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Datasheet for the decision of 14 June 2024

Case Number: T 0023/22 - 3.3.04

Application Number: 10781888.2

Publication Number: 2501799

IPC: C07K14/47, C12P21/00, C12N5/00

Language of the proceedings: EN

Title of invention:
Production of glycoproteins with low N-glycolylneuraminic acid (Neu5Gc) content

Patent Proprietor:
LEK Pharmaceuticals d.d.

Opponent:
Maiwald GmbH

Headword:
Low N-glycolylneuraminic acid/LEK

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
Main request - Novelty - (no)
Auxiliary request 1 - Inventive step (yes) - non-obvious alternative - Sufficiency of disclosure (yes)

Decisions cited:
G 0002/21, T 0939/92

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 0023/22 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 14 June 2024

Appellant: (Opponent)	Maiwald GmbH Elisenhof Elisenstraße 3 80335 München (DE)
Representative:	Maiwald GmbH Elisenhof Elisenstraße 3 80335 München (DE)
Respondent: (Patent Proprietor)	LEK Pharmaceuticals d.d. Verovskova 57 1526 Ljubljana (SI)
Representative:	Dehmel, Albrecht Dehmel & Bettenhausen Patentanwälte PartmbB Herzogspitalstraße 11 80331 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 28 October 2021 concerning maintenance of the European Patent No. 2501799 in amended form**

Composition of the Board:

Chairwoman	M. Pregetter
Members:	D. Luis Alves
	M. Blasi

Summary of Facts and Submissions

I. European patent EP 2 501 799, entitled "*Production of glycoproteins with low N-glycolylneuraminic acid (Neu5Gc) content*", was granted on European patent application No. 10 781 888.2, filed as an international application published as WO 2011/061275.

II. An opposition was filed invoking the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC), under Article 100(a) EPC, as well as the grounds under Article 100(b) and (c) EPC.

III. The opposition division decided that, account being taken of the amendments in the form of auxiliary request 2, the patent and the invention to which it related met the requirements of the EPC (Article 101(3)(a) EPC).

IV. The opponent (appellant) filed an appeal against that decision. The patent proprietor is the respondent to this appeal.

V. In the statement setting out the grounds of appeal, the appellant contested the opposition division's reasoning with regard to sufficiency of disclosure, novelty and inventive step. Documents D15 to D17 were also filed.

VI. In its reply to the statement setting out the grounds of appeal, the respondent filed sets of claims of a main request and auxiliary requests 1 to 6. The main request is identical to auxiliary request 2 held allowable by the opposition division. Auxiliary request 1 is identical to auxiliary request 3 filed on

17 July 2020 in opposition proceedings. Documents D18 to D24 were also filed.

VII. Claims 1, 12 and 13 of the **main request** read:

"1. A medium for the cultivation of eukaryotic cells, the medium comprising as (an) additive(s) DMSO, N-acelylmannosamine (NAcMan), N-acetylglucosamine (NAcGlc), or any combination of two or more of these additives, including the combination of NAcMan and NAcGlc, wherein the medium additionally comprises iron in a concentration ranging from 0.05 to 0.1 mM, 0.070 to 0.09 mM, and 0.075 to 0.08 mM, respectively.

12. Use of DMSO, NAcMan, NAcGlc, or any combination thereof, as additive(s) in a medium for eukaryotic cells for controlling the sialic acid content of a glycoprotein produced by a eukaryotic cell, wherein the glycoprotein exhibits (i) a degree of sialylation that is identical to or higher than the degree of sialylation of the same glycoprotein when produced in the same medium but without the additive(s); and (ii) a content of Neu5Gc that is lower than the content of Neu5Gc of the same glycoprotein when produced in the same medium but without the additive(s) and wherein the medium additionally comprises iron in a concentration ranging from 0.05 to 0.1 mM, 0.070 to 0.09 mM, and 0.075 to 0.08 mM, respectively.

13. Use of DMSO, NAcGlc, or any combination of DMSO, NAcMan and NAcGlc, as additive(s) in a medium for eukaryotic cells for controlling the sialic acid content of a glycoprotein produced by a eukaryotic cell, wherein the glycoprotein exhibits (i) a degree of sialylation that is identical to or higher than the degree of sialylation of the same glycoprotein when

produced in the same medium but without the additive(s); and (ii) a content of Neu5Gc that is lower than the content of Neu5Gc of the same glycoprotein when produced in the same medium but without the additive(s)."

In **auxiliary request 1**, claim 13 has been deleted. Claims 1 and 12 read as claims 1 and 12 of the main request.

VIII. The appellant submitted further arguments with its letters dated 9 October 2023 and 14 May 2024.

IX. The board appointed oral proceedings and, in a communication pursuant to Article 15(1) RPBA, informed the parties of its preliminary opinion that, *inter alia*, claim 13 of the main request was directed to the use of DMSO, NAGlc or any combination of DMSO, NAcGlc and NAcMan "*as additive(s) in a medium for eukaryotic cells*" without any limitation to a use of those compounds "*for controlling the sialic acid content of a glycoprotein*".

X. Oral proceedings took place as scheduled. At the end of the oral proceedings, the Chair announced the board's decision.

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

D2: Baker, K.N. *et al.*, Biotech Bioeng 73(3), 2001, pages 188-202

D4: Schauer, R. *et al.*, Biochemistry and Role of Sialic Acids. In: Biology of Sialic Acids, Chapter 2, 1995, Resenberg A. (ed.), Plenum Press, New York, pages 7-67

D6: WO 2008/128227 A1

D7: Rodriguez, J. *et al*., Biotechnol Prog 21, 2005, pages 22-30

D8: Declaration of Tanja Ficko Trcek including Annex A

D12: Ham, R., Microbiol 53, 1965, pages 288-293

D13: "Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F12" Product information from SAFC Biosciences, 2006, two pages

D14: Shaw, L. and Schauer R., Biochem. J. 263, 1989, pages 355-363

D15: Shenkin, A., e-SPEN Eur e-J Clin Nut Met 3, 2008, pages e255-e258

D16: Kakuta, K. *et al*., Comp Biochem Physiol 118A(1), 1997, pages 165-169

D17: Schrödel, A., Biol Unserer Zeit 37(5), 2007, page 289

D18: Francis, G.L., Cytotechnology 62, 2010, pages 1-16

D19: Kan, M. and Yamane, I., In vitro 20(2), 1984, pages 89-94

D20: Gstraunthaler, G., ALTEX 20(4), 2003, pages 275-281

D21: EP-A2-0 481 791

XII. The appellant's arguments relevant to this decision may be summarised as follows:

Admittance of documents D15 to D21 into the appeal proceedings

Documents D15 to D17 should be admitted into the appeal proceedings. They were filed in response to an argument relating to the iron concentration in foetal calf serum (FCS), brought forward for the first time at oral proceedings before the opposition division.

Documents D18 to D21 should not be admitted into the appeal proceedings, for the following reasons: document D18 did not help to clarify the iron concentration in document D2; documents D19 and D20 concerned serum-free cultures and therefore were not relevant.

Main request
Claim interpretation - claim 13

Claim 13 was to be read as meaning that the feature "controlling the sialic acid content ..." did not limit the use. The purpose of the claimed use was "for production of a glycoprotein". Any effect that the compounds might have on the sialylation and Neu5Gc content of the glycoprotein was a mere discovery.

In claim 13, the means of realisation, within the meaning of decision G 2/88 of the Enlarged Board of Appeal, was the addition of DMSO or NAcGlc to a medium for eukaryotic cells. Therefore, the subject-matter was not novel over any disclosure in the prior art of such a medium for cultivating eukaryotic cells.

Novelty (Article 54 EPC) - claim 13

Each of documents D2, D6 and D7 disclosed cultivation of eukaryotic cells in a medium containing one of the additives set out in claim 13.

Document D7 disclosed a medium comprising DMSO and the production of IFN-beta by CHO cells in this medium (see page 24, right-hand column, "Effect of Media Supplements").

Auxiliary request 1
Inventive step (Article 56 EPC) - claims 1 and 12

Closest prior art

Document D2 disclosed the use of NAcMan as an additive in a medium for the production of glycoproteins in eukaryotic cells. This additive resulted in a decrease in Neu5Gc content of the glycoproteins.

The medium was DMEM-F12, which contained just 1.6 µM iron (see documents D12 and D13). Although the medium was supplemented with FCS, the final iron concentration in the medium was much lower than required by claim 1. It was common general knowledge that iron was a trace element for animals (see document D15). The supplement FCS contributed only 2% to the medium. Considering the iron concentration in FCS to be on average 43 µM (see documents D16 and D17), the final iron concentration in the medium could be calculated to be 2.5 µM, which was approximately 20 times lower than required by claim 13.

The determination in document D16 was based on serum ferritin and transferrin. The respondent questioned the

determination on the grounds that it did not include other proteins known to bind iron. This argument was not supported by the common general knowledge that ferritin and transferrin were the two major proteins storing and transporting iron in blood. Haemoglobin as a source of iron was not relevant in the context of FCS, since haemoglobin should be absent from serum. As regards bovine serum albumin as a source of iron in serum, the reference to document D18 did not support the argument because the cited passage did not refer to iron. Neither of documents D19 and D20 supported the argument either because the first concerned human serum albumin instead of bovine serum albumin and the latter did not mention iron. In any case, it was spurious to allege that other protein sources of iron would contribute more iron to serum than the two major sources to such a degree as to change the overall iron concentration of the medium to 20 times the one calculated.

Technical effect and objective technical problem starting from a lower concentration of iron in the prior art

Document D2 already disclosed that the additive NAcMan resulted in a decrease in the Neu5Gc content of the glycoprotein. Claim 1 was directed to a medium which contained a higher concentration of iron than the medium disclosed in document D2. The technical effect of this difference was a foreseeable disadvantageous modification, namely an increase in the Neu5Gc content of the glycoprotein. Hence, the objective technical problem was to be formulated as the provision of an alternative medium and method for producing glycoproteins in eukaryotic host cells.

Obviousness

Any effect of the iron concentration on the Neu5Gc content was obvious when taking into account the disclosures in either of documents D4 and D14.

Document D4 disclosed that Neu5Gc was exclusively produced by the enzyme CMP-Neu5Ac hydroxylase and that addition of iron salts could increase the enzyme's activity (see pages 28 and 29).

Document D14 disclosed that the activity of this enzyme was indeed stimulated by the addition of iron (see figure 5). Although this teaching was based on the use of purified enzyme, the skilled person would infer from it that increasing the iron concentration in the cell culture medium would have an effect on the Neu5Gc content. Figure 5 suggested using iron concentrations below 0.2 mM.

Technical effect and objective technical problem starting from an undefined concentration of iron in the prior art

If the board considered that the iron concentration in the prior art medium could not be ascertained, then the difference between the claimed and the prior art medium was the range of iron concentrations. However, this difference was not associated with any technical effect.

The experimental results in document D8 were not public before the filing date of the patent. In accordance with decision G 2/21 of the Enlarged Board of Appeal, these should not be taken into account because the

application as filed did not make plausible a technical effect of iron concentrations in this range.

Even if they were considered, they nevertheless did not show the alleged technical effect, i.e. a lower Neu5Gc content while maintaining or increasing sialylation, over the whole scope claimed. For example, for additive NAcGlc, there was no effect at an iron concentration of 0.05 mM. Moreover, document D2 showed that glucosamine had the opposite effect (see tables III and IV). There were no experimental results showing an effect of the combined use of NAcMan and NAcGlc versus NAcMan alone. As regards the effect of iron, it could be seen from figure 1 that, in a medium with the additive NAcMan, a decrease in iron concentration from 0.2 mM to 0.1 mM or to 0.07 mM had no effect.

Since the alleged technical effect was not present over the whole scope of the claim, the objective technical problem was to be formulated as "*an alternative means to increase sialylation and decrease Neu5Gc content*".

Obviousness

The subject-matter did not involve an inventive step because there was no technical effect that could be acknowledged over the whole scope claimed.

Disclosure of the invention (Article 83 EPC) - claim 12

The patent did not show a lower Neu5Gc content with a lower iron concentration in the medium. Therefore, if the claimed use was seen as limited by this feature, then it was not sufficiently disclosed.

XIII. The respondent's arguments relevant to this decision may be summarised as follows:

Admittance of documents D15 to D21 into the appeal proceedings

Documents D15 to D17 should not be admitted into the appeal proceedings. They were filed late because iron concentration was a feature already present in the claims as granted. The iron concentration in the medium disclosed in document D2 became an issue only later in the opposition proceedings, since it was in fact conceded in the notice of opposition that this document did not provide an explicit disclosure of the iron concentration.

Moreover, none of these documents answered the question of what the concentration of iron in the medium was in document D2.

Documents D18 to D21 should be admitted into the appeal proceedings. They were filed as a way of disputing that the iron concentration in the medium in document D2 could be calculated. The reply to the appeal was the first opportunity to file them, because the statement setting out the grounds of appeal was the first time the appellant calculated the iron concentration.

Document D16, filed with the appeal, was crucial to this calculation.

Main request
Claim interpretation - claim 13

In claim 13, the purpose of "controlling the sialic acid content ..." was a limiting feature for the use.

Use "as additive", without any reason for such use, was meaningless. For that reason the claimed use could not be read as being limited merely by the features "DMSO, NAcGlc or any combination of DMSO, NAcGlc or NAcMan as additive(s) in a medium for eukaryotic cells". This therefore could not be the purpose of the claimed use.

Therefore, what followed those features was the intended purpose of the use and was to be read as a limiting feature.

Novelty (Article 54 EPC)

None of documents D2, D6 or D7 disclosed the claimed use because they did not disclose the effect of the compound on the sialylation and Neu5Gc content of the glycoprotein.

Auxiliary request 1
Inventive step (Article 56 EPC) - claims 1 and 12

Closest prior art

Document D2 contained no teaching regarding iron.

The iron concentration calculations submitted by the appellant for the medium disclosed in this document were speculative. Documents D15 to D17 did not support the iron concentration calculations for the supplement FCS: D15 did not relate to FCS and the aim of D16 was to determine the role of ferritin as iron transporter, not to determine the overall iron concentration in FCS. For that purpose, additional iron-binding proteins needed to be considered (see documents D18 to D20).

On the other hand, documents D17 and D21 disclosed that most of the many components of FCS had not yet been identified and that its composition varied significantly from lot to lot (see documents D17, left-hand column, first paragraph and D21, page 2, lines 34 to 36).

In conclusion, the iron concentration in the FCS supplement used in the medium of document D2 was undefined and consequently the overall iron concentration of the medium was undefined as well.

Technical effect and objective technical problem

The medium defined in claim 1 differed from the closest prior art in the range of iron concentration. The technical effect of this difference was a reduced Neu5Gc content in the glycoprotein.

The technical effect of iron concentration was clearly foreshadowed in the application as filed. Therefore, the experimental results in document D8 could be taken into account as evidence of the technical effect. An incremental approach to analysis of the results was not correct for biological systems. The results showed a clear tendency for Neu5Gc content to reduce as iron concentration reduced. Therefore, the range in claim 1 was not arbitrary. Figure 1 showed a clear effect in experiments with "house medium". That an effect of iron concentration was not visible for medium with additive NAcMan was merely a matter of insufficient resolution. Indeed, there was a saturation effect, meaning any effect of additional compounds might not be visible. Nevertheless the effect was visible for medium with NAcMan for an iron concentration of 0.05 mM.

Glucosamine and NAcGlc could not be used interchangeably. Moreover, in document D2, glucosamine was used in combination with uridine as a further additive. Therefore, no conclusions for additive NAcGlc could be drawn from the results with glucosamine in document D2.

Hence, the objective technical problem was to be formulated as the provision of alternative means and methods for producing glycoproteins in eukaryotic cells wherein the glycoprotein shows a decreased Neu5Gc content.

Obviousness

Document D2 did not focus on the enzyme Neu5Ac-hydroxylase within the complex metabolic conversion scheme provided in figure 6. Thus, combining the teaching in document D4 or D14 with that in document D2 was the result of hindsight.

Moreover, document D4 did not disclose reducing the iron concentration in order to modulate the enzyme's activity.

Document D14 did not disclose experiments in complex culture media containing living cells but instead in supernatants of lysed cells. Furthermore, the iron concentration was 0.2 mM, which was above the range in claim 1. Therefore, it was speculative whether an increase or decrease in iron concentration in the culture medium would result in a modified Neu5Gc content.

Disclosure of the invention (Article 83 EPC) - claim 12

No arguments were submitted in this respect.

XIV. The requests of the parties were as follows:

The appellant requested that the decision under appeal be set aside and the patent be revoked in its entirety. Moreover, the appellant requested that auxiliary requests 2 to 4 and documents D18 to D21 not be admitted into the appeal proceedinqs. Further, there were two conditional requests to refer questions to the Enlarged Board of Appeal (points 10 and 29 to 30 of the appellant's letter dated 9 October 2023).

The respondent requested that the appeal be dismissed and the patent be maintained as amended in the form of the main request (the version held allowable by the opposition division) or, alternatively, that the patent be maintained in amended form on the basis of the set of claims of one of auxiliary requests 1 to 6, all filed with the reply to the appeal. Further, it was requested that documents D15 to D17 not be admitted into the appeal proceedings.

Reasons for the Decision

Main request - Claim 13

Claim interpretation

1. Claim 13 reads (underlining by the board):

"Use of DMSO, NAcGlc, or any combination of DMSO, NAcMan and NAcGlc, as additive(s) in a medium for eukaryotic cells for controlling the sialic acid content of a glycoprotein produced by a eukaryotic cell, wherein the glycoprotein exhibits [...]."

2. In a claim drafted with nested purposes, as is the case with claim 13 of the main request before the board, the question arises as to which of the purposes is a limiting feature of the claimed use. In the case at hand, the claim includes the following purposes: "as additive in a medium", "for eukaryotic cells", "for controlling sialic acid content of a glycoprotein", and "wherein the glycoprotein exhibits ...".

3. In its communication pursuant to Article 15(1) RPBA, the board informed the parties of its preliminary opinion that the claim is directed to use of the compounds DMSO, NAcMan or any combination of DMSO, NAcMan and NAcGlc, as additives in a medium for eukaryotic cells.

4. Contrary to the respondent's argument, this definition of purpose is not meaningless. It defines use of the listed compounds for eukaryotic cell culture. Other hypothetical uses such as "as additive in bread", or "as additive in paint" are not covered by claim 13. Since "as additive in a medium for eukaryotic cells" defines a purpose for the use, additional reasons for this use merely define the results of one and the same use without however defining further uses. Accordingly, they do not further limit the claimed use.

Novelty (Article 54 EPC)

5. The appellant raised objections based on the disclosures in each of documents D2, D6 and D7. Novelty over the disclosure in document D7 is considered below. In view of the conclusions reached, it is not necessary to give reasons in relation to the disclosures in documents D2 and D6.

6. Document D7 discloses the use of DMSO as an additive in a culture medium for CHO cells, i.e. eukaryotic cells (see page 24, right-hand column, "Effect of Medium Supplements"). This was not in dispute.

7. In view of the board's claim interpretation set out above (see points 1. to 4.), there is no feature distinguishing the subject-matter of claim 13 from the use disclosed in document D7. In conclusion, the subject-matter of claim 13 is not novel (Article 54 EPC).

8. The respondent's arguments relied on a claim construction which was not adopted by the board and they are therefore not persuasive.

Admittance of documents D15 to D21 into the appeal proceedings

9. Documents D15 to D17 were filed by the appellant with the statement setting out the grounds of appeal. They address the question of the iron content in foetal calf serum (FCS). This question is relevant because the parties were in dispute about the iron content in the closest prior art, i.e in the medium disclosed in document D2.

10. While the respondent requested that these documents not be admitted, it acknowledged that this question was addressed for the first time at the oral proceedings before the opposition division.

11. In view of this, the board considers that the filing of these documents is to be seen as a reaction to the discussion on the contribution of the supplement FCS to the overall iron concentration in the closest prior art medium. The board decided to admit the documents.

12. In these circumstances, the respondent's argument that the documents were not suited to answering the question relating to the iron concentration in that medium was not considered pertinent by the board when deciding on admittance of the documents, as the decision on admittance did not hinge on whether the argument based on those documents was convincing. This latter question therefore became an issue only when the documents and the associated arguments on substance were being considered.

13. Documents D18 to D21 were filed by the respondent with the reply to the appeal. They were used to contest the appellant's calculation of the iron concentration in the medium of document D2, specifically the iron concentration in FCS.

14. The board agrees with the respondent's argument that these documents can be seen as a reaction to the calculation of an iron concentration in FCS, which became an issue for the first time in the appeal proceedings.

15. As noted above, whether the documents support the party's argument is the subject of their assessment on substance.

16. Hence, the board decided to admit documents D18 to D21 into the appeal proceedings.

Admittance of documents D22 to D24 into the appeal proceedings

17. These documents were filed by the respondent to support arguments relating to the assessment of the subject-matter of claims 1 and 12 of the main request and auxiliary request 1 in respect of inventive step. Since the board acknowledged inventive step without taking into account the content in these documents, there is no need for the board to further address their admittance or substance.

Auxiliary request 1
Inventive step (Article 56 EPC)

18. The opposition division held that the claimed subject-matter involved an inventive step. On appeal, the appellant raised objections against independent claims 1 and 12 of this request.

Closest prior art

19. The appellant relied on document D2 as representing the prior art closest to the medium defined in claim 1.

20. Document D2 discloses methods of glycoprotein production in eukaryotic cell culture and in particular studies the impact of additives on the glycosylation profile. Experiments were carried out with two cell

lines. Additive NAcMan resulted in increased sialylation with decreased N-glycolylneuraminic acid (Neu5Gc) content (see abstract and table IV).

21. A major point of dispute between the parties was whether document D2 discloses the iron concentration in the medium.

22. Document D2 discloses that the culture medium is DMEM-F12 supplemented with 2% FCS and various other supplements (see page 189, left-hand column, last paragraph). It is entirely silent on the presence of iron.

23. The appellant referred to documents D12 and D13 to establish the iron concentration in the DMEM-F12-based medium. However, the overall iron concentration in the medium remained a point of dispute because the supplement FCS also contributes iron to the medium. While the appellant submitted documents D15 to D17 in this regard, the board is not convinced for the following reasons that they provide evidence of the iron concentration in the medium specifically used in the experiments reported in document D2.

24. According to the appellant, the concentration of iron in FCS can be derived from document D16.

25. In the board's view, document D16 shows that the iron concentration in FCS varies greatly from lot to lot. The values determined in 13 lots of commercial FCS from a number of suppliers ranged from 1.56 to 3.37 µg/ml (see page 167, left-hand column, last full paragraph). Furthermore, documents D17 and D21 disclose that the composition of FCS varies significantly from lot to lot and its components are mostly still unidentified (see

document D17, left-hand column, first paragraph and D21, page 2, lines 34 to 36).

26. Thus, the board concludes that, even taking into account the fact that document D2 identifies the supplier of FCS, the iron concentration in FCS cannot be ascertained. Additionally, it cannot be determined whether the values for iron concentration as determined in document D16 apply to the specific FCS used. Thus, a calculation of the overall iron concentration in the medium in document D2 remains speculative and the iron concentration cannot be considered implicitly disclosed therein.

Technical effect and objective technical problem

27. In light of the above, the medium defined in claim 1 differs from the closest prior art in that it contains iron in a concentration of 0.05 mM to 0.1 mM.

28. In opposition proceedings, the respondent had submitted document D8 with experimental results to show an effect of the iron concentration on the Neu5Gc content of the glycoprotein. The parties were in dispute as to whether these experimental results could be taken into account as evidence of the technical effect relied upon.

29. The appellant argued that, in accordance with decision G 2/21 of the Enlarged Board of Appeal, document D8 should not be taken into account as evidence of the technical effect.

This line of argument is not convincing because a decrease in Neu5Gc content with lower iron concentration in the medium can be considered encompassed by the technical teaching and embodied by

the invention disclosed in the application as filed, for the following reasons. The aim of the application is to provide cell culture media, and methods of preparing glycoproteins with a low Neu5Gc content while maintaining a high degree of sialylation (see page 1, first paragraph). In the experimental section, the application states that decreasing the iron concentration in the medium resulted in glycoprotein with reduced Neu5Gc content: "*The inventor tested some other medium components like iron, insulin, and glutamine, both in the absence and presence of NAcMan and NAcGlc. She studied the effect of those three components on the Neu5Gc content and degree of sialylation. The result is that a reduced iron concentration entails a reduction of the content of Neu5Gc* [...]" (see example 7, page 27 of the application as filed, second paragraph). While the corresponding experimental results are not provided in full detail in the application as filed, this does not alter the fact that the application as filed outlines the experiments, states that they have been carried out, and draws conclusions based on the experimental results.

30. In accordance with case law, for a technical effect to be relied upon in the formulation of the objective technical problem, it must be present for substantially all the embodiments claimed (see decision T 939/92, Reasons 2.5.4 and 2.6).

31. Document D8 shows the Neu5Gc content of glycoproteins produced in media with or without one of the additives tested, at four different iron concentrations (see figure 1). For media with the additive NAcMan, the Neu5Gc content remains unchanged when the iron concentration is reduced from 0.2 mM (a value outside

the range in claim 1) to 0.1 mM and 0.07 mM (both values inside the range in claim 1). The board concludes that for a substantial part of the range 0.1 to 0.05 mM, the alleged technical effect is not present.

32. The respondent argued that the effect of the additive NAcMan on the Neu5Gc content is such that an additional effect of the reduced iron concentration cannot be discerned. This line of argument is not convincing. While this might in theory be the case, whether it is indeed so remains speculative. The board decides on the basis of the evidence submitted by the parties. In the present case, it was incumbent upon the respondent to select the experimental conditions suitable for demonstrating the technical effect it wished to rely on.

33. Hence, no effect associated with the specific range in claim 1 can be attributed to substantially all its embodiments. Therefore no technical effect going beyond the effects already provided by the prior art medium can be taken into account.

34. In light of the foregoing, the objective technical problem solved by the medium defined in claim 1 is seen as the provision of further media for producing glycoproteins in eukaryotic cells, wherein the glycoproteins have a decreased Neu5Gc content.

Obviousness

35. The appellant argued that the presence of an inventive step could not be acknowledged since no technical effect could be associated with all the embodiments claimed. This argument was taken into account by the

board when formulating the objective technical problem less ambitiously than proposed by the respondent, namely as the provision of further media achieving the same technical effects already achieved in the closest prior art. However, in line with the case law of the boards of appeal, once the technical effect and objective technical problem have been determined, it is still necessary to assess whether the solution claimed was obvious to the skilled person.

The question of obviousness was addressed by the appellant in a different context, namely that of a problem-solution approach starting from a medium with lower iron concentrations than claimed. The appellant argued that, in view of either document D4 or document D14, the skilled person would have modified the iron concentration in order to modify the Neu5Gc content of the glycoproteins. However, the question that had to be addressed in the present context was whether the skilled person seeking a solution to the problem as formulated above, would have modified the closest prior art to arrive at the concentration range in claim 1. The appellant has not argued that, when faced with that problem, the skilled person would have recognised this range of iron concentrations as a possible solution to the problem. In other words, it has not been argued that the concentration set out in claim 1 was well known to the skilled person for producing glycoproteins in eukaryotic cells.

36. In the board's view, neither of documents D4 and D14 cited by the appellant, albeit in a different context, suggest this concentration range either.

36.1 Document D4 concerns the occurrence, function, analysis and biosynthesis of sialic acids. As regards Neu5Gc, it

reports that it is exclusively synthesised via hydroxylation of the sialic acid metabolite CMP-acetylneuraminic acid (CMP-Neu5Ac) via the enzyme CMP-Neu5Ac hydroxylase (see page 26, second and third paragraphs). Regarding the enzyme's activity, it states: "*Several lines of evidence point to the participation of an iron cofactor in the reaction of the hydroxylase. For example, several iron-binding compounds are potent inhibitors of this enzyme [...] Furthermore, the addition of iron salts can stimulate the hydroxylase* [...]" (see page 28, last paragraph). It suggests that the activity of this enzyme is the main factor influencing the level of Neu5Gc in sialylated proteins and that this activity may be tuned so as to obtain the required ratio of Neu5Gc to Neu5Ac in the glycoprotein (see page 29, second paragraph, first and last full sentences). However, although the document sheds light on the role of the enzyme in the incorporation of Neu5Gc into glycosylated proteins, it does not provide any information on the effect of iron on the enzyme's activity in glycosylation during cell culture. Moreover, it does not disclose reducing the iron concentration in the medium. Indeed, what is directly pointed out is that adding chelators results in the reduction of enzyme activity. This document does not give any indication of the desirable iron concentration for cell culture.

36.2 Although document D14 discloses experiments carried out at a concentration of 0.2 mM iron, a value outside the range in claim 1, they use purified enzyme instead of cell culture. The board considers that only with hindsight can this be taken as a suggestion for modifying the medium in document D2 in the way claimed. Moreover, the question arises as to whether the skilled person would turn to this document at all to solve a

problem in the context of glycoprotein production, which is not the subject of document D14.

37. In conclusion, having regard to the cited documents, the subject-matter of claim 1 is not obvious to the skilled person. The same conclusion applies to independent claims 7, 11 and 12, which are directed to methods and uses of the medium defined in claim 1, respectively.

Disclosure of the invention (Article 83 EPC) - claim 12

38. The appellant's objection was that the patent did not demonstrate the effect recited in the claim, namely lowering the Neu5Gc content while maintaining or increasing the degree of sialylation. However, in line with the board's interpretation of claim 13 of the main request, as set out above under points 1. to 4., this effect does not limit the claimed use. Accordingly, whether this effect is attained is not a matter to be assessed under sufficiency of disclosure.

Requests to refer questions to the Enlarged Board of Appeal

39. The appellant's requests were conditional on the board concluding that the subject-matter of claim 13 was novel (see paragraphs 9 and 10 of the appellant's letter dated 9 October 2023) or alternatively on the board concluding that "increasing sialylation" was a limiting feature of claim 13 and that this claim interpretation resulted in the claimed subject-matter being novel with regard to document D7 and being sufficiently disclosed (see paragraphs 28 to 30 of the same letter).

40. The board concluded that the feature in question does not limit the use defined in claim 13. Moreover, the subject-matter of claim 13 is not novel over the disclosure in document D7. Therefore, neither of the conditions is fulfilled. Accordingly, the requests to refer questions to the Enlarged Board of Appeal were rejected by the board.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent in amended form with the following set of claims, and a description and drawings adapted thereto as necessary:

   claims 1 to 12 of auxiliary request 1 filed with the reply to the statement of grounds of appeal.

The Registrar: The Chairwoman:

I. Aperribay M. Pregetter

Decision electronically authenticated