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Datasheet for the decision of 11 April 2024

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Language of the proceedings: EN

Title of invention:
MODULATION OF CELL GROWTH AND GLYCOSYLATION IN RECOMBINANT GLYCOPROTEIN PRODUCTION

Patent Proprietor:
F. Hoffmann-La Roche AG

Opponents:
Schulz Junghans
Patentanwälte PartGmbB
ABG Intellectual Property Law, S.L.

Headword:
Modulation of cell growth and glycosylation in glycoprotein / HOFFMANN-LA ROCHE

Relevant legal provisions:
EPC Art. 123(2), 83, 84, 111
RPBA 2020 Art. 11

Keyword:
Main Request and Auxiliary request 1 - Amendments - added matter (yes)
Auxiliary request 2 - Clarity - (yes)
Auxiliary request 2 - sufficiency of disclosure - (yes)
Remittal (yes)

Decisions cited:
T 0292/85, T 0277/95, T 0422/99, T 1408/04, T 0063/06, T 1582/08, T 0428/09, T 1822/12

Catchword:

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Case Number: T 1113/22 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 11 April 2024

Appellant: (Patent Proprietor)	F. Hoffmann-La Roche AG Grenzacherstrasse 124 4070 Basel (CH)
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Respondent: (Opponent 2)	ABG Intellectual Property Law, S.L. Avenida de Burgos, 16D Edificio Euromor 28036 Madrid (ES)
Representative:	ABG Intellectual Property Law, S.L. Avenida de Burgos, 16D Edificio Euromor 28036 Madrid (ES)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 23 February**

2022 revoking European patent No. 3110961 pursuant to Article 101(3)(b) EPC

Composition of the Board:

Chairwoman	T. Sommerfeld
Members:	D. Pilat
	A. Bacchin

Summary of Facts and Submissions

I. European patent No. 3 110 961 is based on European patent application No. 15 709 430.1, filed as international application published as WO 2015/128314. The patent was opposed on the grounds of Article 100(a) in conjunction with Articles 54 and 56 EPC, and of Articles 100(b) and (c) EPC. The opposition division revoked the patent.

II. The patent proprietor (appellant) lodged an appeal against the decision of the opposition division. With the statement of grounds of appeal (SoG), the appellant re-submitted the main request and auxiliary requests 1 to 10 and submitted new auxiliary requests 11 to 15 as well as new documents D46 to D49.

III. With its reply to the appeal (RSoG), opponent 1 (respondent) requested that the appeal be dismissed or alternatively that the case be remitted to the opposition division for further prosecution. It moreover requested that auxiliary requests 10 to 15 and documents D46 to D48 not be admitted.

IV. With letter dated 16 February 2024, the appellant submitted new auxiliary requests 16 and 17. The respondent replied with letter dated 6 March 2024 and requested that auxiliary requests 16 and 17 not be admitted.

V. In a communication under Article 15(1) RPBA, the parties were informed of the board's provisional opinion on the issues of the case.

VI. The respondent filed comments to the board's communication on 25 March 2024.

VII. Opponent 2 made no submissions on the substance of the case in appeal proceedings. With letter dated 8 April 2024, opponent 2 announced that it would not attend oral proceedings.

VIII. Claim 1 of the main request reads as follows:

"1. A method for production of a recombinant glycoprotein under fermentation culture conditions in a mammalian cell, the method comprising adjusting the concentrations of each of iron, copper, zinc and manganese in the culture medium during the culture to affect biomass generation and/or N-glycan maturity in the expressed glycoprotein wherein the adjustment is:

- increasing the concentration of each of iron, copper, zinc and manganese in order to increase biomass, wherein the concentrations are adjusted to:
  (a) iron - from 15 µM to more than 80 µM;
  (b) copper - from 0.3 µM to more than 2.5 µM;
  (c) zinc - from 20 µM to more than 50 µM; and
  (d) manganese - from 0.01 µM to more than 3 µM;
  and/or
- increasing the concentration of each of zinc and manganese and, optionally, decreasing the concentration of each of iron and copper to increase N-glycan maturity in the expressed glycoprotein, wherein the carbohydrate portion of the expressed glycoprotein has a G0, G1 or G2 structure and wherein the concentrations of zinc, manganese, iron and copper are adjusted to:
  (a) iron - from 0 µM to 25 µM;
  (b) copper - from 0 µM to 0.1 µM;

(c) zinc - from 20 µM to more than 50 µM; and

(d) manganese - from 0.01 µM to more than 3 µM; or

- (i) decreasing the concentration of each of iron, copper, zinc and manganese to increase production of immature non-fucosylated glycoproteins or (ii) increasing the concentration of each of copper and iron and decreasing the concentration of each of zinc and manganese to increase production of immature non-fucosylated glycoproteins wherein the concentrations are adjusted to:

(a) iron - from 0 µM to 35 µM;

(b) copper - from 0 µM to 1 µM;

(c) zinc - from 0 µM to 20 µM; and

(d) manganese - from 0 µM to 0.01 µM; or the concentrations are adjusted to:

(a) iron - from 15 µM to more than 80 µM;

(b) copper - from 0.3 µM to more than 2.5 µM;

(c) zinc - from 0 µM to 20 µM; and

(d) manganese - from 0 µM to 0.01 µM;

wherein a decrease in the concentration of any or all of iron, copper, zinc and manganese in the culture medium is achieved by complexing the iron, copper, zinc and manganese with a chelator and/or by seeding the cells into a fresh medium containing a reduced concentration of any or all of iron, copper, zinc and manganese compared to the medium of the immediately preceding culture phase."

Dependent claims 2 to 7 define preferred embodiments of the method of claim 1.

IX. Claim 1 of auxiliary request 1 differs from claim 1 of the main request in that the method for production of a recombinant glycoprotein under fermentation culture conditions to increase of biomass **"and/or"** to increase N-glycan maturity in the expressed glycoprotein is

limited to either the increase of biomass **"or"** the increase of N-glycan maturity in the expressed glycoprotein.

X. Claim 1 of auxiliary request 2 differs from claim 1 of auxiliary request 1 in that the decrease in the concentration of any or all of iron, copper, zinc and manganese in the culture medium is achieved by seeding the cells into a fresh medium containing a reduced concentration of any or all of iron, copper, zinc and manganese compared to the medium of the immediately preceding culture phase, the alternative of complexing the iron, copper, zinc and manganese with a chelator having been deleted.

XI. The following documents are cited in this decision:

D1: Hossler, P., Advances in Biochemical Engineering /Biotechnology 127 (2012), pages 187 to 219

D2: Raju, T.S., Bio Process International, 2003, pages 44 to 53

D3: Surve, T. and Gadgil, M., Biotechnol Prog 31(2) (2015), pages 460 to 467 (Epub Dec. 29, 2014)

D4: Burgener, A. and Butler, M., in Cell Culture Technology For Pharmaceutical And Cell-Based Therapies, eds. S. Ozturk and W.-S. Hu, 2005, Chapter 3 Medium Development, pages 41 to 79

D5: WO 2006/026445

D15: WO 2011/134919

D16: Qian, Y. *et al.*,Biotechnol. Prog., 2011, Vol. 27, No. 4, pages 1190 to 1194

D17: Bai, Y. *et al.* Biotechnol. Prog., 2011, Vol. 27, No. 1, pages 209 to 219

D31: WO 99/61650

D40: Markert, S. *et al.*, Biotechnol Prog., 2020, Vol 36, 6, e3042, pages 1 to 17

XII. The parties' submissions, insofar as they are relevant to the decision, are discussed in the reasons for the decision, below.

XIII. The appellant (patent proprietor) requested that the decision under appeal be set aside and that the case be remitted to the opposition division or, alternatively, that the patent be maintained on the basis of the claims of the main request or of any of auxiliary requests 1 to 17.

XIV. The respondent (opponent 1) requested that the appeal be dismissed. Alternatively, should the board come to the conclusion that the main request or any of the auxiliary requests met the requirements of Articles 123(2), 84 and 83 EPC, it requested that the case be remitted to the opposition division for further prosecution. It requested that auxiliary requests 10 to 17 not be admitted into the appeal proceedings.

XV. There are no requests on file from opponent 2.

Reasons for the Decision

Main request and auxiliary request 1 - claim 1
Article 123(2) EPC

1. Claim 1 of the main request and of auxiliary request 1 recites that a decrease in the concentration of any or all of iron, copper, zinc and manganese in the culture medium is achieved by complexing the iron, copper, zinc and manganese with a chelator and/or by seeding the cells into a fresh medium containing a reduced

concentration of any or all of iron, copper, zinc and manganese compared to the medium of the immediately preceding culture phase (for the full wording of the claim, see section VIII).

2. Contrary to the conclusions of the opposition division, the board agrees with the respondent that there is no direct and unambiguous basis for the decrease of the metal ion concentrations by complexing metal ions with a chelator without a washing out of the complexed metal ions. The board considers that the passages indicated by the appellant, as discussed in the following, do not provide such a basis.

3. Indeed, the passage on page 33, first full paragraph, of the application as filed discloses that "a decrease in the concentrations of any or all of iron, zinc, copper or manganese can be achieved in an existing or fresh medium by complexation of the metal ion, for example by addition of a metal ion chelator to the culture and washing out of the complexed metal ion". Contrary to the appellant's arguments, the term "for example" in this passage refers to the entire process consisting of adding a metal ion chelator to the culture and washing out of the complexed metal ion. There is no direct or unambiguous disclosure that the second step, which is preceded by an "and", is optional.

4. As to original claim 17, it refers to a decrease in the bioavailable concentration of any or all of iron, copper, zinc and manganese in the culture by complexing the iron, copper, zinc and manganese with a chelator. However the board considers that the expression "bioavailable concentration" has a different meaning to the term "concentration", as the bioavailable iron

concentration will not be decreased when complexed with a chelator, such as transferrin, unlike a free ion. Hence, claim 17 as filed cannot provide a basis either.

5. Finally, since claim 17 is directed to decreasing bioavailable concentration while the passage on page 33 is concerned with decreasing concentration, the two passages cannot be combined. Hence, even if the use of a chelator without a washing out step could be identified as a common denominator between the two passages, as argued by the appellant, the skilled person would not have derived directly and unambiguously, using common general knowledge, from the whole of the application as filed, a method as in claim 1 wherein a decrease in the concentration of any or all of iron, copper, zinc and manganese in the culture medium is achieved by complexing the iron, copper, zinc and manganese with a chelator alone without a step of washing out.

6. For this reason claim 1 of the main request and of auxiliary request 1 contravenes Article 123(2) EPC.

Auxiliary request 2
Claim construction

7. Claim 1 is directed to a method for production of a recombinant glycoprotein under fermentation culture conditions in a mammalian cell. This method comprises adjusting the concentrations of iron, copper, zinc and manganese in the culture medium during the culture to affect biomass generation (a) and/or N-glycan maturity in the expressed glycoprotein (b).

8. The adjustment to affect biomass generation (a) "to increase biomass" is defined in **aspect A** by

"increasing" each of the concentrations of iron, copper, zinc and manganese. For achieving N-glycan maturity in the expressed glycoprotein (b), two alternatives are defined, namely: increasing each of zinc and manganese and optionally decreasing each of iron and copper in order "to increase N-glycan maturity in the expressed glycoprotein, wherein the carbohydrate portion of the expressed glycoprotein has a G0, G1 or G2 structure" (**aspect B)**; or either decreasing each of iron, copper, zinc and manganese (**aspect C**, item (i) in the claim) or increasing copper and iron and decreasing zinc and manganese (**aspect D**, item (ii) in the claim), in order to "increase production of immature non-fucosylated glycoproteins". For each aspect, concentration ranges for each of these elements are given, as shown in the following:

[aspect A]
- increasing the concentration of each of iron, copper, zinc and manganese in order to increase biomass, wherein the concentrations are adjusted to:
(a) iron - from 15 µM to more than 80 µM;
(b) copper - from 0.3 µM to more than 2.5 µM;
(c) zinc - from 20 µM to more than 50 µM; and
(d) manganese - from 0.01 µM to more than 3 µM;
and/or

[aspect B]
- increasing the concentration of each of zinc and manganese and, optionally, decreasing the concentration of each of iron and copper to increase N-glycan maturity in the expressed glycoprotein, wherein the carbohydrate portion of the expressed glycoprotein has a G0, G1 or G2 structure and wherein the concentrations of zinc, manganese, iron and copper are adjusted to:
(a) iron - from 0 µM to 25 µM;
(b) copper - from 0 µM to 0.1 µM;

(c) zinc - from 20 µM to more than 50 µM; and
(d) manganese - from 0.01 µM to more than 3 µM;
[aspect C]
(i) decreasing the concentration of each of iron, copper, zinc and manganese to increase production of immature non-fucosylated glycoproteins or
[aspect D]
(ii) increasing the concentration of each of copper and iron and decreasing the concentration of each of zinc and manganese to increase production of immature non-fucosylated glycoproteins wherein the concentrations are adjusted to:
(a) iron - from 0 µM to 35 µM;
(b) copper - from 0 µM to 1 µM;
(c) zinc - from 0 µM to 20 µM; and
(d) manganese - from 0 µM to 0.01 µM; or the concentrations are adjusted to:
(a) iron - from 15 µM to more than 80 µM;
(b) copper - from 0.3 µM to more than 2.5 µM;
(c) zinc - from 0 µM to 20 µM; and
(d) manganese - from 0 µM to 0.01 µM.

9. The method steps of adjusting the concentrations of iron, copper, zinc and manganese in aspects A to D are further limited by the functional feature relating to the effect to be achieved, which limits the concentrations to be used to those within the concentration ranges that achieve the claimed effect on the expressed recombinant glycoprotein. Each aspect A to D defines a particular effect and the necessary adjustment of concentrations, which must fall within a defined numerical concentration range in order to obtain that effect. Clearly the "increase" or "decrease" of ion concentrations in the claim are process steps that must be carried out. This means that the cells must be cultured in a medium that has a lower

or higher ion concentration than after the claimed decrease or increase (patent, paragraph [0120]). In addition, the adjusted concentration of the respective ion in the medium must achieve the claimed effect associated to aspects A to D. The increase in biomass or N-glycan maturity is in relation to the biomass or N-glycan maturity where no adjustment (increase or decrease) of concentration of the metal ions as claimed has occurred. The claim does not require that the concentrations of ion in the medium prior to the increase be below the ranges defined in the claim. Nor does claim 1 require a starting value for the ion concentrations or impose any extent of adjustment. The increase in biomass must at least be measurable, but the claim does not require it to be statistically significant.

10. Although the claim does not define an upper limit for the concentrations of the ions to be increased, the board considers that the skilled person, understanding the claim in a technically logical sense, would not use concentrations of iron or other claimed trace element ions which were known in the art to be toxic (e.g. document D17, paragraph bridging pages 209 and 210; see e.g. T 1408/04, reasons 1, and T 1582/08, Reasons 16).

11. Thus, the method steps of claim 1 are interpreted by the board, in accordance with both parties, as imposing a functional limitation - achieving the effect - to the numerical limitation of the adjusted concentrations defined in aspects A to D of claim 1.

Article 123(2) EPC

12. In comparison to claim 1 of the main request and of auxiliary request 1, in auxiliary request 2, claim 1

has been amended so as to remove the alternative involving the use of a chelator, which was found to add subject-matter (see points 2. to 6. above).

13. The respondent maintained their objections that the application as filed did not provide a basis for the claimed "and/or" at the end of aspect A, and that it did not disclose the concentration ranges for aspects C and D, which required a decrease in each of iron, copper, zinc and manganese or alternatively an increase in the concentration of each of copper and iron and a decrease in the concentration of each of zinc and manganese.

14. According to the respondent, the conjunction "and" at the end of aspect A of claim 1 implied that an increase in biomass as defined in aspect A and in addition any of the additional technical effects claimed in aspect B, aspect C and aspect D had to be obtained by the same method. The combination of aspects reported on page 7, lines 11 to 16 of the patent application as filed was just one embodiment among others, in which growth had been enhanced before glycoprotein maturity was enhanced. There was no disclosure of a method that applied a number of adjustments in an arbitrary order, as now in the claim.

15. The board notes that the conjunction "and/or" at the end of aspect A can be found in claim 1 as originally filed. Thus, claim 1 as originally filed already encompassed that the ion concentrations were to be adjusted to affect either the biomass and/or N-glycan maturity. How each of these aims was achieved was further defined in original claims 3 and 8 (for the first aim, aspect A) and in original claims 4, 6 and 9 (for the second aim, aspect B). It is immaterial that

the claimed embodiment represents only one embodiment and that the claim is not limited to a sequence of steps, where first the growth is enhanced and then the glycoprotein maturity is enhanced (patent application as filed, page 7, lines 11 to 16), as these limitations do not appear in the above mentioned original claims either. Thus, on their own, claims 1, 3, 4, 6, 8 and 9 provide sufficient basis for these features of claim 1 of the main request.

16. As regards aspects C and D, it is true that original claim 11 defines two sets of concentration ranges for each metal ion, and does not specify whether the metal ion concentrations are to be decreased or increased. However, by reference to claim 7, it is specified that the concentrations of each of copper, iron, zinc and manganese are decreased, in accordance with aspect B of claim 1. Likewise, although claims 7 and 11 as filed provide no basis for increasing the concentrations of copper and iron while decreasing the concentrations each of zinc and manganese, as claimed in aspect D of claim 1 of the main request, the board considers that a basis is present in the application as filed on page 7, third paragraph, third item, alternative (ii), read in combination with page 8, lines 14 to 26.

17. Accordingly, auxiliary request 2 complies with Article 123(2) EPC.

Clarity (Article 84 EPC)

18. According to the respondent, aspect B of claim 1 of the main request and thus of auxiliary request 2 was unclear because the adjustments of the concentrations of iron and copper in aspect B of granted claim 1 were optional, whereas the adjustments of concentration

ranges in granted dependent claim 3 were specified for each of iron, copper, zinc and manganese, including iron and copper. Amended claim 1, which combined granted claims 1 and 3, lacked clarity because it was not clear whether the adjustment of iron and copper was optional or not. As regards aspects C and D, since the numbering of the two sets of concentration ranges (i) and (ii) defined in granted claim 5 was omitted in amended claim 1, any set of concentration ranges (i) or (ii) could be assigned any set of measures claimed, thus rendering also these aspects of claim 1 unclear.

19. The board disagrees with the respondent's views for the following reasons:

Claim 1 of auxiliary request 2 is based on granted claim 1 in combination with granted claim 3 (for aspect B) and 5 (for aspects C and D). Already for the reason that new claim 1 is based on the combination of an independent granted claim with dependent granted claims in their whole (so-called type B amendments in decision G 3/14, OJ 2015, 102, Reasons 2.), clarity is in principle not open to discussion (G 3/14, Reasons 80. and 81.)

20. Furthermore, the board fails to identify any alleged new lack of clarity. The option that the concentration of each of iron and copper is decreased to increase N-glycan maturity in the expressed glycoprotein was present in granted claim 1 and is still present in claim 1 of auxiliary request 2. That claim 1 of auxiliary request 2 further specifies that the concentration of each metal ion for all four ions is adjusted to specific concentrations, including iron and copper, while at the same time a decrease for iron and copper is mentioned to be optional, is not considered

to be inconsistent, because the given ranges in fact do not provide any indication of whether the concentrations of iron and copper are decreased or increased or at all changed. So decreasing the concentrations of these metal ions continues to be optional, as it was in granted claim 1 and in fact also in granted claim 3, and no lack of clarity has been introduced.

21. Similar considerations apply to the amendments related to aspects C and D. While granted claim 1 only generally referred to decreasing the concentrations of all ions (as item (i)) or to increasing copper and iron and decreasing zinc and manganese (as item (ii)), granted claim 5 provided two sets of specific ranges for the concentrations of the ions. For the reasons explained above (point 8. of the reasons above), the board considers that the numbering (i) and (ii) in claim 5 as granted, which was not adopted in claim 1 of auxiliary request 2, cannot be interpreted as referring to items (i) and (ii) of claim 1 as granted. Hence, claim 1 is interpreted, in its broadest possible sense, in that the adjustment of iron, copper, zinc and manganese can be made to any concentration of the two sets of concentration ranges, while at the same time respecting either alternative (i) or (ii) of claim 1 as granted. This is in principle also the interpretation that would have been given to claim 5 as granted, therefore no new lack of clarity has arisen. At the most, one could interpret granted claim 5 as restricting alternatives (i) and (ii) of granted claim 1 to alternatives (i) and (ii), respectively, of granted claim 5, which is however not in agreement with the claims as filed (point 8. of the reasons above). Even if that was the case, the new interpretation of

claim 1 of auxiliary request 2 would still not be unclear.

22. The respondent's objections against auxiliary request 2 under Article 84 EPC are thus not convincing.

Sufficiency of disclosure (Article 83 EPC)

23. In view of the above claim construction, the technical effects of affecting biomass generation and/or N-glycan maturity are technical features of the claim. Hence any failure of the claimed method to deliver these effects is to be dealt under sufficiency of disclosure rather than inventive step (see G 1/03, OJ 2004, 413, point 2.5.2 of the Reasons and Case Law of the Boards of Appeal of the European Patent Office 10th edition 2022, hereinafter "Case Law", II.C.6.1).

24. The respondent argued, in particular at the oral proceedings, that the claimed subject-matter was not sufficiently disclosed because it did not fulfil the criteria set out in decision T 422/99, point 3.2 of the Reasons, according to which the requirements of Article 83 EPC are only met:

(i) if at least one way is clearly indicated in the patent specification enabling the skilled person to carry out the invention, and

(ii) if the disclosure allows the invention to be performed in the whole area claimed

(iii) without undue burden, applying common general knowledge.

25. The respondent moreover argued, in agreement with the opposition division (appealed decision, page 51, last paragraph), that, since the patent did not contain a single example or sufficient information on how the invention could be put into practice, there was only a weak presumption that the invention was sufficiently disclosed. The respondent's burden of proof could therefore be discharged by plausibly arguing that the skilled person would not be able to put the invention into practice over the entire breadth of the claim using common general knowledge (see decision T 63/06, reasons points 3.1.1 to 3.2.2).

26. The board however disagrees that in the present case there is a weak presumption that the invention is sufficiently disclosed. In fact, Examples 2 and 3 predict that increasing and/or decreasing the iron, copper, zinc and manganese concentrations according to aspects A to D of claim 1 will achieve the claimed technical effect of increasing biomass, increasing N-glycan maturity in the expressed glycoprotein or increasing production of immature non-fucosylated glycoproteins. Although, admittedly, Example 2 does not show any step of increasing or decreasing ion concentrations, the effects of zinc, iron, copper, and manganese concentration variations are illustrated on a curve e.g. in terms of viable cell density (VCD) or cell time integral (CTI), see Figures 6C, 6F and 8. On the other hand, Example 3 teaches that the iron concentration could be decreased from day 6 on in order to increase the amount of mature N-glycans with a G0, G1 or G2 (aspect B of the claimed invention). The "Growth" and "Galactosylation" setups (Figures 9A and B) show that a relative increase in concentrations of both zinc and manganese from day 6, corresponding to the concentrations in Table 10, in line with the real-

time measurements in Figures 13C and D, results in an increase in N-glycan maturity of the expressed glycoprotein (Figures 11B and C). In addition, paragraphs [0176] and [0177] of the patent teach that (i) using low concentrations of each one of iron, copper, zinc and manganese or (ii) using the opposite conditions to those required to generate miminum levels of mannosylated glycans, i.e. high concentration of each of copper and iron and low concentration of each of zinc and manganese, according to aspects C and D, results in an increase of the production of immature non-fucosylated glycoproteins as illustrated in Figures 8E, 8F, 8D, 8G and shown for the "Afucosylation" setup in Figures 9A, 11A, 11D, 13A and 13D.

27. Moreover, although the "Growth" setup in Figure 9A does not fall under the scope of aspect A of claim 1 because the iron concentration is decreased rather than increased, it is nevertheless plausible that increasing the concentration of iron immediately after cell culture splitting on day 6 (Figures 6C and 8A), instead of decreasing it, will increase the biomass based on example 2 (Figures 9B and C). Hence, the board concurs with the appellant that there are no serious doubts, let alone supported by verifiable facts, that the technical effect cannot be achieved over the whole breadth of the claims.

28. In view of the information and the examples in the patent, a strong presumption of validity exists. Moreover, the patent clearly indicates at least one way of putting the invention in practice, enabling the skilled person to carry out the invention, and allowing the invention to be performed in the whole area claimed, without undue burden, applying common general

knowledge. The board thus considers that the claimed subject-matter is sufficiently disclosed.

29. The respondent essentially argued that there was no reproducible teaching of the claimed invention, in particular since the examples used specific parameters which could not be reproduced (i). Moreover, the lack of indication of upper range limits for the ion concentrations allowed toxic concentrations to be used or at least would require an undue burden from the skilled person to determine which concentrations still achieved the desired effect (ii). Finally, for each of the aspects of the claim, the evidence in the patent or in the prior art did not support that the recited technical effect could be achieved (iii). These arguments are discussed in detail in the following sections.

(i) No reproducible teaching of the invention claimed

30. It is true that the examples in the patent may not necessarily be reproducible, because information relating to the identity of the cells, media composition and feed platforms is not disclosed, as observed by the opposition division in the appealed decision (page 52, penultimate paragraph). However, the composition of the cell culture media had a critical impact on biomass and glycosylation, as was evidenced e.g. by the fact that manganese increased high mannose glycoforms (i.e. immature forms) on monoclonal antibodies expressed in CHO cells when glucose was absent or limiting (document D3, abstract). The presence of transferrin in the medium also had an impact on the availability of iron (patent, paragraph [0014]; document D4, page 64, penultimate paragraph; appealed decision, page 53, second paragraph).

Moreover, further process parameters such as pH, temperature, inoculation densities, oxygen saturation, shifts in one or more conditions were not disclosed either. According to document D1 (page 196, last paragraph and end of penultimate paragraph), document D5 (paragraphs [0160] and [0173] and document D15 (page 1, second paragraph), such parameters also had an impact on the effect of adjustment of metal ion concentration.

31. However, it is established case law that there is no need for a skilled person to be able to exactly reproduce a patent's examples in order for the claimed invention to satisfy the requirements of sufficiency of disclosure under Article 83 EPC (see Case Law, II.C. 6.1). Determination of the appropriate cell culture parameters for a given cell and a given medium is routine for the skilled person, who would draw from its common general knowledge that sugars in the medium have an impact in the glycosylation of the expressed proteins and that transferrin impacts on the availability of iron in the medium. The respondent has not substantiated its allegation that the claimed technical effects would not be achieved when using any mammalian cell expressing a glycoprotein under any culture conditions and in any culture media, applying the claimed adjustments in trace elements concentration, being functionally limited to those capable of increasing biomass and/or N-glycan maturity in the expressed glycoprotein. Nor has it provided any corroborating evidence that there would be serious doubts substantiated by verifiable facts in the patent and in scientific literature that the claimed technical effects are not obtainable over the scope of the method according to claim 1. Although the Examples of the patent may not be exactly repeatable as such, the

claimed subject-matter can be carried out by the skilled person without undue burden and, in the absence of evidence to the contrary, will lead to the recited effects.

32. The respondent moreover argued that, as emphasized in the patent itself (paragraphs [0184] and [0188]), the timing of the adjustment of the ion concentrations had a critical impact on the technical effect; firstly, according to the patent (paragraph [0121], Table 2), the adjustments of ion concentrations could occur at any time during cultivation and independently from each other; secondly, the timing of the manganese adjustment had a critical impact on N-glycan maturity (patent, paragraph [0170], Figures 5A and 5B); and, thirdly, a critical impact was also attributed to the proprietary cells, which were known to have an heterogeneous glycosylation in a culture medium, where the metal ions could act as co-factor or as inhibitor (document D40, Table 1; document D1, paragraph bridging pages 193 and 194; document D2, paragraph bridging pages 45 and 46, page 52, middle column; patent, paragraphs [0018], [0157] and [0171]).

33. The board considers that, even though the patent does not mention details such as the time of adjustment, the values to which the metal ions must be adjusted within the open-ended range, whether the culture media contains components which could potentially interact with metal ions thereby reducing the concentration of available metal ion, it is well established and routine for the skilled person in the art to determine such parameters and adapt the culture conditions accordingly (patent, Figure 2 and Table 2). With regard to the specificity of the cell lines, Figure 14 of the patent, which refers to four different clones, shows that they

behave in a similar way with regard to the claimed concentration of metal ions over the duration of the process. Although there is no reference to N-glycan maturity in Figure 14, there is no evidence that it would not be achieved either. Similarly, Figure 4 of the patent shows the effect of trace elements on mAb galactosylation for two clones, but there would be no reason to doubt that the predicted results could not be achieved for other clones as well.

(ii) No indication of upper limits for the ranges of ion concentrations

34. As discussed above, the skilled person would immediately exclude such ion concentration values that are known to be toxic to the cells. Since the toxicity of these metal ions formed part of the common general knowledge (e.g. patent, paragraphs [0003] and [0009], (Cu: patent, paragraph [0016] and document D16, page 1190, left column, last sentence, document D31, page 2, penultimate paragraph, page 4, lines 26 to 29, Figure 4; Fe: document D4, page 29, which is page 64 of the reproduced book, penultimate paragraph, document D17, paragraph bridging pages 209 and 210; Mn: patent paragraph [0009]), the skilled person would have excluded detrimental ion concentrations as being clearly outside the scope of practical application of the claimed subject matter. The known toxic ion concentrations would not be regarded by the skilled person as being covered by the claim and cannot therefore justify an objection of insufficiency of disclosure (Case Law, II.C.8.1).

35. Of course, as argued by the respondent, there will be values in between where the skilled person would have to find out whether they still lead to the desired

effect or not. Of note is that if the claimed effect of aspects A to D could not be achieved, then said embodiment would not fall under the ambit of the method of claim 1 because the functional feature of claim 1 would not be met. The skilled person can however perform the invention without having first to determine which values for each ion concentration still fall within the scope of the claim and achieve the claimed effect. Hence the board fails to see that this would represent an undue burden.

(iii) No support in the patent or prior art for the technical effect being achieved in each of the aspects of the claim

Aspect A - increase in biomass

36. The respondent argued that none of Examples 1 to 4 supported the technical effect of aspect A of claim 1. Example 1 did not contain any information relating to iron. Examples 2 to 4 did not contain any indication that an increase in biomass as required in the claim occurred. The patent did not therefore provide any evidence that increasing the concentrations of all four ions as claimed would lead to an increase in biomass; it also did not show that an increase in concentration for any of the ions alone achieved this effect. To the contrary, the patent presented data which were in contradiction with the claimed invention. For example, the parabolic curve in Figures 6C, 6F and 8A to 8G of the patent meant that adjusting an iron concentration according to aspect A of claim 1 beyond a certain concentration yielded a decrease in biomass instead of an increase (Example 2). In Example 3, the VCD and CTI at days 6 to 10 was highest under conditions of the cell "Growth" setup, but the iron concentration was decreased from 47.158 µM to 15 µM or lower after day 6,

i.e. to levels outside the scope of the claim (Example 3, Figures 9B, 9C and 13B, Table 9A). On the other hand, although the "Afucosylation" setup kept the iron concentration at a high level, within the recited range, and increased on day 6 (Figure 13B), while the zinc concentration fell outside the claimed range throughout the entire culture period (Figure 13D), the biomass did not increase when compared to iron conditions not covered by the claim ("Growth" setup). In Example 4, the initial concentrations of all metal ions for the GG (Growth/Galactosylation) setup were within the respective ranges defined for aspect A, while the medium for the GA (Growth/Afucosylation) setup had a zinc ion concentration of 12 µM instead of more than 20 µM as defined for aspect A (Table 12, Figure 12C and D). The biomass obtained for the GA setup was however higher than for the GG setup. The copper concentration had no influence on CTI and accordingly on the biomass (Example 2, Figure 8A). Although, between days 1 to 4, the copper concentration in the "Galactosylation" setup was below the lower limit defined in aspect A while in the "Growth" setup the copper concentration was within the recited range, the biomass obtained by the "Galactosylation" setup was equal or even increased over the "Growth" setup (Example 3, Figure 13A). In contrast to the patent's statement in paragraph [0050] that both VCD and CTI were equally suitable for measuring the biomass, the two parameters led to different results - increase vs. no increase - for said experiment and thus made the lack of sufficient disclosure even more apparent.

37. The board agrees with the respondent that the examples of the patent do not disclose a method of claim 1 which increases the concentrations of each of the concentrations of iron, copper, zinc and manganese and

wherein the concentration of each of these elements are adjusted to the ranges set in aspect A of claim 1. Instead, they show the effects of changes in concentration for each ion in isolation and not together. But exactly because the examples do not fall in the scope of the claim, their results cannot cast doubt on the practicability of aspect A of the claimed method. The fact that an increase of each ion alone does not lead to the desired effect or that the effect is present even for concentrations of one ion falling outside the claimed ranges cannot therefore be taken as evidence that the claimed technical effect is not achieved. The invention is based on the prediction that the claimed technical effects are achieved when the ion concentrations are adjusted as claimed and there is no evidence in the patent or on file that this is not the case.

38. As regards Example 2, the board agrees with the appellant that the parabolic curve of Figure 6C is a predictive function using a quadratic term of iron (iron x iron = iron2) allowing for the modelling of non-linear relationships between variables (paragraph [0175]). Hence the decrease seen in Figure 6C means that the rate of "biomass increase" diminishes as the ion concentration increases (negative quadratic effect) but still that the biomass increases with an increase in ion concentration (positive linear effect). From the parabolic curves, it cannot be concluded that an increase in iron concentration beyond the concentration corresponding to the top of the parabolic curve will result in a biomass decrease. Thus, neither Figure 6C nor Figure 8A can substantiate serious doubts that the method can be performed across the entire scope of claim 1, using claimed concentration of iron beyond the

concentration corresponding to the top of the parabolic curve.

39. Thus, the respondent's arguments fail to establish that a skilled person would not be able to carry out the claimed method across the entire scope of aspect A of claim 1 without undue burden.

Aspect B – Increase of N-Glycan Maturity

40. As regards aspect B, the respondent argued that document D3 provided serious doubts that the claimed increase in N-glycan maturity could be obtained by adjusting the manganese concentration in the range recited in the claim, because document D3 achieved contrary effects: in document D3, manganese was shown to increase high mannose glycoforms (M5), i.e. immature glycoforms (patent, paragraph [0072], lines 29 and 30 and paragraph [0082], line 46) of the monoclonal antibody expressed in CHO under particular conditions (document D3, abstract). Moreover, document D3 showed that the amount and type of sugar in the cell culture medium would compromise the effect of manganese. The respondent further argued that the experimental section did not disclose any process that would encompass an increase in concentration of both zinc and manganese. There was therefore no confirmation that the claimed increase in N-glycan maturity in the expressed glycoprotein, wherein the carbohydrate portion of the expressed glycoprotein had a G0, G1 or G2 structure, was accordingly achieved over the whole breadth of the claim.

41. As regards document D3, the board notes that this document relates to the effect of manganese in a medium under specific conditions on N-glycan maturity of

mammalian cells, the carbohydrate portion of which are non-fucosylated A2 and A2G1 forms, which are not covered by aspect B of claim 1, which refers to fucosylated G0, G1 or G2 structure. Consequently, document D3 cannot substantiate serious doubts that an increase in N-glycan maturity, the carbohydrate portion of which have a G0, G1 or G2 structure, can be obtained by adjusting the concentration of manganese within the range defined in claim 1.

42. As regards the experimental section of the patent, the board agrees with the appellant that the manganese and zinc concentrations are shown to be increased for the "Galactosylation" setup in Example 3 on day 6 (Figures 9A, 13C and 13D). Firstly, the "Galactosylation" setup is in line with the concentration range defined in claim 1 for zinc and manganese. Zinc was around 30 µM and, thus, below the "high" level of Table 10 (50 µM) from day 10 to 14 (Figure 13D). Secondly, the manganese was increased from approximately 0.01 µM to 0,036 µM on day 6 and maintained at approximately 0.01 µM from day 7 to 10 (Figure 13C). Despite the low concentration of zinc and the low level of manganese, the proportion of glycoforms G0 and G1 obtained was highest for the "Galactosylation" setup at days 10 to 14 (Figures 11B, 11C). Example 2 confirms the linear increase in N-glycan maturity for the glycoform G1 starting from level -1 for zinc (13.9 µM) (Figures 8D and 15).

43. The respondent moreover argued that, although it was described in Example 1 that increasing the zinc concentration tended to have a positive effect on relative G1 and G2 abundance, the results depicted in Figures 16A to 16D did not reflect this alleged trend. An increase in zinc concentrations at constant concentrations of copper and manganese (Table 8: level

0 = 0.091 µM) had no effect on immature mannose glycoforms, which would otherwise decline if N-glycans maturation occurred (Figures 7B and 8A). There was no increase in N-glycan maturity when cells were cultured in the "Growth" setup medium having a zinc concentration defined in aspect B compared to the "Afucosylation" setup medium having a zinc concentration outside the claimed range (Figures 11A to 11C). The N-glycan maturation, in terms of G0 and G1 and high mannose content, should be the same from day 10 to day 14 for the "Growth" and "Galactosylation" setups, since they had almost the same zinc and manganese concentrations (Figures 13C and 13D), when compared to the "Afucosylation" setup, but this was not the case (Figures 11A to 11C).

44. The board notes that although the manganese concentration in the "Growth", "Galactosylation" and "Afucosylation" setups was increased on day 6 and fell within the concentration range defined in aspect B of claim 1 (Figure 13C), the zinc concentration in the "Afucosylation" setup is far below the concentration defined in aspect B of claim 1, whereas for the "Growth" setup the zinc concentration was decreased instead of being increased on day 6. These facts are confirmed in paragraph [0178] and Figure 15, in particular Figures 15A, 15D). Since the "Growth" setup is not an embodiment of aspect B of claim 1, because the zinc concentration is not increased, it cannot be compared to the "Afucosylation" setup, whose zinc concentration is outside the claimed range. Even if there is no material difference between the "Growth" and "Galactosylation" setups with regard to the mandatory zinc and manganese concentrations during the referenced culture period of days 10 to 14 (Figures 13C and 13D), the "Galactosylation" setup is the only

embodiment of claim 1 aspect B showing an increase of both manganese and zinc concentrations within the concentration range of claim 1 aspect B on day 6. The comparison of N-glycan maturity obtained with the "Galactosylation" and "Afucosylation" setups, which show a measurable increase in G0 and G1 glycoforms (Figures 11D and 11C), provides evidence that the ion concentration adjustments recited in the claim lead to the claimed effect.

45. Moreover, although the concentration of zinc in Examples 1, 2 and 4 is in line with the concentration used in aspect B of claim 1, the numerical values for G1 or G2 glycoforms on the ordinate of Figure 16A, which correspond to the increase of both manganese and zinc concentrations, show that there is an increase in G1 or G2 glycoforms (patent, paragraph [0168]). The respondent's argument that an increase in zinc concentration does not cause an increase in G1 or G2 glycoforms (Figures 16A to 16D) must fail.

46. While the board agrees with the respondent that there is a decrease of G1 species in Figure 8A when the zinc concentration increases, as defined in aspect B of claim 1, it notes also that an increase in the concentration of manganese increases G1 species. Thus, when both zinc and manganese have high relative concentrations, independently of the copper and iron concentrations, an increase in G1 species is clearly observed (Figures 8C, 8D, 8G). Moreover, the numerical values of G1 species for all the zinc and manganese with a high relative concentrations (+1) in Figures 8A, 8C, 8D, 8G is significantly higher, i.e. above 5,9, than when zinc or manganese or both have a low relative concentrations (-1) i.e. below 2.7. The respondent's

argument relying on a particular and isolated facet of Figure 8A is therefore not persuasive.

47. Although an increase in N-glycan maturity requires a concomitant decrease in immature species, this effect is not observed in Figures 7B, 7G and 7H when the manganese concentration is at 0.091 µM, in line with the claimed manganese concentration, and the zinc concentration is increased. However, when both zinc and manganese are increased and selected to have high relative concentrations, independently of the copper concentration, a decrease in immature species w/o Fuc is observed by its value on the abscissa, albeit in some cases only slightly (Figures 7B, 7G and 7H), as well as a net increase in G1 abundance (Figure 8A). Again, the respondent's argument based on one facet of Figures 7B, 7G and 7H is not persuasive.

48. Paragraph [0174] of the patent reports about the prediction profiler results of Example 2. Depending on its concentration, copper plays a crucial role on the N-glycan maturity. In general, on moderate levels, zinc displayed a negative and copper a positive correlation to the amount of high mannose glycans as depicted in Figure 7. Although there is cross-interaction between zinc and copper, there are no results and evidence in Figure 7 which would plausibly show and support that increasing both zinc and manganese concentrations would result in an increase in immature species w/o Fuc and thus a decrease in N-glycan maturity, contrary to what is claimed in aspect B of claim 1. Furthermore, although paragraph [0174] relates to aspects C and D, rather than aspect B of claim 1, Figure 8G in the patent confirms that aspect B of claim 1 is indeed obtained. The respondent's argument is therefore not convincing.

49. As the decrease of each of iron or copper concentration is optional, it is not necessary to assess whether this adjustment results in an increase in N-glycan maturity as claimed in claim 1.

50. Thus, based on the above evidence there are no serious doubts supported by the verifiable facts in the patent that the claimed technical effect is not obtainable for the entire concentration range in aspect B of claim 1.

Aspects C and D - Increase of Immature Non-Fucosylated Glycoproteins

51. The respondent argued that the increase in the production of immature non-fucosylated glycoproteins was not supported by any experiment in the patent for any of the measures claimed in aspects C and D. Given the iron or copper concentration range claimed, which was infinite, an increase in immature non-fucosylated glycoproteins according to aspects C and D should be obtainable at any iron or copper concentration. However, decreasing the zinc concentration to the relative concentration level (-1) of 13.900 µM, which is within the claimed range (see Table 8), did not lead to an increase in the production of immature non-fucosylated glycoproteins (Figures 7B, 7G, 7H and 7J).

52. The board notes that decreasing both the concentrations of zinc and manganese to a concentration within the claimed range, independently of the selected iron or copper concentrations, is required in aspects C and D of claim 1. Even if the concentrations of both zinc and manganese are decreased, independently of the copper and iron concentrations, a clear increase in immature non-fucosylated glycoproteins is observed (Figures 7B,

7G, 7H and 7J and 8A and 8B), while the manganese concentration does not fall under the claimed range (Table 8). The respondent's argument, relying solely on a particular and isolated facet of Figures 7 and 8, is therefore not persuasive.

53. Thus, based on the above discussion there are no serious doubts supported by verifiable facts that the claimed technical effect is not obtainable for the entire concentration range in aspects C and D of claim 1.

54. For all the reasons given above, sufficiency of disclosure is acknowledged.

Requests for remittal (Article 111 EPC, Article 11 RPBA)

55. No substantive examination of auxiliary request 2 has been carried out by the opposition division under Articles 54 and 56 EPC. In such a situation, the board would therefore perform, by not remitting the case to the opposition division, both first- and last-instance proceedings tasks and would effectively replace the opposition division rather than review the decision under appeal in a judicial manner, as required by Article 12(2) RPBA. Thus, special reasons exist for remitting the case within the meaning of Articles 11 RPBA 2020, which *inter alia* concurs with the parties' requests to remit the case to the opposition division for further prosecution.

56. Consequently, the board considers it appropriate to exercise its discretion under Article 111(1) EPC to remit the case to the opposition division for further prosecution.

Order

For these reasons it is decided that:

1. The appealed decision is set aside.
2. The case is remitted to the opposition division for further prosecution.

The Registrar: The Chairwoman:

L. Malécot-Grob T. Sommerfeld

Decision electronically authenticated