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Datasheet for the decision of 1 July 2021

Case Number: T 1483/15 - 3.3.08

Application Number: 10004393.4

Publication Number: 2213725

IPC: C12N5/02, C12N7/02, C12N1/16, C12N1/20

Language of the proceedings: EN

Title of invention:
Animal protein-free media for cultivation of cells

Patent Proprietor:
Baxalta Incorporated
Baxalta GmbH

Opponent:
F.Hoffmann-La Roche AG

Headword:
Method for cell cultivation/BAXALTA

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

Keyword:
Main request - inventive step (no)
Auxiliary request (AR-MRd) - requirements of the EPC met (yes)

Decisions cited:
T 0783/09, T 1253/07, T 0068/99, T 1511/07

Catchword:

Europäisches Patentamt
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Office européen des brevets

Beschwerdekammern

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Case Number: T 1483/15 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 1 July 2021

Respondent: (Patent Proprietor 1)	Baxalta Incorporated 1200 Lakeside Drive Bannockburn, IL 60015 (US)
Respondent: (Patent Proprietor 2)	Baxalta GmbH Thurgauerstrasse 130 8152 Glattpark (Opfikon) (CH)
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Appellant: (Opponent)	F.Hoffmann-La Roche AG 124 Grenzacherstrasse 4070 Basel (CH)
Representative:	Jaenichen, H.-R. Müller, S. Vossius & Partner Patentanwälte Rechtsanwälte mbB Siebertstrasse 3 81675 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 13 May 2015 concerning maintenance of the European Patent No. 2213725 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	D. Rogers

Summary of Facts and Submissions

I. European patent No. 2 213 725 based on European patent application No. 10004393.4, entitled "Animal protein-free media for cultivation of cells", is based on a divisional application of the earlier European patent application No. 05798575.6 (EP 1 805 298) (hereinafter "the parent application" filed under the Patent Cooperation Treaty on 12 October 2005 and published as WO 2006/045438 on the 4 May 2006). The patent was opposed on the grounds of Article 100(a) in conjunction with Article 56 EPC, and of Articles 100(b) and (c) EPC. The opposition division considered the main request to infringe Article 83 EPC while auxiliary request AR I, submitted during oral proceedings, was held to meet the requirements of the EPC.

II. The patentee lodged an appeal against the decision of the opposition division to maintain the patent in amended form and requested that the decision under appeal be set aside. The patentee (henceforth, the respondent) withdrew its appeal with a letter dated 21 September 2015.

III. The opponent (appellant) lodged an appeal against the decision of the opposition division to maintain the patent in amended form.

IV. Patentee (respondent) replied to the statement of grounds of appeal and filed the request maintained by the Opposition Division as the main request and auxiliary requests AR-MRb, AR-MRc, AR-MRd, AR-MRe, AR1, ARlb, ARlc, ARld, ARle, AR2, AR2b, AR2c, AR2d, AR2e, AR3, AR3b, AR3c, AR3d, AR3e, AR4, AR4b, AR4c, AR4d and AR4e.

V. Oral proceedings took place on 1 July 2021. At the end of the proceedings, the appellant withdrew all auxiliary requests, except auxiliary request MRd.

VI. Claim 1 according to the main request reads as follows:

"1. An animal protein-free cell culture medium, comprising at least one polyamine and at least one protein hydrolysate derived from the group consisting of plants and yeast, wherein the polyamine is present in the culture medium in a concentration ranging from 2 to 5 mg/L and the protein hydrolysate is present in a concentration ranging from 0.05 % (w/v) to 0.25 % (w/v)."

Independent claims 5, 8 and 12 relate to a method for cultivating cells, a method for expressing a target protein and a method for producing a virus, respectively, in which the animal protein-free cell culture medium of claim 1 is used.

VII. Claim 1 according to the auxiliary requests MRd differs from claim 1 of the main request by the following amendment:

AR MRd: "1. [...] wherein the at least one protein hydrolysate is derived from soy and wherein the at least one polyamine is putrescine."

Dependent claims 2 to 4 were deleted and the remaining claims renumbered and their back reference adapted accordingly.

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

D1: WO2004/005493 (published 15 January 2004);

D3: WO01/23527 (published 5 April 2001);

D5: EP 0481791A2 (published 22 April 1992);

D8 Burteau C.C., *et al.* "FORTIFICATION OF A PROTEIN-FREE CELL CULTURE MEDIUM WITH PLANT PEPTONES IMPROVES CULTIVATION AND PRODUCTIVITY OF AN INTERFERON-GAMMA-PRODUCING CHO CELL LINE." In Vitro Cellular & Developmental Biology - Animal vol. 39(7), pp 291-6, Jul-Aug 2003;

D11: Igarashi K and Kashiwagi K. "Polyamines: Mysterious Modulators of Cellular Functions." Biochemical and Biophysical Research Communications; vol. 271(3), pp. 559-64 19 May 2000;

D13: Eun Jung Kim, *et al.* "DEVELOPMENT OF A SERUM-FREE MEDIUM FOR THE PRODUCTION OF HUMANIZED ANTIBODY FROM CHINESE HAMSTER OVARY CELLS USING A STATISTICAL DESIGN." In Vitro Cellular & Developmental Biology. Animal, vol. 34, no. 10, pp. 757–761, (1998);

D14: Katsuta H *et al.* "Effects of Polyamines on the Proliferation of Mammalian Cells in Tissue Culture." Jpn J Exp Med, vol. 45(5), pp. 345-54 (Oct. 1975);

D19 US 2004/0171152 (published 2 September 2004);

D26 Schlaeger, E. *et al.* "SF-1, A LOW COST CULTURE

MEDIUM FOR THE PRODUCTION OF RECOMBINANT PROTEINS IN BACULOVIRUS INFECTED INSECT CELLS."
Biotechnology Techniques, vol. 7, pp. 183-188, (1993).

IX. The submissions made by the appellant, insofar as relevant to the present decision, may be summarized as follows:

Main request (claims 1-15)
Article 123(2) EPC

Claim 1 of the main request required a particular concentration range of the polyamine, i.e. a concentration range between 2 to 5 mg/L; and a particular concentration range of the protein hydrolysate, i.e. a concentration range between 0.05 % (w/v) to 0.25 % (w/v).

Paragraphs [0031] and [0032] of the patent application disclosed lists of five different concentration ranges of the polyamine and of the protein hydrolysate, respectively, while paragraph [0038] of the patent application disclosed the same list of five different concentration ranges of the polyamine and of the protein hydrolysate within the same paragraph. There was no clear order of preference within the listed concentration ranges of the polyamine or of the protein hydrolysate. There was thus no direct and unambiguous disclosure for an animal protein-free medium comprising specifically a combination of the last concentration ranges from each list, as the most preferred ones, in paragraph [0038].

Sufficiency of disclosure (Article 83 EPC)

The appellant contested that the features for carrying out the invention were sufficiently disclosed or defined in the patent to enable the skilled person to reproduce the claimed invention without undue burden.

First, the patent stated that the "quality of commercially available lots of soy hydrolysate varies extremely and as a result, there are large variations in the production of the recombinant proteins ..." (cf. paragraph [0011]). Second the parameters and the conditions of the manufacturing process of the protein hydrolysate were known to have significant effects on the chemical composition of the protein hydrolysate produced, whereby it became impossible to determine whether the selected protein hydrolysate established an inventive step or not. Polyamines were also shown to have different effects on the proliferation of different mammalian cells, for example rat liver cells (RLC10(2)) in a medium containing 0.02 mM spermine (corresponding to 4.05 mg/L) were killed to 100%, whereas putrescine and agmatine had no or little cytotoxicity on these cells, even at concentrations of about 1.0 mM (see Fig. 1 of document D14). This provided evidence that media containing polyamines were cytotoxic, even though they also contained 10% inactivated fetal calf serum. Likewise primary liver cells were killed to 100% at a spermine concentration of about 1 mg/L, whereas JTC-16 cells exhibited markedly high resistance to spermine (see document D14 Fig. 2). This finding was in line with the experiments of the patent which reported the effect of putrescine, ornithine and spermine on the cell specific productivity for CHO cells (see patent application, Fig. 9). The increase of cell specific productivity for the ARH77 cells and BHK cells was only observed for putrescine having only little or no cytotoxicity.

The term "yeast and/or plant derived protein hydrolysate" was undefined and open-ended. Both the source and type of "yeast and/or plant derived protein hydrolysate" had to be selected with respect to the cell and the recombinant protein to be expressed to achieve a consistent production (see documents D19 and D8), for example the most suited "yeast and/or plant derived protein hydrolysate" for growing Vero cells was HyPep 5115 "rice hydrolysate" (see document D19, Table 2), while document D3 showed that only soy hydrolysate led to a significant increase in recombinant protein yield (see page 5,lines 1 to 12). The biological diversity of plants and of the multiple possible parameters and conditions of the manufacturing process among which the skilled person may select from, rendered an extrapolation of an effect on growth and productivity observed for one "yeast and/or plant derived protein hydrolysate" to another "yeast and/or plant derived protein hydrolysate" impossible. Finally, the quality (grade) of the plant-specific protein hydrolysates necessary for carrying out the invention was never indicated in the patent application.

Inventive step (Article 56 EPC)

The patent highlighted that when efficient host systems and cultivation conditions are provided serum and animal proteins are not needed (see paragraph [0005]).

Document D26 represented the closest prior art for the animal protein-free medium of claim 1.

The animal protein-free cell culture medium "IP301" contains a protein hydrolysate, "Yeastolate" in a concentration of 0.4 % (w/v) (40 ml/L), 1 mg/L

spermidine, 1 mg/L spermine.4HC1 (corresponding to 0.581 mg/L spermine) and 1 mg/L putrescine.2HC1 (corresponding to 0.547 mg/L putrescine) in total 2.128 mg/L polyamines and a protein hydrolysate in a concentration of 0.4 % (w/v).

Although the culture medium SF-1, building up on the medium IP301, was indicated to have several advantages, this medium was not an option when recombinant proteins had to be produced for medical purposes.

The difference between document D26 and the claimed cell culture was that the concentration of protein hydrolysate in the media was higher than what claim 1 required. However, the protein hydrolysate concentration claimed did not impart a superior volumetric protein productivity. On the contrary, Figure 2 substantiated that the volumetric FVIII productivity in a medium comprising either 0.25% (w/v) or 0.4% (w/v) soy hydrolysate was comparable. Furthermore, the increased cell specific productivity observed in Figure 5 could not justify a synergistic effect as two control media were missing. First no cell specific productivity was assessed for a medium comprising 0.4% soy hydrolysate and 1 mg/L putrescine and second no cell specific productivity was assessed for a medium comprising 1 mg/L polyamines but without soy hydrolysate.

The experimental results in the examples of the patent could therefore not substantiate the existence of a synergistic effect in media combining polyamines and protein hydrolysates as defined in claim 1.

The technical problem to be solved could therefore only be seen as the provision of a further protein free cell

culture medium, i.e. an alternative animal protein-free cell culture medium to the prior art, or as an improved IP301 medium.

Decisions T 0867/13 of 6 March 2018, point 13 of the reasons and T 1165/06 of 19 July 2007, point 15 of the reasons established that it was the normal task of a skilled person working in a certain field not to remain inactive but to seek alternatives, to be constantly occupied with the elimination of deficiencies, with the overcoming of drawbacks and with the achievement of improvements of known devices and/or products.

Thus, faced with the technical problem to be solved as formulated above, the skilled person would have come across document D3 - belonging to the same technical field as document D26 - which disclosed a cell culture medium without animal protein comprising a DMEM/HAM's F12 base medium and soy hydrolysate at a preferred concentration of 0.25% (w/v) and would have replaced, with a reasonable expectation of success, the yeastolate component of the medium IP301, disclosed in document D26, by a soy hydrolysate disclosed in document D3 at its most preferred concentration so as to obtain a Factor VIII titer about twofold higher than when yeast hydrolysate was used (see document D3 pages 3 and 4, lines 16-21; examples 4 and 8, Tables 2 and 5, soy peptone last column, page 8, lines 9-11 and 14-16). The skilled person would have arrived at the subject-matter of claim 1 without inventive step.

Documents D26 and D3 focused on yeastolate while document D3 focused in addition on soy hydrolysate. The other ingredients of the media were not specifically considered and thus formed part of the common denominator of the culture media's composition.

Document D3 provided direct instructions that soy hydrolysate improved the Factor VIII protein titer in comparison to a media using yeastolate. It provided accordingly an incentive to replace the latter by the first hydrolysate with a reasonable expectation of improving at least the protein's yield.

Auxiliary request MRd (claims 1-12)
Articles 123(2) and 76(1) EPC

Claim 1 comprised added matter as it combined elements from several independent lists. The medium of claim 1 was the result of a selection from a list of polyamines consisting of cadaverine, putrescine, spermidine, spermine, agmatine, ornithine, and combinations thereof (see paragraph [0035]) that was combined with a selection from a second list of plant-derived protein hydrolysate consisting of a cereal hydrolysate and/or a soy hydrolysate (see paragraph [0039]).

Inventive step (Article 56 EPC)

The appellant relied essentially on its inventive step objection raised against the main request and added the following comments.

The difference between the chemically defined medium IP 301 of document D26 and the subject-matter of claim 1 was that the medium comprised soy hydrolysate instead of yeastolate and used a lower concentration of it. Second the medium comprised 2 to 5 mg/L of putrescine instead of polyamines, such as spermine and spermidine.

From document D11 the polyamines used in the medium IP 301 were however known to be equivalent (see page 559, col.1). Putrescine was representative for polyamines.

From the patent application, no effect could be assigned to the presence of putrescine in the medium that was not observed for spermine or spermidine. Figure 9 of the patent application compared the cell specific productivity of GD8/6 cells cultured in media comprising 0.25% (w/v) of soy hydrolysate and either no polyamines - control medium - or putrescine, spermine or spermidine. The media comprising 0.25% (w/v) soy hydrolysate and putrescine or spermine gave similar results (e.g. Qp relative 180% and 149% compared to the control media 100%). Thus, the relative cell specific productivity depended essentially on the concentration of polyamines. The media in Figure 9 supplemented with ornithine, spermine and spermidine included also all putrescine that was introduced with the soy hydrolysate (see Figure 6).

The concentration of the different polyamines in the chemically defined medium IP 301 in document D26, which were all equivalent in their effect, represented only an arbitrary choice the effect of which could be replaced by the use of a single representative polyamine at the corresponding total concentration.

Finally, the alleged synergistic effect, in accordance with paragraph [0018] of the patent could not be taken into account in the absence of a control experiment (see decision T 0605/14 of 7 June 2018).

On the basis of the above analysis and the effect induced by the different polyamines in the culture medium, the difference between the subject-matter of

claim 1 and document D26 were the limitation of the polyamines to putrescine and its concentration had to be increased to the total concentration of polyamines in the medium disclosed in document D26. No specific effect could be attributed to this difference. The media were equivalent.

The technical problem to be solved was therefore, as for the main request, to provide an alternative animal protein-free medium.

Document D3, which belonged to the same technical field as document D26, related to a protein-free and serum-free medium for the cultivation of cells (cf. page 4, paragraph 2). The medium comprised soy hydrolysate which was preferably added to the medium at a concentration of 2.5 g/L (corresponding to 0.25% (w/v)) (see pages 5 and 18, Table 2, page 22, lines 8-11). Example 8 described the culture of recombinant FVIII-CHO cells in a protein-free and serum-free medium containing different hydrolysates in particular a soy or yeast hydrolysate at 0.25% (w/v) (see page 22, lines 7-11).

Since no surprising effect was assigned to the combination of soy hydrolysate and putrescine at the concentrations defined in claim 1, they formed a mere aggregation of features, whereas the alignment of their concentration with those of the claim 1 was the result of common general knowledge.

In summary, starting with the chemically defined medium IP 301 of document D26, the skilled person faced with the technical problem identified above was motivated to combine it with document D3 and replace the higher concentration of yeastolate by the lower concentration

of soy hydrolysate and would have replaced the multiple polyamines present in the medium by its representative putrescine (see document D11 or common general knowledge) at the overall corresponding polyamine concentrations.

X. The submissions made by the **respondent**,(patent proprietor), insofar as relevant to the present decision, may be summarized as follows:

Main request (claims 1-15 as granted)
Article 123(2)

The subject-matter of claim 1 did not contravene Article 123(2) EPC and Article 76 EPC.

Article 83 EPC

An objection of lack of sufficiency of disclosure raised under Article 83 EPC presupposes that serious doubts substantiated by verifiable facts exist (see decision T 19/90).

The skilled person was capable of determining whether a protein hydrolysate was from yeast or plant origin. Further, the patent application provided a clear definition of what was a protein hydrolysate of plant and/or yeast (see paragraph [0040]). Finally, the term "yeast and/or plant derived protein hydrolysate", allegedly undefined, remained unamended in claim 1 and for this reason was not open to objections under Article 84 EPC (see decision G 3/14 (OJ 2015, A102, catchword).

There was furthermore no disclosure, neither in document D8 nor D19, to establish that an animal

protein-free medium comprising one of the recited plant peptones and polyamines rendered the culture of cells in general impossible (see e.g. document D8, Table 2; document D19 Table 2) or that the animal protein-free cell culture medium of claim 1, having a particular type of plant and/or yeast protein hydrolysate and a particular polyamine, some of which were known to be cytotoxic, was suited for culturing some cells only or not suited for culturing cells at all.

Inventive step (Article 56 EPC)

In case document D26 represented the closest prior art for the animal protein-free medium of claim 1, then it taught away from a serum-free medium, as it described two insect cell media: IP301 and SF-1.

IP301 contained 0.4% yeastolate (a yeast hydrolysate), as well as 1 mg/L putrescine 2HCl (i.e. about 0.547mg/L putrescine), 1 mg/L spermidine, 1 mg/L spermine 4HCL (i.e. about 0.58 mg/L spermine) and 0.4% yeastolate, which amounted in total to 2.128 mg/L polyamines. SF-1 medium contained 1/10 of the polyamines and 0.4% yeastolate. It also contained 0.5% primatone, a meat extract, and lactalbumin, a milk-derived protein. The SF-1 medium was not an animal protein-free medium and contained moreover only 1/10th of the polyamine present in the IP301 medium (i.e. 0.2128 mg/L). However, both media were tested with regard to the production of a recombinant protein (sTNFRa) which demonstrated that the SF-1 medium had several major advantages over the medium IP 301, in particular a very low batch to batch variation (see page 188, line 2).

The skilled person was taught in document D26 that the medium SF-1 was preferred over the medium IP301. Since

the preferred medium was not an animal-protein free medium as required in claim 1, it was not a realistic starting point for the development of an improved animal protein free medium. Thus, document D26 taught away from the present invention.

The skilled person, considering the integral teaching of document D26, would not have turned to the poorer medium IP301, but would have started from the preferred animal protein containing SF-1 medium. Starting from the SF-1 medium, there was however no teaching in document D26 (or in any other document) which would have motivated the skilled person to omit from this medium both lactalbumin and primatone in order to increase the amount of polyamines and to reduce the amount of hydrolysate present in this medium, in the hope of providing a medium allowing cells to have an increased cell specific productivity and reduced lot-to-lot variability.

Even if the medium IP301 was used as the starting point, the skilled person had no motivation to focus on the hydrolysate and to further reduce its concentration in order to fall within the claimed range in the hope of providing an improved medium resulting in increased cell specific productivity and reduced lot-to-lot variation, as plausibly demonstrated in the patent, even less so with a reasonable expectation of success. They were many other ingredients/components in this medium, like salt and amino acids and their respective concentrations, the skilled person could focus on.

The patent application proposes the medium of claim 1 as solution to the technical problem of providing an improved medium resulting in increased cell specific productivity and reduced lot-to-lot variation.

The culture medium comprising the soy hydrolysate of lot K119-1 had a 3.5 fold higher Factor VIII volumetric productivity (1750 [U/L/D]) than the culture medium comprising the soy hydrolysate of lot M022453 (500 [U/L/D]) (see Figure 3 of the patent and Figure 9).

There was no motivation or pointer in document D3 to look at the medium's protein hydrolysate so as to allow cells to be cultured with an improved cell productivity and so as to achieve more consistent and predictable production conditions, irrespective of the particular hydrolysate lot-to-lot variation. Nor was there a suggestion to combine document D26 with document D3 and to pick a soy hydrolysate to bring it within the claimed concentration range (see page 5, lines 21 to 22). A culture medium comprising both yeast and soy hydrolysates were not excluded too (see document D1, claim 7).

Document D3 related to a protein-free and serum-free medium for the cultivation of cells. The medium comprised soy hydrolysate (see page 5). However, the production of Factor VIII using the medium of the examples failed to mention which specific soy hydrolysate was used and whether putrescine was in the medium or not. Thus, starting from document D26, there was no motivation or pointer in document D3 to change the medium of D26 towards the claimed invention in the hope of providing a medium allowing an increased cell specific productivity and reduced lot-to-lot variation.

Auxiliary request MRd (claims 1-12)
Articles 123(2) and 76(1) EPC

A basis for the subject-matter of claim 1 could be found in items 1 and 4, and claims 1 and 4 of the patent application as well as in paragraphs [0031] and [0032], in particular in view of its preferred ranges.

Inventive step (Article 56 EPC)

The respondent relied on the inventive step objections raised against the main request and added the following comments.

Document D14 disclosed that putrescine had different metabolic functions and activities and was for this reason alone not representative for all the other polyamines (see Fig.1). Document D5, for example, used only putrescine to support cell growth. Even the patent showed that GD8/6 cells cultured in a medium comprising putrescine at a concentration between 2 to 5 mg/L and 0.25% (w/v) soy hydrolysate achieved a cell specific productivity of 910 [mU/ 10^6 cells/ day] (see Figure 9 of the patent) that was much higher than what was achieved by the other polyamines.

The difference between the chemically defined medium IP 301 of document D26 and the animal protein-free medium defined in claim 1 was the use of soy hydrolysate and putrescine at the indicated concentration ranges.

The effect underlying this difference was that the medium allowed for an efficient cell specific productivity and to overcome the inhibitory effect on the protein production yield owing to the lot-to-lot variation of the protein hydrolysate (see Figures 3A and 3B, 4A and 4B; Figure 5 first row QP 2190 +/- 168 [U/L/D] for soy hydrolysate 0.25% (w/v) + putrescine. 2HCl 1 mg/L having a margin of error of +/-170 whereas

for a same medium but lacking putrescine the margin of error was about +/- 500; in the second this corresponded in relative terms to +/- 251 vs +/- 79).

The effect of the medium comprising putrescine and soy hydrolysate on the cell specific productivity and lot-to-lot variability independence was demonstrated for Factor VIII, IgG1 and EPO proteins (see Figure 7; Figure 8, Figure 9, rows 1 to 4; Figure 3A and 3B).

The technical problem to be solved was therefore the provision of an improved animal protein-free medium that allowed for an increased cell specific productivity of a heterologously expressed protein as well as a more consistent productivity in culture.

The skilled person found no motivation in document D5 for improving the animal protein-free culture medium to allow for an increased cell specific productivity of a heterologously expressed protein as well as a more consistent productivity in culture. Likewise, document D3 did not mention the presence of putrescine in any of the media at all.

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

XII. The respondent requested that the appeal be dismissed or to set aside the decision under appeal and to maintain the patent upon the basis of auxiliary request AR-MRd, submitted under cover of a letter dated 9 February 2016.

Reasons for the Decision

Main request (claims 1-15)

Articles 123(2) and 76(1) EPC

1. The granted patent results from a divisional application of the earlier parent application EP 05798575 (EP1805298). The claims of the parent application are included as "items" in the description of the divisional application (cf. paragraph [0078| of the patent application). It follows that if the subject-matter of the claims of the patent lacks a basis in the patent application, it also lacks a basis in the parent application.

2. In accordance with established jurisprudence, the relevant question to be decided in assessing whether or not claim 1 encompasses subject-matter extending beyond the content of the application as filed, is whether the skilled person would derive the subject matter directly and unambiguously from the application as filed.

2.1 The appellant argued that there was no direct and unambiguous disclosure of an animal protein-free medium comprising the specific combination of the most preferred concentration ranges from each list in paragraph [0038].

2.2 The board agrees with the findings and the reasons put forward in item 13.3 of the decision under appeal. In line with the principles laid down in decisions T 783/09 of 25 January 2011 (see points 5.5 to 5.7 of the reasons) T 1253/07 of 15 December 2010 (see points 2.3 of the reasons) and T 68/99 of 12 June 2003 (see points 3.2.1 and 3.2.2 of the reasons), the skilled person would have considered that the specific combination of concentration ranges of polyamines and protein hydrolysate, highlighted in paragraph [0038] as "most preferred", characterises a further preferred

embodiment of the animal protein-free cell culture medium, and the best way to achieve the technical effects that the invention intends to provide (see also decision T 1511/07 of 31 July 2009, point 2.2 of the reasons).

2.3 The combination of these two most preferred ranges in claim 1 does not contravene Article 123(2) and Article 76 EPC.

Sufficiency of disclosure (Article 83 EPC)

3. The appellant raised an objection of insufficiency of disclosure based on the fact that, first a skilled person could not determine whether a protein hydrolysate was from plant and/ or yeast, second that a protein hydrolysate and its quality was technically undefined in terms of its components, depending inter alia on the parameters of the production process used, and third that polyamines were cytotoxic at some concentrations of claim 1 rendering the culture of cells impossible.

4. The board agrees with the findings and the reasons put forward in item 16.3.1 of the decision under appeal. An objection of lack of sufficiency of disclosure raised under Article 83 EPC presupposes that serious doubts substantiated by verifiable facts exist (see decision T 19/90).

4.1 The board considers a skilled person undoubtedly capable of determining whether a protein hydrolysate is from yeast or plant origin. The patent provides furthermore a clear definition of what is a protein hydrolysate of plant and/or yeast (see paragraph [0042]). Finally, the term "yeast and/or plant derived

protein hydrolysate", allegedly undefined, remained unamended in claim 1 and for this reason is not open to objections under Article 84 EPC (see decision G 3/14 (OJ 2015, A102, catchword).

4.2 There is no disclosure, neither in document D8 nor D19, that an animal protein-free medium comprising one of the recited plant peptones and polyamines renders the culture of cells in general impossible (see e.g. document D8, Table 2; document D19 Table 2) or that the animal protein-free cell culture medium of claim 1, having a particular type of plant and/or yeast protein hydrolysate and a particular polyamine, some of which were known to be cytotoxic, is suited for culturing some cells only or not suited for culturing cells at all.

4.3 Finally, the objection that the culture medium of claim 1 comprising a "yeast and/or plant derived protein hydrolysate" and a polyamine within a specific concentration range did not achieve the technical effect underlying the invention, because some polyamines were cytotoxic for some cells and the chemical composition of the protein hydrolysate was largely dependent on the manufacturing process parameters used, is an objection to be treated under Article 56 EPC since this effect is not mentioned in claim 1 (see Decision G 1/03 OJ 2004, 413, point 2.5.2 of the Reasons).

4.4 In the absence of verifiable facts and corroborating evidence that the invention disclosed in the patent cannot be achieved without undue burden, the appellant's objection of insufficiency of disclosure must therefore fail.

4.5 The board concludes that the claims of the main request meet the requirements of Article 83 EPC.

Inventive step (Article 56 EPC)

5. Claim 1 relates to "[A]n animal protein-free cell culture medium, comprising at least one polyamine and at least one protein hydrolysate derived from the group consisting of plants and yeast, wherein the polyamine is present in the culture medium in a concentration ranging from 2 to 5 mg/L and the protein hydrolysate is present in a concentration ranging from 0.05 % (w/v) to 0.25 % (w/v).

6. It was common ground between the parties that either document D26 or D3 represent the closest prior art for the subject-matter of claim 1.

6.1 Document D26 discloses two insect cell media. The SF-1 medium, stated to have several major advantages over the IP301 medium, and the animal protein-free cell culture medium "IP301" which contains a yeast protein hydrolysate ("Yeastolate") in a concentration of 0.4% (w/v), 1 mg/L spermidine, 1 mg/L spermine.4HCl (corresponding to 0.581 mg/L spermine) and 1 mg/L putrescine.2HCl (corresponding to 0.547 mg/L putrescine). The total polyamine concentration of 2.128 mg/L in the medium IP301 falls within the concentration of claim 1.

6.2 The only difference between medium IP301 of document D26 and the subject-matter of claim 1 is that the concentration of the yeast protein hydrolysate ranges from 0.05% (w/v) to 0.25% (w/v).

6.3 According to the respondent, the effect underlying this difference was to allow for an efficient cell specific productivity and to overcome the inhibitory effect on the protein production owing to the protein hydrolysate lot-to-lot variation (see paragraphs [0013] and [0014] of the patent). This effect was illustrated in examples 5 and 7 and Figures 1A, 3A, 3B and 4B. Figure 9 further shows the absolute cell specific productivity in the presence or absence of various polyamines.

6.3.1 The technical problem to be solved was therefore the provision of an improved animal protein-free medium that allows for an increased cell specific productivity of a heterologously expressed protein as well as a more consistent productivity in culture.

6.4 Appellant contested that the claimed medium led to a superior effect compared to other media, e.g. volumetric FVIII-productivity for 0.25% (w/v) or 0.4% (w/v) soy hydrolysate. The patent was missing comparative and essential control data (see Figure 2).

6.5 The board considers that the patent does not provide evidence that the sole difference with respect to the medium IP 301 of document D26 has the effects of increasing cell specific productivity and reducing variability of cell culture characteristics owing to lot-to-lot variability of the protein hydrolysate. The experimental results of the patent are limited to media comprising soy hydrolysate and selected polyamines. Even if the patent establishes that the technical effect is achieved for the specific media tested, the patent remains silent about what effect, having regard to the breadth of claim 1, extending to any protein or yeast hydrolysate and polyamines, and lacking any functional limitation, the replacement of yeastolate at

a concentration of 0.4% (w/v) with soy hydrolysate at a concentration of 0.25% (w/v) can have. The patent provides no evidence that the above identified and sole distinguishing feature has actually a technical effect that should be taken into account when formulating the technical problem. The burden of proof is on the party claiming an effect to show that this effect is truly achieved across the entire area claimed.

6.5.1 Consequently, the problem underlying the claimed invention is defined as the provision of another/ alternative animal protein-free cell culture medium.

6.5.2 The solution to that problem is the animal protein-free medium according to claim 1.

Obviousness

6.6 It remains to be decided whether the claimed solution to the problem underlying the patent as defined above is obvious in view of the state of the art.

6.7 Document D26 discloses two insect cell media. The SF-1 medium and an animal protein-free cell culture medium "IP301"

6.7.1 The respondent submitted that the skilled person would not have considered medium "IP301" as a suitable starting point for the assessment of inventive step. The skilled person was indeed clearly taught that SF-1 was preferred over the IP301 medium (see document D26, page 188 points 1 to 4).

6.8 The board is not convinced by this argument as the subject-matter of claim 1 is an animal protein-free medium and in consequence must be completely free of

animal proteins (see paragraphs [0012] and [0017]). In view of this requirement, the skilled person would have disregarded the SF-1 medium, as starting medium for the assessment of inventive step, as it contains animal proteins such as lactalbumin and primatone, and would have focused on the chemically defined medium "IP301", shown to be equally suitable for the production of recombinant sTNFR-α in SF-9 insect cell line (see abstract and Fig. 3A).

6.9 The respondent argued that even if the skilled person would have started with medium IP301, it had no motivation to focus on the protein hydrolysate, from among all the medium ingredients, and even less to reduce its concentration so as to fall within the claimed range in the hope of providing a medium wherein cells increase their specific productivity and reducing their lot-to-lot variation, and even less so with a reasonable expectation of success.

6.10 The board cannot share the respondent's view. First, the skilled person starting with a cell culture medium in document D26, would only have started with an animal protein-free cell culture and thus with medium IP 301. Second, faced with the technical problem of providing an alternative culture medium, as no technical effect could be assigned to the presence of 0.25% (w/v) of plant and yeast protein hydrolysate instead of 0.4% (w/v) of yeastolate, the skilled person would have come across and considered the content of document D3.

6.11 Document D3 relates to an animal protein-free cell culture medium (DMEM/HAM's F12), preferably comprising soy hydrolysate in a concentration between 1 to 5 g/L, corresponding to 0.1% (w/v) to 0.5% (w/v) (see page 8, lines 8 to 22). The soy hydrolysate was preferably

added to the medium at a concentration of 2.5 g/L (corresponding to 0.25% (w/v)) (see page 18, Table 2, page 22, lines 8-11). Example 8 describes the culture of recombinant FVIII producing CHO cells in a protein-free and serum-free medium containing different hydrolysates in particular a soy or yeast hydrolysate at 0.25% (w/v) (see page 22, lines 7-11).

6.11.1 The reduction and the replacement of 0.4% (w/v) yeastolate in the medium IP 301 by either yeast or soy hydrolysate at a concentration of 0.25% (w/v), as disclosed in document D3 (Table 5), represents therefore an obvious alternative to a person skilled in the art trying to solve the above mentioned technical problem.

6.12 It follows that claim 1 lacks an inventive step in view of document D26 in combination with document D3.

6.13 Thus, the main request lacks an inventive step.

Auxiliary request AR-MRd (claims 1-12)
Articles 123(2) and 76(1) EPC

7. The appellant argued that claim 1 comprised added matter, as it combined elements from several independent lists. The medium of claim 1 was the result of a selection from a list of polyamines consisting of cadaverine, putrescine, spermidine, spermine, agmatine, ornithine, and combinations thereof (see paragraph [0035]), and a selection from a second list of plant-derived protein hydrolysate consisting of a cereal hydrolysate and/or a soy hydrolysate (see paragraph [0039]).

7.1 The respondent asserted that a basis for the subject-matter of claim 1 could be found in items 1 and 4, claims 1 and 4 and in paragraphs [0031] and [0032] of the patent application.

7.2 The board agrees with the findings and the reasons put forward in item 13.3 of the decision under appeal that the combination of the concentration ranges of the polyamine and the protein hydrolysate is the result of selecting the most preferred concentration ranges (see also item 2.2 above), while all the examples as well as paragraph [0039] provide a direct and unambiguous disclosure that the most preferred plant-derived protein hydrolysate was soy hydrolysate.

7.3 The combination of these two most preferred ranges with soy hydrolysate does not contravene Article 123(2) and Article 76 EPC.

Inventive step (Article 56 EPC)

8. Document D26 is considered to represent the closest prior art with regard to the subject-matter of claim 1.

8.1 The difference between the chemically defined medium IP 301 of document D26 and the subject-matter of claim 1 is that it has first a lower concentration of soy hydrolysate instead of yeastolate and second a putrescine concentration ranging from 2 to 5 mg/L, instead of several polyamines such as spermine and spermidine.

8.2 As regards appellant's argument that polyamines were equivalent and that putrescine is representative of polyamines' activities, the board considers the following:

8.2.1 Document D11 refers to polyamines as necessary for normal cell growth. The authors note that although the mechanisms of regulation of intracellular polyamines are clarified, an understanding, at the molecular level, of the role of polyamines in cell growth is still lacking (see document D11 page 559, col.1, first sentence; abstract and page 559, col.2, first full paragraph). Thus, from this teaching the board cannot ascertain and deduce that all polyamines, present in a cell culture medium at a particular concentration, are equivalent in their effect on cell growth activities.

8.2.2 Neither document D14, which examined biological effects of polyamines on mammalian cells, nor document D5 which used only putrescine to support cell growth, provide evidence that putrescine and polyamines are equivalent and that putrescine is a representative polyamine for the other polyamines.

8.2.3 Hence, there are no reasons for considering the different polyamines in the chemically defined medium IP 301 to be equivalent in their effects, let alone to replace them by one polyamine, here putrescine, at the corresponding total concentration.

8.3 The board notes that the patent application discloses GD8/6 cells cultured in a medium comprising putrescine at a concentration between 2 to 5 mg/L and 0.25% (w/v) soy hydrolysate. The cell specific productivity of 910 [mU/ 10^6 cells/ day] is clearly higher than what was achieved by any other polyamine (see Figure 9 of the patent).

8.3.1 The board cannot share appellant's view that no effect can be assigned to the presence of putrescine in the

medium that was not observed for spermine or spermidine.

Figure 9 of the patent application shows for example that a medium comprising 0.25% (w/v) soy hydrolysate and putrescine at a concentration ranging from 2 mg/L to 5 mg/L has a relative cell specific productivity $Qp_{relative}$ of 180% while the same medium but comprising spermine has a relative cell specific productivity $Qp_{relative}$ of 149%. The control medium, without soy hydrolysate and putrescine, has a $Qp_{relative}$ of 100%. These results demonstrate that the cell specific productivity, be it relative or absolute, is higher for putrescine than for spermine or ornithine.

8.3.2 The board agrees with the appellant that the media comprising 0.25% (w/v) of soy hydrolysate comprise inherently, based on Figure 6 of the patent application, 1.4 mg/L of putrescine.

Even considering this fact, the media comprising putrescine and another polyamine do not qualify as media with a putrescine concentration ranging from 2 to 5 mg/L. Even leaving aside the putrescine concentration range mentioned in claim 1, all the media comprising putrescine and another polyamine have a lower cell specific productivity, be it relative or absolute, compared to the medium comprising only putrescine. Thus, the cell specific productivity increases even more for media comprising 0.25% (w/v) soy hydrolysate and putrescine at a concentration ranging from 2 mg/L to 5 mg/L.

8.4 The board concurs with the respondent that media, as defined in claim 1, overcome the inhibitory effects impacting the production yield of a product due to the

lot-to-lot variation of soy hydrolysates. The reduced variability was demonstrated for Factor VIII, IgG1 and EPO proteins (see Figure 7; Figure 8, Figure 9, rows 1 to 4; Figure 3A and 3B). The presence of putrescine and different lots of soy hydrolysate in the media at the concentration ranges of claim 1 leads to a reduced variability in cell specific productivity and in specific cell growth rate which is reflected by a lower margin of error (see Figures 3A and 3B, 4A and 4B; Figure 5 first and second rows compare the media comprising soy hydrolysate 0.25% (w/v) + putrescine.2HCl 1 mg/L and lacking putrescine: QP = 2190 +/- 168 versus 959 +/- 497 [U/L/D] and Qp = 1473 +/- 79 versus 631 +/- 251 [$mU/10^6$ cells/day]).

8.5 In view of the considerations set out above, the board concludes that the claimed medium differs from the closest prior art medium in at least two features, namely the presence of putrescine in a concentration ranging from 2 to 5 mg/L and of a soy hydrolysate in a concentration ranging from 0.05% (w/v) to 0.25% (w/v).

8.6 These differences result in a medium conferring increased cell specific productivity and reduced lot-to-lot variability.

8.7 In consequence, the technical problem is defined as the provision of an improved animal protein-free medium with an increased cell specific productivity of a heterologously expressed protein as well as a more consistent productivity in culture.

8.7.1 Since no synergistic effect is taken into account in the formulation of the objective technical problem, the reference to decision T 0605/14 of 7 June 2018 and the lack of some control experiments is obsolete.

8.8 In the light of the experimental data disclosed in the patent (set out in e.g. Figures 3A, 3A, 4A, 4B and 7 to 9) the board is satisfied that the animal protein-free medium of claim 1 solves this technical problem.

Obviousness

8.9 It remains to be assessed whether or not the skilled person starting from the closest prior art medium IP 301 and faced with the technical problem identified above would have arrived at the claimed method in an obvious manner.

8.10 Document D26 discloses medium IP301 containing yeastolate (a yeast hydrolysate), as well as 1 mg/L putrescine 2HCl (i.e. about 0.547mg/L putrescine), 1 mg/L spermidine, 1 mg/L spermine 4HCL (i.e. about 0.58 mg/L spermine) and 0.4% yeastolate, which amounts in total to 2.128 mg/L polyamines. There is no disclosure in document D26 that would prompt the skilled person to seek to improve the chemically defined medium IP301. The only improved medium disclosed in document D26 is the serum-free SF-1 medium comprising 0.5% primatone, a meat extract, and lactalbumin, a milk-derived protein and thus not an animal protein-free medium.

8.11 Appellant submitted that it was obvious for the skilled person to modify the IP301 medium disclosed in document D26 in view of document D3, belonging to the same technical field.

8.12 Document D3 discloses a cell culture medium without animal protein comprising a DMEM/HAM's F12 base medium and soy hydrolysate at a preferred concentration of 0.25% (w/v). Example 8 shows that the titer of Factor

VIII is about twice as high when soy hydrolysate is used than when yeast hydrolysate is used (see document D3 pages 3 and 4, lines 16-21; examples 4 and 8, Tables 2 and 5, soy peptone last column, page 8, lines 9-11 and 14-16).

8.13 However, nowhere does document D3 disclose or suggest the presence of putrescine in a medium, let alone at a specific concentration range, which also contains soy hydrolysate at a concentration as defined in claim 1.

8.14 Thus, starting from the medium IP 301 disclosed in document D26 the skilled person faced with the technical problem formulated above would not have been motivated to combine it with document D3.

8.15 In conclusion, the combination of document D26 with document D3 or with any other less relevant prior art document on file would not have led the skilled person to the medium of claim 1 and thereby to the claimed subject-matter of the auxiliary request AR-MRd in an obvious way.

8.16 Thus, the first auxiliary request AR-MRd fulfils the requirements of Article 56 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 with the following claims and a description to be adapted:

- Claims: Nos 1 to 12 of auxiliary request AR-MRd filed under cover of a letter dated 9 February 2016.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated