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Datasheet for the decision of 19 March 2024

Case Number: T 0856/22 - 3.3.08

Application Number: 15800498.6

Publication Number: 3150699

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Language of the proceedings: EN

Title of invention:
Medium containing uridine and N-acetyl-D-mannosamine

Patent Proprietor:
JCR Pharmaceuticals CO., LTD.

Opponent:
Maiwald GmbH

Headword:
Medium containing uridine and ManNAc/JCR

Relevant legal provisions:
EPC Art. 56, 83
RPBA 2020 Art. 12(4), 12(6)

Keyword:
Inventive step - (yes)
Sufficiency of disclosure - (yes)
Late-filed evidence - error in use of discretion at first instance (no) - should have been submitted in first-instance proceedings (yes)

Decisions cited:
G 0007/93, T 0117/86, T 0671/03, T 1852/11, T 1955/13, T 1525/17

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Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 0856/22 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 19 March 2024

Appellant: (Opponent)	Maiwald GmbH Elisenhof, Elisenstrasse 3 80335 München (DE)
Representative:	Maiwald GmbH Elisenhof Elisenstraße 3 80335 München (DE)
Respondent: (Patent Proprietor)	JCR Pharmaceuticals CO., LTD. 3-19, Kasuga-cho Ashiya-shi, Hyogo 659-0021 (JP)
Representative:	Müller-Boré & Partner Patentanwälte PartG mbB Friedenheimer Brücke 21 80639 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 25 January 2022 concerning maintenance of the European Patent No. 3150699 in amended form**

Composition of the Board:

Chair	B. Claes
Members:	A. Schmitt
	A. Bacchin

Summary of Facts and Submissions

I. The appeal lodged by the opponent (appellant) is against the interlocutory decision of the opposition division according to which European patent No. 3 150 699 (the patent) in the version of auxiliary request 1, and the invention to which it relates, meet the requirements of the EPC.

II. The patent, entitled "*Medium containing uridine and N-acetyl-D-mannosamine*", was granted on the basis of European patent application No. 15 800 498.6, which was published in accordance with Article 153(4) EPC as EP 3 150 699 A1 (the application), and which had been filed as an international application published as WO 2015/182792.

III. The opposition proceedings were based on the grounds for opposition in Article 100(a) EPC, in relation to inventive step (Article 56 EPC), and those in Article 100(b) and (c) EPC.

IV. With the statement of grounds of appeal the appellant filed, *inter alia*, document D21, which contained new experimental data.

V. In reply to the appeal, the patent proprietor (respondent) filed a set of claims of the main request (identical to the set of claims of auxiliary request 1 considered in the decision under appeal) as well as sets of claims of five auxiliary requests.

The independent claims of the main request, claims 1, 6 and 9, read as follows:

"1. A medium comprising uridine at a concentration of 0.5 to 10 mM and N-acetyl-D-mannosamine at a concentration of 1 to 15 mM for expressing a glycoprotein by culturing mammalian cells."

"6. A method for production of a glycoprotein, comprising culturing mammalian cells transformed with an exogenous DNA encoding the glycoprotein in the medium according to one of claims 1 to 5."

"9. A method for production of a glycoprotein comprising culturing mammalian cells transformed with an exogenous DNA encoding a glycoprotein or mammalian cells in which an endogenous promoter located upstream of an endogenous gene is substituted by a promoter inducing strong gene expression in the medium according to claim 5, wherein preferably the exogenous DNA encoding the glycoprotein is a human-derived DNA."

VI. The board summoned the parties to oral proceedings in accordance with their requests and issued a communication pursuant to Article 15(1) RPBA.

VII. The following documents are referred to in the present decision:

D1 X. Gu and D. I. C. Wang, Biotechnology and Bioengineering, 1998, 58(6), 642-648

D2 Markely (2011) "High-throughput quantification of glycoprotein sialylation", Ph.D. thesis, Massachusetts Institute of Technology, Dept. of Chemical Engineering; https://dspace.mit.edu/handle/1721.1/65762

D9 N. S. C. Wong *et al.*, Biotechnology and Bioengineering, 2010, 107(2), 321-336

D18 Experimental results submitted by the respondent in the opposition proceedings

D19 J.C. Egri and J. K. Browne, British Journal of Cancer, 2011, 84 (Supplement 1), 3-10

D21 Experimental results submitted by the appellant with the statement of grounds of appeal

VIII. The arguments of the parties relevant to the present decision are set out in the reasons for the decision.

IX. The parties' requests relevant to the present decision were as follows.

The appellant requested that the decision under appeal be set aside and the patent be revoked, that document D21 be admitted and considered in the appeal proceedings and that the opposition division's decision to admit document D18 into the opposition proceedings be overruled.

The appellant further requested that the following questions be referred to the Enlarged Board of Appeal:

"*How can a party prove that a certain argument was indeed made during oral proceedings in first instance Opposition proceedings when neither the minutes nor the Decision of the Opposition Division mentions that argument?*

How can the RPBA 2020 Art. 12 be exercised in the absence of any opportunity for the party to prove (with certainty) that a specific line of argumentation was indeed brought forward [sic] *during oral proceedings in first instance Opposition proceedings?*"

The respondent requested that the appeal be dismissed (main request), or, in the alternative, that the patent be maintained in amended form with the set of claims of one of auxiliary requests 1 to 5 filed with the reply to the appeal, that document D21 not be admitted and considered in the appeal proceedings and that the appellant's new line of argument according to which the claimed medium was not limited to a particular point in time in the culturing process not be admitted into the proceedings.

Reasons for the Decision

Consideration of document D18 in the appeal proceedings

1. The appellant submitted essentially that the opposition division had exercised its discretion to consider document D18 in the opposition proceedings according to the wrong principles, had committed a procedural violation by stating that document D18 could not have been filed earlier and had not provided reasons why (the data of) document D18 should be admitted into the proceedings.

2. It is established case law of the Boards of Appeal that, on appeal against a decision taken by an opposition division in exercise of its discretion, it is not for the board to review all the facts and circumstances of the case as if it were in that

division's place and decide whether or not it would have exercised discretion in the same way. A board may overrule the way in which the opposition division exercised its discretion only if it concludes that it did so according to the wrong principles, without taking the right principles into account or in an arbitrary or unreasonable way, thereby exceeding the proper limits of its discretion (on this point, see, in particular, G 7/93, Reasons 2.6).

3. Document D18 was filed by the respondent before the final date for making written submissions set by the opposition division under Rule 116 EPC. The opposition division therefore had discretion to admit and consider document D18, in particular if it was considered *prima facie* relevant, as is evident from point 14.1 of the decision under appeal. The opposition division thus applied the correct criterion of *prima facie* relevance (see also Case Law of the Boards of Appeal, 10th edition 2022 (Case Law), IV.C.4.5.3a)), even if its additional comment that document D18 could not have been filed earlier might be incorrect. Furthermore, the appellant did not request that the oral proceedings before the opposition division be postponed when document D18 was admitted, and no violation of the appellant's right to be heard - resulting from the admission of document D18 - is apparent. The opposition division therefore correctly admitted document D18 into the opposition proceedings.

4. The EPC does not provide a legal basis for retroactive exclusion of - in appeal proceedings - documents, requests or evidence already correctly admitted into the opposition proceedings, all the more so if the impugned decision is based on them, as is the case for document D18 (see, e.g., decisions T 1852/11,

Reasons 1.3, and T 1525/17, Reasons 4.3; Case Law, V.A.3.4.4). In view of the very aim of appeal proceedings to review the decision under appeal, such submissions are automatically part of the appeal proceedings (see Article 12(2) RPBA).

5. Document D18 is therefore part of the appeal proceedings.

Admittance and consideration of document D21 (Article 12 RPBA)

6. According to Article 12(2) RPBA, a party's appeal case must be directed to the requests, facts, objections, arguments and evidence on which the decision under appeal was based. Any part of a party's appeal case which does not meet this requirement is to be regarded as an amendment that may be admitted only at the discretion of the board unless the party demonstrates that this part of its appeal case was admissibly raised and maintained in the proceedings leading to the decision under appeal (see Article 12(4) RPBA). Evidence which should have been submitted in the proceedings leading to the decision under appeal should (in principle) not be admitted by the board unless the circumstances of the appeal case justify its admittance (Article 12(6) RPBA).

7. With the statement of grounds of appeal, the appellant submitted experimental data (document D21) in support of their view that the alleged technical effect of the claimed medium was not achieved for all embodiments encompassed by the claim. The respondent objected to the admittance of the new data in document D21 substantially for being filed late and lacking *prima facie* relevance.

8. According to the appellant, submission of the data in document D21 had not been necessary prior to the oral proceedings in opposition when the opposition division in fact unexpectedly changed its mind on this aspect compared to its preliminary opinion. In the appellant's opinion, document D21 had thus been filed at the earliest opportunity.

9. Under Rule 76(2)(c) EPC the notice of opposition must contain a statement of the extent to which the European patent is opposed and of the grounds on which the opposition is based, as well as an indication of the facts and evidence presented in support of these grounds. This provision implies that opponents are required to submit all their facts, evidence and arguments in support of the grounds on which the opposition was based within the opposition period (see, e.g., decisions T 117/86, Reasons 4 and 6, and T 671/03, Reasons 2.1.1.).

10. It is indeed the responsibility of each party in *inter partes* cases to facilitate due and swift conduct of the proceedings, in particular by submitting any evidence supporting an objection as early and completely as possible (see, e.g., decision T 1955/13, Reasons 4.3.3), for instance, at the time when the objection was raised or when it was rebutted, to allow the opposition division to base its decision on any relevant evidence. In the case at hand, the objection that the alleged technical effect of the claimed medium was not achieved for all embodiments encompassed by the claim was raised by the then opponent in the notice of opposition (section E.I) and was immediately rebutted by the then patent proprietor in their reply to the opposition (sections 7.1.1 to 7.1.4). Document D21 therefore should have been filed during the opposition

proceedings to allow the opposition division to consider it and base its decision thereon.

11. Neither the opposition division's preliminary opinion nor a change in this opinion in the final decision can justify filing such evidence only on appeal. Despite the fact that the opposition division accepted the opponent's arguments in its preliminary opinion, it also clearly indicated that the issue still needed to be discussed in the oral proceedings (see points 9.4.1.3 and 10. of the opposition division's preliminary opinion). The fact that, during the oral proceedings in opposition, the opposition division ultimately accepted the patent proprietor's repeated arguments and acknowledged that the patent provided credible evidence in support of a synergistic effect of the claimed medium cannot be regarded as an unexpected and surprising development of the case that could justify the filing of document D21 only in the appeal proceedings.

12. Nor could the filing of document D21 have been triggered by an amendment of the subject-matter in question due to, for instance, the filing of amended claims by the then patent proprietor. After receipt of the opposition division's preliminary opinion the patent proprietor continued its arguments concerning the presence of the technical effect relied upon, based on the experiments provided in the patent.

13. Furthermore, the appellant did not submit that the filing of document D21 was triggered by the admittance of document D18 during oral proceedings before the opposition division. In any case, had the appellant intended - in the opposition proceedings - to react to the admittance of that document by filing the

experimental data now filed as document D21, it would have been their duty to request a postponement of the oral proceedings - either before or even during the oral proceedings - following the opposition division's decision to admit it. However, none of these measures was taken.

14. Consequently, the circumstances of the appeal case do not justify admitting document D21 into the appeal proceedings. The board therefore decided not to admit and consider document D21 in the appeal proceedings under Article 12(6) RPBA.

Main request
Claim construction - claim 1

15. A medium comprising uridine is claimed. The scientific term "uridine" refers to a defined nucleoside containing uracil attached to ribofuranose with the CAS registry number 58-96-8. The term "uridine" therefore denotes a single precisely defined chemical molecule and - contrary to the appellant's allegation - neither encompasses esters of (pyro-, tri-)phosphoric acid with uridine, such as the molecules UMP, UDP, UTP, nor nucleotide-sugars, such as UDP-N-acetylglucosamine or UDP-N-acetylgalactosamine.

16. This interpretation is not changed by the fact that paragraph [0026] of the patent discloses that uridine "*may be the salt thereof*". Nor is it changed by the disclosure in the first full paragraph of the left-hand column on page 331 of document D9. Indeed, a salt consists of positively charged cations and negatively charged anions. A uridine "salt" hence does not encompass molecules in which uridine is covalently bound to another molecule such as (a) phosphate

group(s) or a sugar molecule. The passage on page 331 of document D9 states that "*uridine (or UTP) ... is one of the limiting substrates ...*". This sentence means that either uridine or UTP may be a limiting substrate, but not - as alleged by the appellant - that uridine and UTP denote the same chemical compound.

17. It is not necessary to include the CAS registry number for uridine in the claim since the term "uridine" in itself refers to this single chemical compound.

18. The board is also not persuaded by the appellant's arguments that the expression "medium comprising uridine" encompasses a medium, in which uridine is only present internally within cells that are present in the medium, i.e. that the claimed medium does not necessarily comprise externally added (free) uridine. As established by the case law of the Boards of Appeal (see, e.g., Case Law II.A.6.1), a claim should be interpreted in a technically sensible manner taking into account the whole disclosure of the patent and with a mind willing to understand.

19. The appellant's claim interpretation is, however, contrary to the skilled person's common understanding of this type of expression and contrary to the teaching in the patent, which discloses the *addition* of external uridine to the medium in the examples (e.g., paragraphs [0035], [0055] and [0062]). The only technically sensible manner of interpreting the feature "a medium comprising uridine" in claim 1 is therefore that the uridine is present in the medium as a free molecule.

20. The consideration in point 18. above also applies to the further claim-interpretation issue raised by the

appellant in the context of the disclosures in documents D1 and D9. The appellant submitted that, as the claimed medium could comprise cells and was not restricted to a particular point in time in the culturing process and as the cells in the media disclosed in documents D1 and D9 contained uridine and ManNAc (Figure 2 of document D1 and Figure 4 of document D9), the disclosed media *comprised* (intracellular) uridine and ManNAc.

21. Moreover, at disclosure level and contrary to the submission of the appellant, the "uridine" allegedly present in these media is actually intracellular UDP-N-acetylglucosamine and UDP-N-acetylgalactosamine in the case of document D1 (Figure 2) and intracellular UMP, UDP and UTP in the case of document D9 (Figure 4). None of these molecules is in fact encompassed by the term "uridine" (see point 15. above). The appellant's submission in this context is hence equally unconvincing. Therefore, even if the appellant's claim interpretation - namely that the claimed medium could comprise cells at any point in time during the culturing process - were accepted, documents D1 and D9 would still not disclose media comprising both uridine and ManNAc.

22. Since the appellant's argument that the claimed medium is not limited to a particular point in time in the culturing process, even if considered, does not change the board's conclusion concerning claim interpretation, and since during the oral proceedings before the board the respondent no longer maintained that the appellant's new line of argument was late-filed (see minutes, page 4, first paragraph), it is not necessary to discuss any objection to its admittance.

23. Consequently, as the board's claim construction takes into account all of the appellant's arguments, a referral to the Enlarged Board of Appeal on the question of proof that a submission had already been made during opposition proceedings - as requested by the appellant (see section IX.) - is not necessary for the board to reach a decision.

Sufficiency of disclosure (Article 83 EPC)

24. The medium of claim 1 comprises two components in defined concentration ranges and must be suitable for expressing a glycoprotein by culturing mammalian cells. Claims 6 and 9 concern methods for the production of a glycoprotein that define neither the nature nor the extent of the glycosylation in the glycoprotein (see section V. for full wording of the claims).

25. Hence, even if the experiments disclosed in the patent were not reproducible with respect to the described extent of glycosylation, as asserted by the appellant in view of the results shown in document D18 compared to those in Figure 3 of the patent, this would mean neither that the skilled person could not provide the claimed medium nor that the claimed methods would not result in the production of a glycoprotein, which is the only technical effect recited in the claims. The question of whether a reproducible synergistic effect was achieved is not relevant for the assessment of sufficiency of disclosure of the claimed invention since such a synergistic effect is not expressed in the claims.

26. The appellant's arguments as to why, in its opinion, the invention defined in the claims was not

sufficiently disclosed in the patent are therefore not persuasive. The requirements of Article 83 EPC are met.

Inventive step (Article 56 EPC)

27. Taking into account the board's claim construction (see above points 15. and 18.), the appellant maintained two problem-solution approaches, one starting from a medium disclosed in document D9 representing the closest prior art, and another starting from common general knowledge documented in document D1 representing the closest prior art.

Document D9 as closest prior art

28. In document D9, the effects of various nucleotide sugar precursors on the glycosylation of recombinant glycoproteins produced in CHO cells were analysed. The cells were fed with either a sugar alone or a sugar in combination with cytidine or uridine (conditions 1 to 7 in Table II and Figure 3 of document D9). The tested combinations were 20 mM ManNAc and 10 mM cytidine ("condition 7"), and 10 mM galactose or glucosamine with 5 mM uridine ("condition 3" and "condition 5"). Each of these conditions increased sialylation of recombinant IFN-γ compared to a medium not containing any of these molecules (Figure 3 of document D9). However, no synergistic effect on IFN-γ sialylation was achieved by any of these conditions (first full paragraph of right-hand column on page 329 of document D9).

29. The claimed medium differs from the medium of "condition 7" - which was considered by the appellant as the most suitable starting point for the assessment of inventive step and thus suitable to represent the

closest prior art - in the concentration of ManNAc (1 to 15 mM instead of 20 mM) and in the presence of 0.5 to 10 mM uridine instead of 10 mM cytidine.

Technical effect and objective technical problem

30. According to paragraph [0042] of the patent, the technical effect of this difference is a synergistic increase in sialylation (see also point 39. below). It was not disputed by the appellant that the patent demonstrates a synergistic effect on the sialylation of recombinant FSH when 3 mM uridine and 8 mM ManNAc were added to the medium compared to the outcome with either of these additives alone (Figure 9 of the patent).

31. However, according to the appellant, such a synergistic effect was not credible across the whole breadth of the claim, because such an effect had not been demonstrated for the sialylation of recombinant human erythropoietin (rhEPO), which had also been analysed in the experimental section of the patent (paragraphs [0030] to [0043] and Figures 3 and 4). Indeed, no difference in sialylation of the sum of the rhEPO isoforms analysed in this example was observed when cultivated in a medium containing 3 mM uridine and 8 mM ManNAc compared to cultivation in a medium that only contained 8 mM ManNAc if the standard deviations were taken into account and if, as proposed by the appellant, the effect of 3 mM uridine on rhEPO sialylation was set as zero instead of being set as a decrease, as shown in Figure 4 of the patent.

32. However, a synergistic effect of two components means that the effect attained by the two components must be greater than the simple sum of the two components' separate effects. The decrease in sialylation of rhEPO

observed when culturing the rhEPO-expressing cells in 3 mM uridine compared to when culturing them in a medium without any additive cannot therefore be ignored. Taking due account of this, at the least a trend towards a synergistic effect of a combination of 8 mM ManNAc and 3 mM uridine in the culturing medium is evident from Figure 4 of the patent, as also concluded in paragraph [0042] of the patent.

33. This synergistic effect is in fact confirmed in the experimental data submitted as document D18, where the sialylation of rhEPO using the same culturing conditions as those described in the patent was analysed. As is evident from Figure 2 of document D18, culturing the rhEPO-expressing cells in 3 mM uridine and 8 mM ManNAc synergistically increased rhEPO sialylation.

34. Hence, despite the observed differences in the *extent* of sialylation of the two glycoproteins FSH and rhEPO when expressed in the various media tested in the patent and the differences in the extent of rhEPO sialylation observed under the experimental conditions tested in the patent and in document D18, each available experiment actually supports a synergistic effect of 3 mM uridine and 8 mM ManNAc on the sialylation of a glycoprotein. Based on these available data, the opposition division rightly acknowledged this technical effect.

35. Furthermore, contrary to the appellant's assertion, a synergistic increase in the sialylation of rhEPO can be acknowledged if it is achieved by the sum of all rhEPO isoforms expressed by the cells. Indeed, it is not required for the sialylation to be synergistically increased for each separate rhEPO isoform (Figure 3 of

the patent and Figure 1 of document D18), as long as the overall effect on all rhEPO isoforms expressed by the cells is a synergistic increase in sialylation of this protein (Figure 4 of the patent and Figure 2 of document D18).

36. In this context, the appellant pointed to the fact that 14 different rhEPO isoforms existed (Figure 1 of D19) and that, in the patent, only three rhEPO isoforms had been analysed. The effect hence had not been shown to be present for the sum of all 14 rhEPO isoforms. Since the sialylation was not increased synergistically in each of the three rhEPO isoforms that had been analysed (Figure 3 of the patent and Figure 1 of D18), it could not be concluded - according to the appellant - that it was present for the sum of all 14 rhEPO isoforms.

37. It is true that only three rhEPO isoforms were analysed separately in the patent. However, these rhEPO isoforms have the lowest isoelectric points, i.e. the highest number of sialic acid molecules (paragraph [0039] of the patent) and thus the highest extent of sialylation, and a synergistic effect of uridine and ManNAc on the sialylation of the sum of the ratios of these three rhEPO isoforms was observed (paragraph [0042] of the patent and point 32. above). Based on this teaching, the skilled person would not have expected the same not to be true for the sum of all rhEPO isoforms. On the contrary, in the absence of any evidence or verifiable supporting facts, the appellant's arguments do not go beyond mere speculation and are not sufficient to rebut the credible teaching in the patent. They must hence be dismissed.

38. Finally, the appellant asserted that, in the absence of a direct comparison between the sialylation of a

glycoprotein expressed in the medium conditions of document D9 and that in the claimed medium, no technical effect associated with the difference could be acknowledged.

39. However, document D9 explicitly states that none of the tested sugar-nucleotide combinations, including "condition 7", had a synergistic effect on sialylation (first full paragraph of right-hand column on page 329 of document D9, and point 28. above). A synergistic effect on sialylation is therefore a technical effect associated with the claimed medium as compared to the media used in document D9.

40. Hence, based on the evidence on file and in the absence of any evidence to the contrary, a synergistic effect of uridine and ManNAc on the sialylation of a glycoprotein when added to a cell culture medium in the concentration ranges recited in the claim can be acknowledged. Since this effect is not present for any of the media of document D9, the opposition division rightly found that the claimed medium constitutes an improvement compared to the media of document D9. The objective technical problem can therefore be formulated - as proposed by the opposition division - as the provision of an improved medium for expressing a glycoprotein by culturing cells, and not - as proposed by the appellant - as a mere alternative.

Obviousness

41. Neither document D9 nor document D1 contains any teaching which could have led to the realisation by the skilled person that the combination of uridine and ManNAc, let alone the combination thereof in the concentrations recited in the claim, would provide a

medium for expressing a glycoprotein by culturing cells that is improved in terms of sialylation compared to such media that only comprise one of these compounds. The claimed medium was therefore not obvious to the skilled person.

42. As is evident from Table I in document D9, which summarises the results of previous nucleotide sugar precursor feeding experiments on glycosylation, and from the experiments conducted in document D9 (Table II and Figure 3), combinations of uridine with various sugars were previously tested as additives in cell culture media for expressing a glycoprotein by culturing cells. However, the tested combinations that included uridine either did not increase or only marginally increased sialylation of the expressed glycoproteins (2nd, 4th and 6th entry in Table I; "condition 3" and "condition 5" in Table II and Figure 3). These data therefore do not point to the use of uridine, let alone in combination with ManNAc, for providing an improved medium for expressing glycoproteins.

43. The appellant submitted that document D9 disclosed that a combination of ManNAc and a *nucleoside* provided for the highest increase in sialylation ("condition 7" in Figure 3 of document D9). However, the nucleoside in this medium is cytidine and not uridine. Since a synergistic effect of the combination of ManNAc and uridine is acknowledged (see point 40. above), the replacement of cytidine with uridine in the medium of "condition 7" cannot be regarded as a mere arbitrary modification, as asserted by the appellant, despite the fact that cytidine and uridine are both pyrimidine nucleosides.

44. Moreover, the fact that uridine is the only alternative pyrimidine nucleoside available to the skilled person does not, in itself, provide sufficient incentive for the skilled person to replace cytidine with uridine in the medium of "condition 7", especially as the data in document D9 actually point to cytidine as the more effective nucleoside for increasing sialylation (see Figure 3 of document D9). Thus, based on the data in document D9, the skilled person would not have replaced cytidine in the medium of "condition 7" with uridine in the expectation of a synergistic effect on sialylation.

45. The appellant also pointed out that document D9 taught that uridine might be "*one of the limiting substrates for nucleotide sugar synthesis in this study*" (see first full paragraph of the left-hand column on page 331), which constituted a further incentive to include uridine in media for expression of a glycoprotein.

46. However, this passage of document D9 does not discuss sialylation, but only the synthesis of nucleotide sugars, and an increase in nucleotide sugars is not associated with an increase in sialylation. This is evident from the paragraph that bridges the left-hand and right-hand columns of page 331 of document D9, which discloses that the combined feeding of, *inter alia*, uridine and glucosamine "*was effective in increasing nucleotide sugar pools as compared to feeding the sugar precursors alone, though it did not lead to a synergistic increase in IFN-γ sialylation*". The passage on page 331 cited by the appellant does not therefore provide the skilled person with an incentive to combine ManNAc and uridine in a medium for improving the sialylation of a glycoprotein.

47. Nor does the teaching in document D1 concerning the subject of the biosynthetic pathway for CMP-sialic acid synthesis (Figure 1) provide such an incentive for using uridine (as a replacement for cytidine) in the medium of "condition 7" in document D9 for improving the sialylation of a glycoprotein. Indeed, uridine is not mentioned in the biosynthetic pathway depicted in Figure 1 of document D1, and the mere fact that CTP is synthesised from UTP does not (in any way) prompt the skilled person to contemplate using uridine instead of cytidine for the purpose of solving the objective technical problem either (see also points 49. to 51. below).

48. Consequently, starting from the disclosure in document D9 and taking into account the teaching in document D1, the skilled person would not have arrived at the claimed medium in an obvious manner.

Common general knowledge represented by the disclosure in document D1 as closest prior art

49. Document D1 describes the commonly known intracellular pathways for sialic acid biosynthesis and conjugation with glycoproteins (Figure 1). According to the appellant, the fact that ManNAc was the first specific precursor of sialic acid, CMP-sialic acid was synthesised from sialic acid and CTP, and CTP was formed from UTP, indicated that ManNAc and uridine were the "two most immediate precursors" of sialic acid. It was therefore obvious to feed the cell culture with ManNAc and a uridine source in order to increase sialylation of a protein of interest.

50. It can be inferred from the biosynthetic pathway shown in Figure 1 of document D1 that uridine is not one of

the two "most immediate" precursors of sialic acid or sialylation. In fact, Figure 1 does not mention uridine and it indicates that synthesis of sialic acid from ManNAc requires ATP and that sialylation requires CMP-sialic acid, which is synthesised from sialic acid and CTP. This is also confirmed in Figure 2.5 of document D2, which shows the sialylation pathway in mammalian cells. Document D2 discloses uridine in this pathway as a precursor of UMP and, identically to Figure 1 of document D1, states that CTP and sialic acid are the two most immediate precursors of sialylation.

51. The appellant's argument that the skilled person's common general knowledge of the biological pathway leading to sialylation of glycoproteins in a cell would, on its own, have led the skilled person to combine, in particular, uridine and ManNAc in a medium for expressing a glycoprotein by culturing mammalian cells is not therefore persuasive, irrespective of the presence of a synergistic effect of uridine and ManNAc on the sialylation of glycoproteins.

52. An inventive step of the claimed method cannot therefore be denied on the basis of the skilled person's common general knowledge concerning the intracellular pathways for sialic acid biosynthesis and conjugation with glycoproteins, as represented by the disclosure in document D1.

Conclusion on inventive step

53. In view of the above considerations, the medium of claim 1 and the methods of claims 6 and 9 that use this medium involve an inventive step (Article 56 EPC).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

L. Malécot-Grob B. Claes

Decision electronically authenticated