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Datasheet for the decision of 11 December 2025

Case Number: T 0091/24 - 3.3.04

Application Number: 16806055.6

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IPC: C07K14/745, C07K5/02

Language of the proceedings: EN

Title of invention:
Improved media for the expression of recombinant vitamin K-dependent proteins

Patent Proprietor:
CSL Behring Lengnau AG

Opponent:
WALLINGER RICKER SCHLOTTER TOSTMANN

Headword:
Expression of vitamin K-dependent proteins/CSL BEHRING LENGNAU

Relevant legal provisions:
RPBA 2020 Art. 12(4)
EPC Art. 84, 83, 54, 56

Keyword:

Amendment to case - admitted (yes)
Claims - clarity after amendment (yes)
Novelty - (yes)
Sufficiency of disclosure - (yes)
Inventive step - (yes)

Decisions cited:

G 0002/88, G 0006/88, T 1179/07, T 0308/17, T 1343/19, T 0385/21, T 1913/21, T 1616/22, T 1701/22, T 2140/22, T 2192/22

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Case Number: T 0091/24 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 11 December 2025

Appellant I: (Patent Proprietor)	CSL Behring Lengnau AG Biotech Innovation Park 2543 Lengnau BE (CH)
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Appellant II: (Opponent)	WALLINGER RICKER SCHLOTTER TOSTMANN Patent- und Rechtsanwälte Partnerschaft mbB Zweibrückenstraße 5-7 80331 Munich (DE)
Representative:	Hoffmann Eitle Patent- und Rechtsanwälte PartmbB Arabellastraße 30 81925 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 16 November 2023 concerning maintenance of the European Patent No. 3 383 894 in amended form**

Composition of the Board:

Chairwoman	M. Pregetter
Members:	B. Rutz
	R. Romandini

Summary of Facts and Submissions

I. The appeals by the patent proprietor (appellant I) and the opponent (appellant II) lie from the decision of the opposition division that European patent No. 3 383 894, entitled "*Improved media for the expression of recombinant vitamin K-dependent proteins*", in amended form in accordance with auxiliary request 2B, met the requirements of the EPC.

II. The opposition proceedings were based on the grounds of Article 100(a) EPC, in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), and of Article 100(b) EPC.

III. In the decision under appeal the opposition division admitted documents D19 to D20 filed before the final date for making written submissions under Rule 116(1) EPC. Document D21 was not admitted into the proceedings because it was not *prima facie* relevant for assessing inventive step. For document D22 no decision on admittance was taken.

IV. With its statement of grounds of appeal the patent proprietor re-filed sets of claims of the main request and of auxiliary requests 1 (1A in opposition proceedings), 2, 2A, 2B and 3 to 30 (identical to the requests filed during opposition proceedings) and new auxiliary requests 31 to 49.

V. With its statement of grounds of appeal the opponent submitted as Annex A comments on auxiliary requests 3 to 30 as filed during opposition proceedings.

VI. The parties replied to each other's appeals. The opponent filed further documents D23 to D26.

VII. The board summoned the parties to oral proceedings, as requested, and informed them of its preliminary opinion in a communication pursuant to Article 15(1) RPBA 2020.

VIII. With a letter dated 3 December 2025, appellant I filed further auxiliary requests 50 to 53.

IX. With a letter dated 5 December 2025, appellant I filed annotated Figures 1B and 1C of the patent.

X. Oral proceedings were held on 11 December 2025. Before the end of the oral proceedings appellant I made auxiliary request 46 its main request and withdrew its appeal and the requests ranking higher than auxiliary request 2B, on which the decision under appeal was based. Appellant I therefore became respondent and appellant II remained as appellant.

XI. Claim 1 of the main request (former auxiliary request 46) reads as follows:

"1. A method for enhancing the activity of a recombinant vitamin K-dependent protein comprising the following steps:

a) providing mammalian host cells comprising an expression system expressing the recombinant vitamin K-dependent protein,

b) culturing the cells in a cell culture medium comprising one or more cell culture enhancing reagent(s), and

c) separating and/or isolating and/or purifying the recombinant vitamin K-dependent protein from the cell culture,

wherein the one or more cell culture enhancing reagent(s) is/are selected from the group consisting of
L-glutathione provided to obtain a concentration of 0.5-13 mmol/L in the cell culture;
alpha-ketoglutaric acid provided to obtain a concentration of 5-50 mmol/L in the cell culture;
succinic acid provided to obtain a concentration of 2-50 mmol/L in the cell culture;
oxaloacetic acid provided to obtain a concentration of 5-50 mmol/L in the cell culture;
malic acid provided to obtain a concentration of 5-50 mmol/L in the cell culture; and
fumaric acid provided to obtain a concentration of 2-50 mmol/L in the cell culture."

Claim 14 of the main request reads as follows:

"14. Use of a cell culture medium comprising L-glutathione at a concentration of 0.5-13 mmol/L, alpha-ketoglutaric acid at a concentration of 5-50 mmol/L, succinic acid at a concentration of 2-50 mmol/L, oxaloacetic acid at a concentration of 5-50 mmol/L, malic acid at a concentration of 5-50 mmol/L, and/or fumaric acid at a concentration of 2-50 mmol/L for enhancing the activity of a recombinant vitamin K-dependent protein in a mammalian cell."

XII. At the end of the oral proceedings the Chairwoman announced the board's decision.

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

D1 J. Vatandoost et al., "*Expression of Biologically Active Human Clotting Factor IX in Drosophila S2 Cells: γ-Carboxylation of a Human Vitamin*

K-Dependent Protein by the Insect Enzyme", Biotechnology Progress 28(1), 2012, 45-51

D2 WO 2007/075976 A2

D3 WO 2006/101474 A1

D4 WO 2011/003153 A1

D7 WO 2007/036291 A2

D8 WO 03/029442 A1

D9 WO 2005/035748 A1

D19 Z. Yun et al., "*Effect of Antioxidants on the Apoptosis of CHO Cells and Production of Tissue Plasminogen Activator in Suspension Culture*", Journal of Bioscience and Bioengineering 91(6), 2001, 581-5

D21 WO 2014/110433 A1

D23 I. Jordan and J. Kaplan, "*The mammalian transferrin-independent iron transport system may involve a surface ferrireductase activity*", Biochemical Journal 302, 1994, 875-879

D24 D. Trinder and E. Morgan, "*Mechanisms of ferric citrate uptake by human hepatoma cells*", Gastrointestinal and Liver Physiology 38, 1998, G279-G286

D25 G. W. Bates et al., "*The Kinetics and Mechanism of Iron(III) Exchange between Chelates and Transferrin*", The Journal of Biological Chemistry 242(12), 1967, 2810-2815

D26 N. Vijayasankaran et al., "*Animal cell culture media*", Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, 1-15

XIV. The detailed arguments of the parties are addressed in the Reasons for the Decision below. In brief:

The appellant argued with regard to the main request (former auxiliary request 46) that it should not be admitted into the appeal proceedings as it could and should have been filed during first-instance proceedings. The claims failed to comply with the requirements of the EPC for the reasons discussed in conjunction with auxiliary request 37, or auxiliary request 7 in Annex A to the grounds of appeal (see opponent's reply to the patent proprietor's appeal, table in paragraph [0142]).

With regard to auxiliary request 37 the appellant argued that it "[s]*hould not be admitted into the appeal proceedings and fails to comply with the requirements of the EPC for the reasons discussed in conjunction with AR7 in Annex A to our grounds of appeal. AR7 was also not admitted by the OD.*" (ibid.).

With regard to auxiliary request 7 the appellant argued that the "*reasons and objections set out in relation to AR5, i.e. why this request should not be admitted into the appeal proceedings and fails to meet the requirements of the EPC (see also reasons set-out in detail for AR2B), apply* mutatis mutandis *to AR7. AR7 additionally amends use claim 11 to define that the specified concentrations are used to enhance the activity of a recombinant vitamin K-dependent protein in a mammalian cell. This amended use gives rise to clarity objections under Art. 84 EPC for the reasons discussed above in the context of AR3.*" (see Annex A to opponent's statement of grounds of appeal)

With regard to auxiliary request 3 the appellant argued that *inter alia* the feature "*in a mammalian cell*" in use claim II was "*not represented as limiting use feature in any of the claims as granted and serve* [sic] *in the context of a use claim category a different purpose than in a method claim, these amendments are open to the assessment of clarity (G3/14) and fail the requirements of Art. 84 EPC.*"

During oral proceedings in appeal the appellant referred to its written submissions with regard to insufficient disclosure. The board considers this to refer to the arguments put forward with regard to auxiliary request 2B as stated in Annex A to the statement of grounds of appeal and in paragraph [0142] of the opponent's reply to the proprietor's appeal. The arguments which are applicable to the main request (former auxiliary request 46) are:

- the patent only contained examples showing that the total activity of Factor VII in the cell supernatant was increased without normalising for protein concentration
- there was no common activity of all vitamin K-dependent proteins, and it was not specified in relation to what comparator the activity was enhanced
- when aiming at performing the claimed method for other vitamin K-dependent proteins and in different settings (host cells, culture conditions, time points etc.) and thus over the whole scope of the claims, the skilled person was not able to reliably perform such measuring to determine whether the activity of a given recombinant vitamin K-dependent protein expressed was enhanced under the chosen set-up by the concentrations used, as required by

the claim

Claim 1 related to "activity" only. Yield-associated effects were therefore included.

Claim 14 lacked clarity. It was not clear to what technical feature the term "in a mammalian cell" referred. In the relevant art, there was no unequivocally generally accepted meaning for enhancing the activity of the vitamin K-dependent protein (VKDP) in a, i.e. within a, mammalian cell.

During oral proceedings in appeal the appellant argued that the subject-matter of claim 1 lacked an inventive step over the combination of document D8 with document D19 or document D7 and over the combination of document D4 and document D19 or document D7. In writing with regard to auxiliary request 2B the appellant had also referred to documents D9, D21, D2 and D3 as potential closest prior art.
The respondent argued that the main request (former auxiliary request 46) should be admitted because it represented a direct reaction to developments at various stages of the long opposition proceedings.

Claim 1 was novel and inventive because documents D1 and D8/D9 did not disclose cell culture enhancing reagents within the recited concentration ranges. Document D1 did not disclose mammalian cell cultures. Document D21 only disclosed citric acid, which was no longer mentioned in the claim.

The claimed subject-matter was also not obvious having regard to the secondary documents cited by the appellant. Document D19 related to non-vitamin K-dependent proteins (such as tPA) and to increased

viability (or decreased apoptosis), which was not relevant for enhancing activity of VKDPs. Document D4 presented a different solution, namely co-expression of a "processing factor", and therefore taught away from the claimed solution. Document D7 was not concerned with VKDPs or with methods for improving the production of such proteins. It mainly related to antibody production and would thus not have been considered relevant.

Claim 14 was clear because there was only one technically sensible meaning, namely that the use was for enhancing the activity of a VKDP expressed in a mammalian cell culture.

XV. The appellant (opponent) requested that
- the decision be set aside and the patent be revoked
- the main request (former auxiliary request 46) and auxiliary requests 3 to 45 and 47 to 53 not be admitted into the appeal proceedings
- document D21 be admitted into the proceedings because it was *prima facie* relevant, i.e. the opposition division had not exercised its discretion properly
- documents D23 to D26 be admitted into the proceedings

XVI. The respondent (patent proprietor) requested that the patent be maintained on the basis of the main request filed as auxiliary request 46, or alternatively on the basis of auxiliary request 2B, auxiliary requests 3 to 45, or auxiliary requests 47 to 53, with auxiliary requests 2B to 49 having been filed with the statement of grounds of appeal and auxiliary requests 50 to 53 having been filed with the letter dated 3 December 2025.

Reasons for the Decision

Main request (filed as auxiliary request 46)
Admittance (Article 12(4) and (6) RPBA)

1. The request was submitted with the statement of grounds of appeal. The provisions of Article 12(4) and (6) RPBA apply.

2. The present main request differs from auxiliary requests discussed or submitted during the opposition proceedings in combining the following aspects:
   - it does not contain a claim defining a bioreactor
   - it does not contain a claim in the format of claim 24 as granted ("first use claim")
   - it does not list citric acid as a cell culture enhancing reagent

3. The respondent argued that the main request was a direct reaction to the opposition division's finding at the second oral proceedings that claim 25 of auxiliary request 2 ("bioreactor claim", deleted in auxiliary request 2B and in the present main request) was anticipated by the disclosure of document D8, and that claim 23 of auxiliary request 2A ("first use claim", deleted in auxiliary request 2B and in the present main request) was insufficiently disclosed (see items 25 and 29 of the decision, respectively). This differed from the preliminary opinion of the opposition division annexed to the summons to the second oral proceedings dated 21 December 2022 (see items 8, 9 and 12 thereof). Auxiliary requests not listing citric acid were pending in opposition proceedings ("Series 3", auxiliary requests 8 to 14, see Annex to statement of grounds of appeal, "Overview of claim requests"). The necessity of

providing a claim set combining these elements stemmed from developments during the first and second oral proceedings before the opposition division.

4. The appellant counter-argued that the claim request could and should have been filed during the opposition proceedings. Even if based on auxiliary requests filed earlier during opposition proceedings, it had been requested that these earlier auxiliary requests (i.e. auxiliary requests 3 to 19) not be admitted, and in fact the opposition division did not take a decision on their admittance (see minutes of the second oral proceedings in opposition, point 10). Moreover, they were not convergent and gave rise to new objections.

5. The board admitted the request during oral proceedings for the following reasons.

6. The claim request differs from auxiliary request 14 filed by letter dated 5 July 2023 during the opposition proceedings only in that the first use claim ("Use of L-glutathione ...") and the bioreactor claim have been deleted. Auxiliary request 14 was not formally admitted during the opposition proceedings due to the higher-ranking request 2B being allowed (see minutes of the second oral proceedings, dated 16 November 2023, points 2 and 10). Auxiliary request 14, however, can be considered admissibly raised and maintained in the proceedings leading to the appeal (see Article 12(4) RPBA). It was filed within the time limit set in the second communication by the opposition division under Rule 116(1) EPC ("*The final date for making written submissions and/or amendments (R. 116 EPC) is 5 July 2023*."). This is supported by the last point in the annex to that communication, which referred to late filing in the context of Rule 116 EPC:

"*If the parties wish to comment on this note, they should submit any such comments to the EPO – and directly to the other parties – before the indicated date (cf. Guidelines D-VI, 3.2, and E-II, 5, as well as Rule 116 EPC). It is pointed out that late filed facts or evidence might be refused.*"

7. Furthermore, by restricting the claimed method to defined concentration ranges of the cell culture enhancing reagents and deleting citric acid, auxiliary request 14 responded to a number of objections under novelty and inventive step.

8. The patent proprietor could therefore have expected that auxiliary request 14, which was filed before the date set in accordance with Rule 116(1) EPC, would have been admitted into the proceedings if needed to address any objections dealt with in that request.

9. The further amendment to auxiliary request 14 made in the main request (former auxiliary request 46), i.e. deletion of the first use claim and the bioreactor claim, does not increase the complexity of the case and does not raise new issues. It merely entails the deletion of two independent claims which have already been discussed and deleted in a higher-ranking request (auxiliary request 2B) during the opposition proceedings. Bringing a pending auxiliary request into line with the amendments made to a higher-ranking claim request that led the opposition division to a finding of compliance with the EPC is considered to be normal party behaviour and increases procedural economy.

Admittance of documents D19 to D21 and D23 to D26

10. Documents D19 and D20 form part of the appeal proceedings as they have been referred to in the decision under appeal and the request by the respondent not to admit them was withdrawn during the oral proceedings in appeal. The board admitted document D21 into the proceedings, but deems it not necessary to provide reasoning because the request by the respondent not to admit D21 was withdrawn during the oral proceedings in appeal and its admittance has no impact on the outcome of the appeal. Documents D23 to D26 are related to the feature "citric acid" which is not relevant to the decision. No decision on their admittance was therefore taken.

Claim interpretation - claim 1

11. Although the purpose of the claimed method is indicated as "*for enhancing the activity of a recombinant vitamin K-dependent protein*", the method is for producing recombinant vitamin K-dependent proteins (VKDPs). The claim lists a number of steps which are commonly known to generate a product ("*recombinant vitamin K-dependent protein*") from a different starting material ("*host cells comprising an expression system*"):

    a) providing host cells comprising an expression system;

    b) culturing the cells;

    c) separating and/or isolating and/or purifying the protein

12. According to established case law (see e.g. decisions T 1179/07, Reasons 2.1.2 to 2.1.4; T 308/17, Reasons 8 and 13; T 385/21, Reasons 5.4; T 1913/21, Reasons 15; T 1616/22, Reasons 4 and 5; T 2192/22, Reasons 6 to

16), this means that the purpose of the method ("*for enhancing the activity*") only limits the claim in so far as it requires the method to be suitable to achieve that purpose. The claimed method is thus only defined by the mandatory steps a), b) and c) listed above and its suitability to enhance the activity of a recombinant VKDP.

13. The "*activity*" which is to be enhanced is not defined in the claim and no comparator, threshold or time specification is provided, i.e. it is not defined when, to what degree and compared with what the activity is enhanced. The respondent argued in this regard that the activity had to be compared with the situation in the absence of the "*cell culture enhancing reagent(s)*". The respondent also argued that the activity data "*must not be interpreted by merely picking single values at certain time points* [...], *while disregarding the overall teaching of the figures*" (point 26, reply to the appeal). The board is unable to derive such narrow interpretation from the claim wording. Also passages in the description which invoke "*comparable conditions, but where the cell culture medium does not comprises* [sic] *a TCA intermediate and/or L-glutathione*" (see e.g. paragraphs [0072] and [0073] of the patent) cannot be seen to limit the claim in this regard because they relate to "*specific embodiments*" which comprise measuring "*chromogenic activity*".

14. The board agrees with the appellant that enhancing the activity of a VKDP as required in the claim is not limited to "*specific activity*", i.e. activity per mass of protein. Neither the wording of the claim nor the description of the patent would support this narrow interpretation. The examples in the patent, for example, point to a different interpretation as the

chromogenic activity measured was not normalised to protein mass or concentration (see Figures 1 to 6, parts B and C).

15. The board concludes that the claim does not differentiate between enhancement of specific and total activity of VKDPs.

Claim 14

16. In contrast to claim 1, which is directed to a method of production, claim 14 defines a (true) use claim. The claim relates to the use of a product (a cell culture medium) to achieve an effect, namely "*enhancing the activity of a recombinant vitamin K-dependent protein*". In accordance with decisions G 2/88 (Headnote 3.) and G 6/88 (Headnote) of the Enlarged Board of Appeal, the purpose is thus a limiting functional feature of the claim.

Amendments (Article 123(2) and (3) EPC)

17. The appellant has raised no objections under Article 123 EPC against the set of claims of the main request and the board sees no reasons for doing so either.

Clarity (Article 84 EPC) - claim 14

18. The appellant objected to the expression in claim 14 "*in a mammalian cell*", which was not present in the claims as granted. This amendment rendered the claim unclear because it gave rise to several irreconcilable interpretations, e.g. whether a cell culture medium was used within a mammalian cell or whether the activity was enhanced within the cell or only after secretion.

19. The board does not agree. The skilled person reading claim 14 with their common general knowledge would understand that "*in a mammalian cell*" refers to the protein mentioned in the claim directly before said expression. They would furthermore understand that the protein has to be expressed and ultimately be accessible, i.e. be present in a form separable from the expression system, because VKDPs exert their activity extra-cellularly, e.g. as part of the blood clotting system (see paragraphs [0004] and [0005] in the patent). This is also the teaching of the patent as a whole, which deals exclusively with the extracellular activity of VKDPs (see e.g. paragraphs [0004] and [0008]). The skilled person would thus exclude artificial and technically unreasonable interpretations, such as the use of a cell culture medium within a mammalian cell or enhancing the activity of a VKDP inside a mammalian cell. The only reasonable and technically sound interpretation is therefore that the claimed use is to enhance the activity of a VKDP which is produced in a mammalian cell.

20. The amendment therefore complies with Article 84 EPC.

Novelty (Article 54 EPC)

21. The claimed subject-matter is novel over the disclosure of document D1 because the method of claim 1 and the use of claim 14 are limited to mammalian cells, while document D1 discloses insect cell expression systems.

22. The claimed subject-matter is novel over the disclosure of documents D8 and D9 because none of the cell culture enhancing reagents with their respective concentration

ranges are disclosed in the context of a defined culture medium in D8 or D9 (see also point 34. below with regard to the glutathione concentration disclosed in D8).

23. The claimed subject-matter is novel over the disclosure of document D21, which does not disclose any of the specified cell culture enhancing reagents and their concentration ranges.

Sufficiency of disclosure (Article 83 EPC)

24. The appellant has not specifically substantiated its objections under sufficiency of disclosure for the invention to which the claims of the main request relate (see minutes of the oral proceedings before the board of appeal). In its statement of grounds of appeal it limited its arguments to the method of auxiliary request 2B and in particular to the functional limitation "*provided in an amount effective for enhancing the activity of the recombinant vitamin K-dependent protein*" in the method claim.

25. This wording is no longer present in claim 1: rather, the concentrations of the cell culture enhancing reagents are defined by ranges. The appellant has not explained why these ranges which correspond to those experimentally tested in the patent (see Figures 1 to 6) would not achieve the stated purpose, i.e. enhancing the activity of a recombinant vitamin K-dependent protein.

26. The following further objections were put forward with regard to auxiliary request 2B. They will be considered in the following in so far as they apply to the

invention to which the claims of the main request relate. The appellant alleged that the patent

- did not show any enhancement of the specific activity of VKDPs
- did not show activity enhancement for all VKDPs and in different settings (host cells, culture conditions, time points etc.)
- did not provide activity assays for all VKDPs

27. The board considers none of these arguments pertinent. As discussed under "*Claim interpretation*" above, the claim is not limited to enhancing the specific activity of VKDPs, but also covers enhancing the total activity of VKDPs in a cell culture (see point 14. above).

28. The appellant has not provided evidence that the claimed method would not be effective for other VKDPs or that identifying the appropriate conditions for the expression of those VKDPs would pose an undue burden to the skilled person.

29. Nor has the appellant provided evidence that the skilled person would not be able to identify activity assays for other VKDPs.

30. The board therefore considers the claimed invention to be sufficiently disclosed.

Inventive step (Article 56 EPC)
Starting from document D8

31. The appellant considered document D8 to represent a suitable starting point for assessing inventive step. The respondent did not agree because this document did not relate to enhancing the activity of VKDPs, and thus related to a completely different purpose from the

claimed invention. This was also the position taken by the opposition division in the decision under appeal (see point 28.7).

32. The board does not agree, and, in line with established case law (see e.g. T 1343/19, Reasons 39; T 1701/22, Reasons 4 to 5.2; T 2140/22, Reasons 1.9 to 1.9.5), and in accordance with the wording of Article 56 EPC, finds that any state of the art can provide a starting point for assessing inventive step.

33. D8 discloses all method steps of the claimed method, i.e.

a) providing mammalian host cells (e.g. CHO) comprising an expression system expressing the recombinant vitamin K-dependent protein (e.g. Factor VII, see Examples 1 and 2),
b) culturing the cells in a cell culture medium comprising one or more cell culture enhancing reagent(s) (e.g. glutathione, see Tables 3 and 5, CHO-K, 318-X and 318-U media; page 18, lines 4 to 9), and
c) separating and/or isolating and/or purifying the recombinant vitamin K-dependent protein from the cell culture (page 19, lines 3 to 20)

34. The difference between the claimed method and the disclosure of D8 is the specific concentration range of glutathione ("*0.5-13 mmol/L*") within the cell culture medium. Document D8 discloses a range of 0-50 mg/L glutathione as part of a generic medium in which all ingredients are provided with ranges 0-X mg/L (see Table 3, page 16). This, however, cannot be considered a direct and unambiguous disclosure of a cell culture medium suitable for the expression of VKDP because it requires selection from a host of ingredients, some of

which are essential for the expression of VKDPs (e.g. vitamin K). A cell culture medium comprising glutathione in a concentration of 50 mg/L (corresponding to 0.1627 mM) can therefore not be used as a starting point when assessing inventive step either. Rather, any one of the three defined media (CHO-K, 318-X, 318-U) which contain glutathione in a concentration of 2.5 mg/L or 5 mg/L, corresponding to 0.008135 mM and 0.01627 mM, respectively (see Tables 3 and 5 on pages 14 to 18) represents a direct and unambiguous disclosure of cell culture media for expressing VKDP (see page 18, lines 4 to 9, and examples 1 and 2).

35. The effect achieved by the higher glutathione concentration in the method as claimed is increased activity of VKDP. Data supporting such an effect are provided by measurements using a chromogenic assay (see Figures 1B and 1C in the patent). It is apparent from these data that at concentrations above the tested 0.469 mM and below the tested 15.000 mM of glutathione (see e.g. days 9 and 10) the chromogenic activity of FVII is increased compared with the absence of glutathione, while the viable cell density is largely unchanged or even reduced (see Figure 1A). The range of 0.5 to 13 mM glutathione is thus not arbitrarily chosen, but is related to an effect. This was also indirectly acknowledged by the appellant when it considered that the disclosure in D8 of "*50 mg/L glutathione corresponding to approx. 0.1627 mmol/L, is effective in achieving an enhancement in activity of FVII*" (see statement of grounds of appeal, points [31] and [157]). The patent also indicates that "*the presence of L-glutathione, a TCA intermediate or sodium pyruvate in the cell culture medium has only minor impact on the viable cell density (VCD) (Figures 1A,*

2A, 3A, 4A, 5A, 6A, 7A and 8A)" (paragraph [0077]) and that "*at most concentrations tested, L-glutathione has no effect on the viable cell density*" (paragraph [0082]). This shows that the effect is not brought about merely by the presence of more cells in the culture being able to produce VKDP.

36. The question of whether this reflects an increased specific or total activity (see point 14. above) can be left unanswered as both would provide an improvement over the method disclosed in D8.

37. The board therefore concludes that the disclosure of the patent renders it credible that VKDP activity is enhanced.

38. The objective technical problem can thus be formulated as the provision of a cell culture method which enhances the activity of expressed VKDP.

39. The appellant argued that the skilled person would consult document D19, which taught that a concentration of glutathione of 10 mM (see page 582, left-hand column, under the heading "*Cells and media*") achieved better viability of CHO cells because it prevented apoptosis (see abstract and Figure 1). D19 concluded on page 584, right-hand column, last paragraph that "*nontoxic and cheap antioxidants such as VCP and GSH* [glutathione] *should have great potential for application to practical production processes of pharmaceuticals using mammalian cells*". The skilled person would therefore apply the higher concentration of glutathione taught in D19 to the culturing method of D8.

40. The board does not agree for two reasons. First, the skilled person, looking for increased activity of VKDPs, had no reason to consult a document which was not concerned with VKDPs and their activity, but only related to the viability of CHO cells. Second, even if the skilled person had considered D19, they would have found no indication therein that by raising the concentration of glutathione in the medium the activity of VKDPs could be increased independent of cell viability.

41. The claimed method is therefore not obvious over the combination of the disclosures of D8 and D19.

42. Document D7 was cited as a further document which could be combined with D8. It discloses a cell culture medium for the expression of recombinant proteins, in particular antibodies (see Examples; page 66, lines 13 to 15; page 77, lines 4 to 5) and finds that a number of supplements, including succinic acid, malic acid, α-ketoglutaric acid, fumaric acid and oxalacetic acid, increase cell growth and/or productivity and/or reduce toxic metabolite formation. One of the disclosed cell culture media contains sodium succinate in a concentration of 1 g/L (approximately 6.2 mM), which leads to an increase in product (antibody) concentration of 127% in CHO cells (see Examples 1 and 2; Table 3).

43. However, in view of the technical problem of enhancing the activity of VKDPs, the skilled person would not find any indication in D7 that this could be achieved with the listed media, which are only disclosed as generally improving protein expression measured as protein concentration, but not improving activity of VKDPs. In this regard it was common general knowledge

that VKDP activity was particularly sensible to γ-carboxylation and therefore required specific measures (see page 14, lines 17 to 21 of D8; page 1 of D4 and paragraphs [0002] and [0003] in the patent). Even if the skilled person had consulted D7, they could not have had a reasonable expectation of success that the proposed concentration of succinic acid would increase the activity of VKDPs, because D7 does not mention VKDPs and provides examples only for antibodies which are structurally and functionally very different.

44. Therefore the combination of D8 with D7 does not render the claimed method obvious either.

45. The claimed subject-matter is inventive when starting the assessment from document D8.

46. The appellant has not indicated any differences between documents D8 and D9 which might lead to a different conclusion when starting the assessment of inventive step from document D9.

Starting from document D4

47. Document D4 relates to methods of expressing VKDPs wherein the medium contains reduced menadione sodium bisulfite (rMSB), a vitamin K analogue. The difference between the claimed method and the disclosure of D4 is the presence of glutathione in a defined concentration range in the cell culture medium. The board notes in this regard that the claims do not exclude the presence of rMSB and that media used in the examples of the patent also include rMSB (see paragraph [0080]). The effect of the difference is that the expression of active VKDP is increased when glutathione in the

concentrations indicated is added (see Figures 1B and 1C in the patent).

48. The objective technical problem is thus the same as that starting from D8.

49. In this case too, the skilled person had no reason to turn to document D19, which is concerned with the general improvement of viability of CHO cells, or to D7, which is concerned with improving the expression of recombinant proteins, in particular antibodies.

50. The claimed subject-matter is thus not obvious when starting from document D4 either.

51. The appellant argued with regard to both D19 and D7 that the skilled person would always be interested in improving known methods and would therefore have considered the disclosed media, which were shown to improve cell viability and/or production of proteins. In applying this to the cell culture of D8 or D4 the skilled person would, as a bonus effect, have achieved enhanced activity of VKDPs and thus have arrived at the claimed method.

52. The board disagrees, because although the skilled person could have modified the cell culture medium disclosed in document D8 or D4 in many ways, e.g. as taught in D19 or D7, they would not have done so when attempting to solve the objective technical problem defined above because they had no reasonable expectation of enhancing the activity of VKDPs (see points 40. and 43. above).

53. Starting from any of documents D2, D3 or D21, the same findings apply *mutatis mutandis*. In view of the

objective technical problem and in view of the fact that these documents have fewer features in common with the claimed method than documents D8 or D4, the skilled person would not arrive at the invention as claimed either.

54. The subject-matter of claim 14 is equally inventive, as none of the cited prior art teaches that any of the compounds listed in the claim enhance the activity of VKDPs.

Conclusion

55. The claims of the main request and the invention to which they relate meet the requirements of the EPC.

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 on the basis of the main request filed as auxiliary request 46 with the statement of grounds of appeal and the description and drawings to be adapted if necessary.

The Registrar: The Chairwoman:

I. Aperribay M. Pregetter

Decision electronically authenticated