanwälten mbB<br>Beselerstraße 4<br>22607 Hamburg (DE) |
| **Appellant:** (Opponent) | Sara Holland<br>Potter Clarkson LLP<br>The Belgrave Centre<br>Talbot Street<br>Nottingham NG1 5GG (GB) |
| **Decision under appeal:** | **Interlocutory decision of the Opposition Division of the European Patent Office posted on 13 September 2018 concerning maintenance of the European Patent No. 1957630 in amended form.** |

**Composition of the Board:**

| | |
|---|---|
| **Chairman** | B. Stolz |
| **Members:** | M. Montrone |
| | A. Bacchin |

## Summary of Facts and Submissions

I. The appeal lies against the decision of an opposition division to maintain the European patent No. 1 957 630 in amended form. The patent is based on European patent application No. 06847508.6, which has been published as International patent application WO 2007/070315 (the "patent application").

II. The opposition division held in the decision under appeal that the main request added subject-matter, while auxiliary request 1 lacked novelty. Auxiliary request 2 was withdrawn during the oral proceedings. Auxiliary request 3 was held to comply with the requirements of the EPC.

III. Both parties (patent proprietor and opponent) appealed the decision. Since, however, the patent proprietor did not submit a statement of grounds of appeal, their appeal was found inadmissible under Article 108, third sentence, EPC in conjunction with Rule 101(1) EPC.

IV. In the following, the opponent will be addressed as appellant and the patent proprietor as respondent.

V. With their statement of grounds of appeal, the appellant submitted arguments as to why the subject-matter of auxiliary request 3 added subject-matter, was insufficiently disclosed, and lacked an inventive step. Furthermore, a new document was submitted.

VI. In reply, the respondent submitted auxiliary requests I to XIII.

VII. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion.

VIII. In their replies, the appellant provided further arguments under lack of inventive step, while the respondent provided counter arguments.

IX. Oral proceedings before the board were held on 1 July 2022 in the form of a videoconference.

X. Independent claims 1 and 17 of the main request read:

"1. A method for producing an erythropoietic composition comprising sialylated erythropoiesis-stimulating glycoproteins, comprising the steps of: growing a manganese-responsive CHO host cell which produces an erythropoiesis stimulating glycoprotein in a culture medium containing an amount of manganese effective to increase the sialylation of said erythropoietic composition produced by said manganese-responsive host cell, wherein the concentration of manganese in said culture medium ranges from about 0.4 to about 40 µM, and wherein the erythropoiesis-stimulating glycoproteins comprise

(a) the amino acid sequence of SEQ ID NO:3 (erythropoietin) or erythropoietic fragments thereof; or

(b) the amino acid sequence of SEQ ID NO:2 (darbepoetin) or erythropoietic fragments thereof.

17. A culture medium comprising CHO host cells producing erythropoiesis stimulating glycoproteins and manganese at a concentration from about 0.4 to about 40

μM, wherein the erythropoiesis-stimulating glycoproteins comprise

(a) the amino acid sequence of SEQ ID NO:3 (erythropoietin) or erythropoietic fragments thereof;

(b) the amino acid sequence of SEQ ID NO: 2 (darbepoetin) or erythropoietic fragments thereof".

XI. The following documents are referred to in this decision:

D1: WO 95/05465 (published 23 February 1995);

D3: Raju T.S., *et al.*, Biochemistry, 2001, Vol. 40, 8868-8878;

D5: Crowley J.D., *et al.*, "Enzymes and Proteins Containing Manganese: An Overview", in "Metal Ions in Biological Systems", Vol. 37, "Manganese and its Role in Biological Processes", Marcel Dekker, Inc., 2000, eds. A. Sigel and H. Sigel, 209-278;

D17: WO 00/65070 (published 2 November 2000);

D18: WO 01/81405 (published 1 November 2001);

D21: PhD thesis of Stefan Nahrgang, 2002, 1-151, Ecole Polytechnique de Lausanne;

D36: Crowell K.C., *et al.*, Biotechnology and Bioengineering, 2006, Vol. 96(3), 538-549;

D47: Excerpt of "Erythropoietins and Erythropoiesis", 2003, Ed.: G. Molineux, M.A. Foote, S.G. Elliott, 133-150.

XII. The appellant's submissions, insofar as relevant to the present decision, may be summarised as follows:

*Main request*

*Added subject-matter*

The range "*from about 0.4 to about 10 µM*" cited in claims 7 and 19, and in paragraphs [0014], [0021], and [0037] of the patent added subject-matter. According to the decision T 985/98, the combination of limit values from a preferred range (here: "*0.1 to 10 µM*" in claim 11 as filed) with a general range (here: "*0.01 to 40 µM*" in claim 1 as filed) was allowed only, if the new range was from "*0.01 to 10 µM*" or "*0.1 to 10 µM*", but not from "*0.4 to 10 µM*". Nor had the lower limit of "*0.4*" in the contested range a basis in the paragraph bridging pages 13 and 14 of the application as filed. This passage disclosed lists of various lower and upper limits of manganese ($Mn^{2+}$) concentrations without pointing to a preferred range or value.

*Sufficiency of disclosure*

Claim 1 referred to a method for producing a composition comprising sialylated erythropoietin (EPO) or darbepoetin (DPO), or their respective erythropoietic fragments. This aim was achieved by "*growing a manganese-responsive CHO host cell*" in a culture medium characterised by the $Mn^{2+}$ concentration range "*from about 0.4 to about 40 µM*". This range was indicated as being *"effective to increase the sialylation*" of EPO and DPO.

Based on the application as filed, it was for several reasons not plausible that EPO/DPO with increased sialylation was obtained by the method across the whole breath of the claim.

- The application as filed did not determine the absolute increase of sialylation per EPO/DPO molecule, but a relative increase only. Sialylation of EPO/DPO was dependent on two different and independent glycosylation reactions. The first attached oligosaccharide chains to specific amino acids within EPO/DPO at so-called "N-sites" and/or "O-sites", without adding any sialic acid residues to these chains. Sialic acid was attached terminally to the oligosaccharide chains only as a last step during the second glycosylation reaction. Thus, an increase in "O-site" occupancy as shown in Figures 4 to 6, and 10 of the patent application did not necessarily result in an increased sialylation, because both reactions were independent from each other. Consequently, the results of Figures 4 to 6, and 10 were not suitable as evidence that $Mn^{2+}$ increased the sialylation level of EPO/DPO. An increased sialylation level of EPO/DPO was shown in Figures 1 to 3, 8, and 9 of the patent application only.

- The method used for determining an increased sialylation level of EPO/DPO was inappropriate for this purpose. This method separated by chromatographic means high from low sialylated EPO/DPO. Figures 1 and 2 of the patent application disclosed the results for lower and higher sialylated EPO fractions after their elution from the chromatography column. Only 31.39% of the total amount of sialylated EPO was eluted from the

column, which left 68.61% bound to it, i.e. the majority irrespective of EPO's sialylation level. Furthermore, Figure 3 disclosed that the increase in sialylated EPO was from 32.8% to 36.3% (see earlier patent application, page 28, line 1), i.e. very low (3.5%). In view of the huge loss of total EPO/DPO that remained bound to the column, such a low increase was statistically insignificant.

- A comparison of Figures 5 and 6 of the patent application showed that the use of 0,4 µM $Mn^{2+}$ did not increase O-site occupancy (as an indicator of sialylation) of DPO. This was evident from the lower O-site occupancy at 0,4 µM $Mn^{2+}$ in Figure 6 when compared to the control in Figure 5. Likewise the results of the experiments shown in Figures 8 and 9 were inconsistent. While Figure 8 disclosed a decrease of highly sialylated EPO compared to the control when 40 µM $Mn^{2+}$ was used, Figure 9 disclosed a reduction of lower sialylated EPO. This data went against the teaching of the patent application which reported that a decrease in higher sialylated EPO was correlated with an increase of lower sialylated EPO.

- Solely Figures 1 to 3, 8, and 9 of the patent application disclosed the sialylation level of EPO/ DPO, and were, hence, suitable for supporting a potential $Mn^{2+}$ effect on sialylation. These Figures, however, disclosed results from experiments that used 4 µM $Mn^{2+}$ only (see Figures 1 to 3), or showed that 40 µM $Mn^{2+}$ had no effect on increasing the sialylation level of EPO (see Figure 8). Thus, the patent application did not provide experimental evidence that $Mn^{2+}$ achieved an

increased sialylation across the whole concentration range cited in claim 1.

- The patent application disclosed the use of two CHO cell lines in the claimed method. However, one of these cell lines only produced EPO/DPO with increased sialylation. Example 3 mentioned that $Mn^{2+}$ had no effect on the fraction of lower sialylated DPO in a CHO cell line adapted for growth in suspension culture in large tanks and/or adapted to suspension culture in serum-free medium. The same was true for the higher sialylated DPO fraction, since the patent application taught that the amount of lower sialylated EPO/DPO correlated with that of higher sialylated EPO/DPO. Thus, not all CHO cell lines were suitable for the claimed method. The patent application did, however, not disclose criteria for finding suitable CHO cells. In these circumstances, the skilled person was left with trial and error in finding these cells which amounted to undue burden.

- The claimed method was not limited to a particular harvest cycle. However, the patent application did not disclose an increase of sialylated EPO/DPO after each harvest cycle, in particular not after the first and the second harvest. Example 3 (see page 26, lines 10 to 12, page 27, line 21 to page 28, line 2), for example, solely mentioned an increase after the third harvest (see paragraph bridging pages 27 and 28). Although Figure 3 disclosed data for the first and second harvest too, the increase was insignificant in view of the majority of EPO/DPO that remained bound to the column material (see above). Furthermore, post-published evidence of the respondents disclosed

that the addition of $Mn^{2+}$ to culture medium had no effect during the first and the second harvest cycle (see document D36, page 547, column 1, first paragraph).

- Furthermore, an increased sialylation was not achievable over the whole scope claimed because claim 1 lacked a reference point for determining such an increase.

- The application as filed stated on page 13, third paragraph, and page 30, second paragraph that 40 µM $Mn^{2+}$ was toxic for the cells as shown by a drop of 17% of secreted DPO. Thus, also for toxicity reasons, $Mn^{2+}$ was not suitable to achieve an increased sialylation across the whole concentration range claimed.

*Inventive step*

The skilled person was a molecular biologist or team of biologists that were familiar with EPO and DPO, the proteins' production in cell culture, the process of sialylation, and the beneficial effect of sialic acids on EPO/DPO's serum-half life. Moreover, all enzymes involved in the sialylation process were known, including the enzymes' dependency on $Mn^{2+}$ as essential co-factor (see e.g. document D5, Table 1). It belonged thus to the common general knowledge of the skilled person that $Mn^{2+}$ played a key role in sialylation (see document D21, page 18). In addition, it was common general knowledge that the culturing of EPO-producing CHO cells required medium containing trace amounts of $Mn^{2+}$ (about 0.05 to 0.06 µM: see e.g., documents D1, and D18). Moreover, this culturing required sterile conditions, and was a complex task. For these reasons

the skilled person avoided deviations from the established EPO/DPO production process, unless such deviations were necessary (see document D47, pages 15, 133, and 144).

Document D1 represented the closest prior art. The claimed method lacked an inventive step in light of the teaching of this document alone taking the skilled person's common general knowledge into account. Alternatively, the claimed method was obvious in light of document D1's teaching combined with documents D3, or D17.

The claimed method differed from document D1 only in using higher $Mn^{2+}$ concentrations. Even if the technical problem was the provision of an improved method for producing a composition of EPO/DPO, or biologically active fragments thereof, with an increased sialylation, the use of $Mn^{2+}$ in the claimed concentration range was obvious to the skilled person. This was so because it was common general knowledge that $Mn^{2+}$ was an essential co-factor for the enzymes involved in sialylation (see e.g. document D5). The addition of simply more $Mn^{2+}$ to the culture medium was therefore straightforward, and obvious to the skilled person.

Alternatively, the skilled person starting from the method disclosed in document D1 in seek of further ways for increasing the sialylation level of EPO/DPO would have turned to document D3. This document disclosed that the sialylation of TNFR-IgG as a model glycoprotein was increased by an *in vitro* treatment using 5 µM $Mn^{2+}$ in combination with two transferases. Since this document provided a proof of concept that $Mn^{2+}$ increased sialylation, the skilled person would

have added 5 µM $Mn^{2+}$ to the culture medium of document D1 too. This was an obvious step, because no switch to an *in vitro* process was required, which avoided additional method steps and manipulations under sterile conditions. Moreover, the whole process remained in the area of the skilled person's normal practise. Any switch from a pure cell culturing process to an *in vitro* manipulation as mentioned in document D3 did run counter to the concept of the skilled person as set out in the case law (see e.g. T 867/13). The skilled person seeking modifications of the prior art along routine procedures followed merely a "*try-and-see*" exercise by adding $Mn^{2+}$ to the cell culture in the concentration mentioned in document D3 (see T 111/00). In particular, since document D3 disclosed $Mn^{2+}$ as a key factor in increasing sialylation, and lacked any teaching that prevented the skilled person from following this route. The relevant questions to be posed were: "*Would a skilled person be motivated by the prior art to embark on developing the claimed invention?*" "*Or is there anything that would have prevented him/her from taking this approach and achieving it by routine experimentation?*"

Alternatively, the claimed method was obvious in light of the combined teaching of documents D1 and D17. Document D1 disclosed that EPO/DPO had O- and N-sites for sialylation. Not all of these sites were occupied, due to protein heterogeneity (see document D1, page 13, line 28 to page 14, line 3, page 19, lines 5 to 18). Starting from document D1 in seek of a further way to improve the N-site occupancy of EPO/DPO, the skilled person would have turned to document D17. This document disclosed that the N-site occupancy in the t-PA glycoprotein was further increased by adding $Mn^{2+}$ to the culture medium (see page 1, lines 6 to 8, page 5,

lines 1 to 5, and page 15, lines 7 to 22) in the concentration range of 10 nM to 100 µM, which comprised the range cited in claim 1 (see page 5, line 10, page 14, lines 28 to 32, and claims 7 and 8). Example 4 in document D17 disclosed in this context that various concentrations of $Mn^{2+}$ from 3 nM to 100 µM increased the N-site occupancy of t-PA. 100 µM $Mn^{2+}$ was mentioned as being "*still an effective concentration*" (see page 24, line 16 to page 25, line 3). This statement in document D17 motivated the skilled person to use these $Mn^{2+}$ concentrations in the process of document D1 too. By using the concentration range of 10 nM to 100 µM $Mn^{2+}$, the skilled person would have automatically arrived at a solution falling within the claimed method. This required solely standard optimisations of the culture conditions disclosed for t-PA for other glycoproteins, as suggested by document D17 (see page 14, lines 20 and 21). This was a mere try and see exercise. In particular, since reasons for not applying document D17's teaching to document D1 were lacking.

XIII. The respondent's submissions, insofar as relevant to the present decision, may be summarised as follows:

*Main request*

*Added subject-matter*

The contested range in claim 1 found a basis in claims 11 and 12 as filed based on the principles established in decision T 985/98.

*Sufficiency of disclosure*

The results disclosed in the patent application were consistent, and rendered it plausible that the claimed

increased sialylation of EPO/DPO was achievable across the whole breadth of claim 1. O-site occupancy of EPO/DPO was a reliable marker for an increased sialylation of EPO/DPO. Such an increased O-site occupancy was the prerequisite for the subsequent sialylation of oligosaccharide chains attached to this site, i.e. the attachment of a terminal sialic acid to these chains. The correlation between O-site occupancy and increased EPO/DPO sialylation was plausible, since the latter depended entirely on the first (see page 37, lines 11 to 14). The results disclosed in Figures 4 to 6 and 10 had thus to be taken into account in support of the claimed effect.

There was no inconsistency between the data disclosed in Figures 8 and 9 since $Mn^{2+}$ reduced the fraction of lower sialylated EPO/DPO, while it increased the recovery of highly sialylated forms.

As regards the CHO cells, the ultimate paragraph of Example 3 on page 28 did not state that $Mn^{2+}$ had no effect on a fraction of higher sialylated DPO, i.e. the effect claimed. This paragraph mentioned the fraction of lower sialylated DPO only. The reasons why $Mn^{2+}$ had no effect on this fraction were unknown, the observation was maybe an artefact. In any case, no conclusions could be drawn from this isolated statement in Example 3 on the suitability of $Mn^{2+}$ in increasing sialylation of EPO/DPO in $Mn^{2+}$-responsive CHO cells in general.

As regards toxicity, the application as filed mentioned explicitly that the quality of the EPO/DPO proteins, i.e. their sialylation level improved by adding $Mn^{2+}$ in the cited concentration range. If 40 µM $Mn^{2+}$ was used the increase in sialylation was highest, but on the expense of some protein quantity (see page 13, lines 19 to 23). This product loss was irrelevant for the claimed effect in terms of quality. Moreover 40 µM $Mn^{2+}$

was not toxic for the cells since proteins were still produced.
Furthermore, the application as filed disclosed in Figure 3 that an increased sialylation was achieved after each harvest cycle. It was irrelevant that this was not explicitly mentioned in the paragraph bridging pages 27 and 28 of the patent application too, which described the results shown in Figure 3.
The chromatographic method separated highly sialylated EPO/DPO from non-, or low sialylated EPO/DPO. This resulted in protein losses. However, these losses neither rendered the method inappropriate for separating these two fractions from each other, nor did they invalidate the results obtained by the method. All experiments were carried out by using this method, and the results demonstrated consistently a dose effect of $Mn^{2+}$ in increasing the sialylation of EPO/DPO.

*Inventive step*

Document D1 represented the closest prior art. It disclosed that sialylation of EPO/DPO increased upon introducing additional glycosylation sites into the proteins. The $Mn^{2+}$ concentration used in the culturing of CHO cells was far lower (between 0.05 µM to 0.06 µM) than in the claimed method.
The claimed method differed from the method of document D1 in using higher $Mn^{2+}$ concentrations in the culture medium. This had the beneficial effect that the sialylation of EPO/DPO increased further.
The technical problem was therefore the provision of an improved method for increasing the sialylation of EPO/DPO.
The claimed method as a solution to this problem was based on an inventive step. It was common general knowledge that $Mn^{2+}$ was an essential co-factor of the

enzymes involved in the sialylation of glycoproteins. However, it was unknown that $Mn^{2+}$ affected dose-dependently the sialylation level of glycoproteins, let alone of EPO/DPO. In the absence of any pointer in document D1, and the relevant art that $Mn^{2+}$ had a direct dose effect on the sialylation of EPO/DPO, the skilled person taking common general knowledge into account would not have added more $Mn^{2+}$ to a culture medium to arrive at the claimed method. This required hindsight knowledge of the claimed method.

Nor would the skilled person have arrived at the claimed method in an obvious manner by combining the teaching of documents D1 and D3. Document D3 disclosed an *in vitro* approach for increasing the sialylation of a glycoprotein. Since this approach used isolated enzymes under optimised conditions in an artificial situation, the method of document D3 differed fundamentally from the *in vivo* process disclosed in document D1. Already for this reason the skilled person would not have turned to document D3. Furthermore, although document D3 disclosed that 5 µM $Mn^{2+}$ together with two enzymes increased sialylation under *in vitro* conditions, the document was silent on the relevance of $Mn^{2+}$ in achieving this effect, except for being an essential enzymatic co-factor. This would have required the disclosure of experiments comparing the effect of various $Mn^{2+}$ concentrations on sialylation, which were however lacking. The sole pointer derivable from document D3 for increasing the sialylation of glycoproteins was the use of the *in vitro* process as an additional method step. This included the industrial size production of sialylated proteins (see abstract, and last paragraph). Thus if document D3 was consulted at all, the skilled person would have followed this route which taught away from the claimed method.

The skilled person was also not in a try-and-see situation as defined by the case law. This required that document D3 suggested a potential dose effect of $Mn^{2+}$ in increasing sialylation, which was not the case.

Lastly, the claimed method was inventive over the combined teaching of documents D1 and D17 too. Document D17 explicitly taught that concentrations exceeding 0.1 µM $Mn^{2+}$ had no further beneficial effect on the sialylation level of the glycoprotein t-PA (see page 24, line 36 to page 25, line 3). Thus the skilled person had no motivation to add $Mn^{2+}$ in concentrations exceeding this value to increase EPO/DPO's sialylation further. This was so despite document D17 mentioning that concentrations up to 100 µM $Mn^{2+}$ were effective, but compared to 0.1 µM $Mn^{2+}$ none of these concentrations resulted in any further improvement. The use of $Mn^{2+}$ at the optimal concentration of 0.1 µM was also close to the $Mn^{2+}$ concentration contained in the culture medium of document D1 (0.05 µM to 0.06 µM). Rather the skilled person faced with the problem defined above would have looked at the other alternatives disclosed in document D17 for achieving an increased sialylation. These included, for example, the use of iron, or a temperature shift, which both achieved higher sialylation levels compared to $Mn^{2+}$ (see page 22, lines 25 to 29, and page 25, lines 7 and 8). Therefore document D17, like document D3, rather taught away from the claimed method, by disclosing that $Mn^{2+}$ concentrations falling within the claimed range did not achieve the desired effect, contrary to other alternatives. Again there was no try-and-see situation, because document D17 did not provide pointers for using $Mn^{2+}$ in the claimed concentration range to increase EPO/DPO's sialylation level further.

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

XV. The respondent requested that the appeal be dismissed or, alternatively, that the appealed decision be set aside and the patent be maintained on the basis of one of auxiliary requests I to XIII, all filed in reply to the statement of grounds of appeal.

## Reasons for the Decision

*Main request*

*Added subject-matter*

1. If references are made to the application as filed they all refer to the patent application (WO 2007/070315).

2. The appellant submitted that the feature "*from about 0.4 to about 10 µM*" added subject-matter in claims 7 and 19, and in paragraphs [0014], [0021], and [0037] of the patent.

3. The board does not agree. In essence the appellant asserts that the application as filed provides no pointer to select the lower limit "*0.4*" in the range cited in claims 7 and 19, and the respective paragraphs of the patent indicated above.

3.1 According to the criteria set out in decision T 985/98, values of the lower and upper limits of a preferred narrower and a more general range can be combined without adding subject-matter. Claims 11 and 12 as filed disclose the ranges "*from about 0.1 to about 10 µM*", and "*from about 0.4 to about 4 µM*", respectively.

The disclosure of these two ranges in the claims as filed provides a clear pointer to the skilled person that their use is preferred.

3.2 Therefore in line with the case law, the range "*from about 0.4 to about 10 µM*" cited in claims 7 and 19, and the respective paragraphs of the patent can be directly and unambiguously derived from the ranges mentioned in claims 11 and 12 as filed.

4. The main request complies with the requirements of Article 123(2) EPC.

*Insufficiency of disclosure*

5. In the following, all references are made to the patent application (WO 2007/070315) and not to the patent, since amended sets of claims have to be assessed for their compliance with Article 83 EPC.

6. Claim 1 is directed to a method for the production of an erythropoietic composition comprising at least the sialylated erythropoiesis-stimulating glycoproteins erythropoietin (EPO) or darbepoetin (DPO), or erythropoietic fragments thereof. EPO and DPO are structurally characterised by the amino acid sequences of SEQ ID NO: 3 or 2, respectively. The method of claim 1 is further characterised by culturing a manganese ($Mn^{2+}$)-responsive CHO host cell which produces these glycoproteins in a culture medium that contains "*an amount of manganese effective to increase the sialylation*". This effect is achieved by using $Mn^{2+}$ in the concentration range "*from about 0.4 to about 40 µM*".

7. Thus, the method of claim 1 is directed to the recombinant production of structurally characterised sialylated EPO or DPO glycoproteins or functionally active fragments thereof. This purpose is achieved by culturing $Mn^{2+}$-responsive CHO cells expressing these glycoproteins in a medium with a defined $Mn^{2+}$ concentration (range "*from about 0.4 to about 40 µM*"). As a result thereof EPO/DPO are obtained having an increased sialylation level ("*an amount of manganese effective to increase the sialylation*"). Since the feature "*increase the sialylation*" in claim 1 is not further specified, the claim is construed to encompass any increase in sialylation (low and high) compared to a control that contains $Mn^{2+}$ at concentrations lower than the ones cited in the claim.

8. The case law has established (see decision G 1/03, OJ EPO 2004, 413) that, if there is a lack of reproducibility of the claimed invention (i.e. a failure of the claimed features to deliver the aimed effect), this may be relevant under the requirements of sufficiency of disclosure or inventive step. If the technical effect is expressed in the claim, there is a lack of sufficiency of disclosure. Otherwise, there is a problem of inventive step (see G 1/03, Reasons, point 2.5.2).

9. In this case, the technical effect at which the method aims (the production of EPO/DPO with increased sialylation) is mentioned in claim 1. In light of the case law, the question of whether the claimed method is suitable for producing the glycoproteins of interest across substantially the whole breadth of the claim is therefore one of sufficiency of disclosure.

10. According to the appellant the patent application failed for several reasons to plausibly convey that increased sialylation of EPO/DPO was achieved across the whole breadth of claim 1: O-site occupancy was not a suitable marker for increased EPO/DPO sialylation; the method used for determining sialylated DPO/EPO was inappropriate, and the results obtained therefrom unsuitable in supporting a potential effect of $Mn^{2+}$ on increasing sialylation; the data disclosed in the patent application were inconsistent; the patent application did not demonstrate the claimed effect (1) across the whole concentration range of $Mn^{2+}$, (2) for all CHO cell lines, and (3) for all harvest cycles; furthermore (4) the claimed method lacked a reference point for determining an increase; lastly 40 µM $Mn^{2+}$ was toxic to CHO cells.

11. The board is not convinced that any of the lines of argument submitted by the appellant provide serious doubts substantiated by verifiable facts in support of their allegations that the effect of an increased sialylation of EPO/DPO is not achievable across the whole range of claim 1.

12. As regards the suitability of O-site occupancy as a marker for increased sialylation, the sialylation of EPO/DPO concerns the last step of protein glycosylation ("*terminal glycosylation*"). This step adds a sialic acid as terminal residue to oligosaccharides chains that have been attached to EPO/DPO at so called N-sites (a nitrogen atom of the amino acid asparagine (Asn)), and O-sites (an oxygen atom of the amino acids serine (Ser) and threonine (Thr)) during a first glycosylation that is functionally independent from the terminal glycosylation (i.e. sialylation, see page 2, lines 19 to 22 of the patent application).

12.1 The percentage of attached oligosaccharides to EPO/DPO is experimentally determined as N-site and O-site occupancy rate (see, e.g. Figure 4). Thus sialylation of EPO/DPO as a terminal glycosylation occurs only, if in previous glycosylation reactions oligosaccharides are attached to N- and O-sites of EPO/DPO. In other words, an N-site and O-site occupancy of oligosaccharides is the necessary prerequisite for EPO/DPO's sialylation. Due to this absolute dependency of sialylation on N- and O-site occupancy, both reactions are linked by a linear relationship.

12.2 This direct relationship renders it plausible that the N- and O-site occupancy rate is a suitable marker for indicating sialylation, since it can be reasonably assumed that an increased occupancy correlates with an increased sialylation level. If O-site occupancy of EPO/DPO increases (as determined, for example, in Example 4, and shown in Figures 4 to 6), the likelihood rises that O-linked oligosaccharides are sialylated too. In view thereof it is irrelevant that both glycosylations (i.e. attachment of oligosaccharides and sialylation) are independent from each other. In particular in the present case where the appellant has not submitted any experimental evidence to the contrary.

12.3 Accordingly, all results disclosed in Figures 1 to 10 of the patent application are equally suitable for demonstrating directly, or indirectly (by an increased O-site occupancy) a potential dose effect of $Mn^{2+}$ on the sialylation of EPO/DPO.

13. As regards the suitability of the chromatographic method used to determine differences in EPO/DPO's

sialylation level, the method used is based on differing binding properties of higher and lower sialylated EPO/DPO on an anion exchange chromatographic column. While higher sialylated EPO/DPO bind strongly to the column material, lower sialylated and non-sialylated EPO/DPO flow through the column (see patent application, page 20, lines 10 to 15). Thus, the method separates fractions of EPO/DPO according to their sialylation level.

13.1 Since all experiments have been carried out with this method, and all results show consistently a dose effect of $Mn^{2+}$ on the sialylation level of EPO/DPO (see below), the board has no reason to doubt that the method is suitable for reliably detecting differences in the sialylation level of EPO/DPO.

13.2 The appellant has not submitted any experimental evidence that the chromatographic method of the patent application is not suitable for the purpose indicated above. The appellant's arguments rather concern the efficiency of recovering EPO/DPO from the chromatographic column than casting doubts on $Mn^{2+}$'s effect in increasing the sialylation of EPO/DPO. However, these arguments do not convince the board, because the EPO/DPO losses due to the chromatographic material, and $Mn^{2+}$'s dose effect on sialylation are independent and not related to each other.

14. As regards the alleged inconsistency of the experimental data disclosed in the patent application, the appellant compared the results in Figure 5 of the patent application which show an increased O-site occupancy of DPO in the presence of 4 µM $Mn^{2+}$ relative to a control after the first harvest (88% vs 86%), with Figure 6 for 0.4 µM $Mn^{2+}$ which results in a rate of

85.5%, i.e. a value lower than the control value in Figure 5.

14.1 There is no evidence on file that the results of Figures 5 and 6 were obtained from the same experiment. Rather the results were obtained from different experiments because Figure 6 assesses the effect of various $Mn^{2+}$ concentrations on O-site occupancy, contrary to Figure 5 which uses 4 µM $Mn^{2+}$only. However, results from different experiments cannot be compared with each other since experimental conditions are not always exactly reproducible. Thus, Figures 5 and 6 do not show inconsistent results.

14.2 As regards the alleged inconsistency between the results of Figures 8 and 9 of the patent application, these Figures disclose an inhibitory effect of supplemented amino acids ("AA") in enriched media on the sialylation level of EPO. The board finds that the results of these figures are consistent in themselves. Figure 8 discloses that the addition of amino acids to the medium inhibits the formation of highly sialylated EPO compared to a control medium lacking these amino acids (see Figure 8, first two bars). The addition of $Mn^{2+}$ in various concentrations to the medium still containing the amino acids reverses at least partially the inhibitory effect, since the amount of highly sialylated EPO increases again compared to the medium containing the amino acids only (see Figure 8, bars two to five).

14.3 An inhibitory effect mediated by amino acids in enriched medium on the formation of higher sialylated EPO seems to be indirectly derivable from Figure 9 too. Figure 9 discloses that the amount of lower sialylated EPO increases in the presence of inhibitory amino acids

("AA") compared to a control lacking these amino acids (see bars, one and two). This increase might be caused by the formation of less higher sialylated EPO (see Figure 9, bars one and two, compared to Figure 8, bars one and two). The inhibitory effect of the amino acids is partially reversed by adding various concentrations of $Mn^{2+}$. The addition of $Mn^{2+}$ reduces again the amounts of lower sialylated EPO compared to a medium containing solely the amino acids (see Figure 9, bars two to five). This might indicate that the amount of higher sialylated EPO is correspondingly increased (see Figure 8, bars three to five).

15. As regards the alleged lack of data in the patent application in support of $Mn^{2+}$'s dose effect across the whole concentration range cited in claim 1, the appellant submitted that Figure 8 disclosed that 40 µM $Mn^{2+}$ had no effect in increasing the sialylation level of EPO.

15.1 This is not convincing. Figure 8 discloses that the addition of 40 µM $Mn^{2+}$ to a medium containing inhibitory amino acids in part reverses an inhibitory effect (see above). The average amount of higher sialylated EPO after adding 40 µM $Mn^{2+}$ is slightly increased compared to the amount obtained in the presence of amino acids alone (see Figure 8, bars two and three). Moreover, the functional feature "*an amount of manganese effective to increase the sialylation*" in claim 1 is relative and, solely requires an(y) increased sialylation be it low or high (see above).

15.2 Furthermore, Figure 6 of the patent application discloses that the O-site occupancy of DPO increases dose-dependently across the whole range of $Mn^{2+}$ cited in claim 1.

16. As regards the suitability of $Mn^{2+}$-responsive CHO cells in general for producing EPO/DPO with increased sialylation, the respective passage on page 28, lines 9 to 12 of the patent application states as follows: "*In experiments carried out with a line of CHO cells adapted for growth in suspension culture in large tanks and or CHO cells adapted to suspension culture in serum-free medium, no effect of manganese on the fraction of lower sialylated darbepoetin was observed*" (emphasis added). This statement concerns lower sialylated DPO only, while it is silent on higher sialylated DPO. Therefore this isolated statement cannot cast sufficient doubts on the suitability of CHO cells in general for the claimed purpose. Conclusions cannot be drawn from this statement on the amount of higher sialylated DPO, because it cannot be established that higher sialylated DPO are affected at all. In this situation and in the absence of any experimental evidence from the appellant's side, CHO cells in general are suitable for the claimed method.

17. As regards the harvest cycles, Figure 3 of Example 3 of the patent application discloses an increased O-site occupancy of DPO produced in CHO cells after the first, the second and the third harvest cycle. The cells grow in a culture medium that contains 4 µM $Mn^{2+}$. Example 3 of the patent application mentions in the paragraph bridging pages 27 and 28 solely an increased O-site occupancy after the third harvest. The Example is silent on the occupancy rate of DPO after the first and the second harvest. This is however irrelevant in view of the fact that Figure 3 discloses an increased sialylation of DPO after each harvest, even if the increase after the first cycle is very small. However, as set out above, the feature "*an amount of manganese*

*effective to increase the sialylation*" in claim 1 is not specified. Thus the claim encompasses any increase in sialylation compared to a control that contains $Mn^{2+}$ at concentrations lower than the cited ones. In this context the data provided in the patent application is sufficient.

18. The lack of a reference point in claim 1 rather concerns the definition of the claimed method (Article 84 EPC) than sufficiency of disclosure. Claim 1 of the main request differs from claim 1 as granted merely in an amended $Mn^{2+}$ concentration range (0,4 to about 40 µM instead of 0,01 to about 40 µM). The appellant's objection is not directed against the amended lower limit of the concentration range, but to a feature that is lacking in claim 1 as granted too (i.e. a reference point for an increase). However, objections under Article 84 EPC are not a ground of opposition (see G 03/14, published in OJ 2015, 102, catchword).

19. As regards the toxicity of 40 µM $Mn^{2+}$, the passage cited in Example 4 on page 30, lines 11 to 13 of the patent application states: "*However, 40 µM manganese adversely affected levels of protein production, resulting in a total relative decrease of 17% in the mg of darbepoetin produced over the three harvest cycles combined*" (emphasis added). Although the overall amount of DPO produced in CHO cells decreases at 40 µM $Mn^{2+}$, Figure 6 discloses that the rate of O-glycosylated DPO (as an indirect marker of sialylation) increases dose-dependently from 0,4 µM to 40 µM $Mn^{2+}$. Thus, Figure 6 discloses that $Mn^{2+}$ increases sialylation of DPO across the whole concentration range of claim 1. Since this corresponds to the effect likewise mentioned in claim 1, the appellant's submission regarding a toxic effect of 40 µM $Mn^{2+}$ on protein production is not convincing.

Although 17% less of DPO is produced in the presence of 40 µM $Mn^{2+}$ due to unfavourable cellular growth conditions, the produced 83% of DPO is of a higher desired quality (increased sialylation).

20. Consequently, the main request complies with the requirements of Article 83 EPC.

*Inventive step*

21. It is uncontested that document D1 represents the closest prior art.

21.1 This document discloses *inter alia* a method for producing human EPO and analogs thereof (including DPO) that comprise at least one additional glycosylation site to incorporate more or increased levels of oligosaccharide chains. As a consequence thereof, the analogs have a higher sialic acid content (increased sialylation) vs non-modified EPO. DPO, for example, contains two additional N-glycosylation sites compared to EPO (see page 5, lines 10 to 22, and page 13, line 28 to page 15, line 8, Table 3, page 44, "*N47*", Example 1). In other words, document D1 discloses a structural approach to increase EPO's sialylation level. Document D1 discloses that EPO/DPO proteins with increased sialylation have advantageous properties, for example, an increased serum half-life (see page 17, line 28 to page 18, line 9, lines 19 to 27).

21.2 Example 1 of document D1 discloses that recombinant EPO or DPO is produced *inter alia* in CHO cells cultured in DMEM/F12 medium supplemented with and without fetal calf serum (FCS). It is uncontested that this medium has a $Mn^{2+}$ concentration between 0.05 µM and 0.06 µM (see document D1, page 21, lines 30, and 31, statement

of grounds of appeal, page 12, point 3.4, first paragraph, and decision under appeal, page 15, second paragraph).

22. It is likewise uncontested that the claimed method differs from the method in document D1 in that the culture medium contains $Mn^{2+}$ in a concentration range between about 0,4 µM to about 40 µM instead of 0.05 µM to 0.06 µM, i.e. higher $Mn^{2+}$ concentrations are used.

23. However, the appellant disputed that the use of $Mn^{2+}$ in the whole concentration range cited in claim 1 increased the rate of higher sialylated EPO/DPO for the reasons indicated above under sufficiency of disclosure. The board is not convinced by these arguments (see above).

24. Therefore, the objective technical problem is the provision of an improved method for producing a composition of EPO/DPO, or biologically active fragments thereof, with increased sialylation.

25. The method of claim 1 provides a solution to this problem, for the reasons likewise indicated above under sufficiency of disclosure.

*Obviousness*

26. It remains to be assessed whether or not the skilled person, starting from document D1 and facing the problem defined above, would have arrived at the method of claim 1 in an obvious manner.

27. The appellant submitted that the claimed method was obvious for the skilled person based on the teaching of document D1 alone taking common general knowledge into

account. Alternatively, the claimed method was obvious in light of the combined teaching of document D1 with D3, or D17.

28. As mentioned above, document D1 discloses a structural approach to increase EPO's sialylation level by modifying its amino acid sequence (provision of EPO analogs). Document D1, however, neither discloses nor suggests an optimisation of CHO culturing conditions to produce EPO/DPO with an even further increased sialylation, in particular by adding more $Mn^{2+}$ to the culture medium, let alone in the cited concentration range.

28.1 The appellant submitted that the skilled person faced with the problem identified above, would have simply added $Mn^{2+}$ to the culture medium in a concentration falling within the cited range, because $Mn^{2+}$'s key role in sialylation was common general knowledge, including its function as an essential co-factor for the enzymes involved in sialylation.

28.2 The board does not agree. The skilled person knows that the culture medium in document D1 contains a $Mn^{2+}$ concentration that is sufficient for EPO/DPO's sialylation, i.e. it's function as a co-factor for the enzymes involved in sialylation. This is uncontested. However, neither document D1 nor the prior art summarising the skilled person's common general knowledge (see e.g. document D5) contains any pointer that $Mn^{2+}$, besides this function as an essential co-factor, has an additional dose effect on increasing the sialylation of glycoproteins.

28.3 In this situation the skilled person trying to increase EPO/DPO's sialylation has no expectation that the

addition of $Mn^{2+}$ in an amount that exceeds the medium's normal concentration would solve this problem. Let alone in the cited concentration range. Rather, the use of increased $Mn^{2+}$ concentrations requires hindsight knowledge of the claimed method.

29. In a further line of argument the appellant submitted that the claimed method was obvious for the skilled person starting from the method of document D1 combined with document D3's teaching.

29.1 Again the board does not agree. Document D3 discloses an *in vitro* method to increase the sialylation level of TNFR-IgG as a model glycoprotein for improving its serum half-life (see abstract). This method requires that TNFR-IgG is first produced and then purified. In a further process step the protein is treated *in vitro* with two transferases ($\beta$-1,4-galactosyltransferase, and α-2,3-sialyltransferase), either separately or combined in the presence of 5 µM $Mn^{2+}$ (see page 8869, column 2, first and third paragraph, Table 1 on page 8870). Document D3 suggests using the *in vitro* method for an industrial production of therapeutic glycoproteins (see abstract, and page 8875, column 2, last paragraph).

29.2 This explicit suggestion in document D3 contradicts the appellant's argument that the skilled person would not have switched from a pure cell culturing process as disclosed in document D1 to a process that comprises cell culturing and *in vitro* steps for increasing EPO/DPO's sialylation. Rather the skilled person would not ignore this suggestion, except there is a clear reason to do so. Such a reason, however, is lacking from document D3. Moreover, document D3, like document D1, is silent on any dose effect of $Mn^{2+}$ in increasing sialylation of glycoproteins. Therefore, the skilled

person combining the teaching of documents D1 and D3 would not arrive at a method falling within the scope of claim 1.

30. In a further line of argument, the appellant submitted that the claimed method lacked an inventive step in light of the combined teaching of document D1 and D17.

30.1 The board does not agree. Document D17 discloses that N-site occupancy (i.e. sialylation) of the glycoprotein t-PA produced in a mammalian cell culture is increased by various strategies, including the addition of $Mn^{2+}$ to the medium as a co-factor for optimal enzyme activity (see page 1, lines 6 to 10, and page 5, lines 1 to 3, 10 and 11). Example 4 discloses on page 24, lines 24 to 26 that $Mn^{2+}$ is added in concentrations 10 nM (0.01 µM), 100 nM (0.1 µM), 1 µM, 10 µM, and 100 µM.

30.2 $Mn^{2+}$ causes dose-dependently an increased N-site occupancy of t-PA (see Figure 10). Example 4 states in this context on page 25, lines 1 to 3 that a "*positive titration effect was observed between 3 nM and 100 nM. No further improvement occurred when increasing the concentration up to 100 µM, which is still an effective concentration*" (emphasis added). This corresponds with Figure 10B, which shows that concentrations above "*100 nM MnCl2*" (i.e. 0.1 µM $Mn^{2+}$) do not increase t-PA's sialylation level further. Maximally, an increase of 2.5% is achieved (see page 25, line 1).

30.3 Document D17 tests also other metals, including iron ($Fe^{2+}$), and a temperature shift in increasing t-PA's sialylation level (see Example 1, in particular page 22, lines 25 to 30, and page 25, lines 6 to 8). The temperature shift achieves an increase between 5% and 8% (see page 22, lines 27 to 29: an increase from 38%

to 43% = 5%, and from 38% to 46% = 8%), $Fe^{2+}$ achieves a maximum increase of 4% (see page 25, line 8).

30.4 In the board's view, the skilled person reading the statement in document D17 on page 25 (see point 30.2, above) that the maximum increase of sialylation is achieved at 0.1 µM $Mn^{2+}$ with no further improvements at higher concentrations, would not ignore this teaching, except the document provides sound reasons for this. Since these reasons are, however, not provided the skilled person trying to increase the sialylation level further would instead turn to the other strategies mentioned in document D17 (see point 30.3). In doing this, the skilled person would likewise not arrive at the method of claim 1.

30.5 The appellant submitted that the disclosure in document D17 of using $Mn^{2+}$ in the preferred range of "*10 nm to 100 µM*" (see page 14, line 33, and claim 8 combined with claim 7) motivated the skilled person to use $Mn^{2+}$ across this entire range. The board agrees with the appellant that normally the mentioning of a feature in a document as being preferred motivates the skilled person to apply this teaching for an intended purpose. However in this case, the skilled person seeking to increase sialylation of EPO/DPO would not ignore the experimental data and the explicit teaching in Example 4 of document D17 that $Mn^{2+}$ concentrations above 0.1 µM do not achieve this effect. This argument therefore does not convince the board.

31. In their last line of argument, the appellant submitted that the skilled person combining the teaching of document D1 with either D3 or D17 was at least in a so called "try-and-see" situation, such that the skilled person would have inevitably arrived at the claimed

subject-matter. He or she would have therefore necessarily arrived at a $Mn^{2+}$ concentration falling within the cited range after performing standard optimisations.

32. The board does not agree either. In the absence of a pointer in any of the available documents that $Mn^{2+}$ concentrations falling within the cited range might have an additional dose effect on sialylation, the skilled person was not in a "try-and-see" situation. None of the prior art teachings clearly envisages a way of proceeding in the light of the problem to be solved, for example, by suggesting that $Mn^{2+}$ used in concentrations exceeding the amounts normally required for its function as an essential co-factor has a dose effect on sialylation, the presence of which then only has to be verified by routine methods. In the present case, the skilled person is not in such a position because, for the considerations set out above, $Mn^{2+}$'s dose effect on increasing sialylation was not known and therefore the one way among the many possible ways of solving the problem was not foreshadowed. Already for this reason the argument must fail. In this situation the skilled person could have tried to optimise many parameters to achieve the desired effect (see e.g. T 1172/06, Reasons, point 14.13). As set out above, an arrival at the claimed method in these circumstances requires hindsight knowledge of the claimed method.

33. Thus, the method of claim 1 is not obvious in light of document D1 alone combined with common general knowledge, or in combination with the teaching of document D3, or D17. The same applies for the culture medium of claim 17.

34. Therefore, the main request complies with the requirements of Article 56 EPC.

## Order

## For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:


L. Malécot-Grob B. Stolz

Decision electronically authenticated