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Datasheet for the decision of 2 July 2019

Case Number: T 1255/13 - 3.3.08

Application Number: 03808535.3

Publication Number: 1576182

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Language of the proceedings: EN

Title of invention:
PRODUCT QUALITY ENHANCEMENT IN MAMMALIAN CELL CULTURE PROCESSES FOR PROTEIN PRODUCTION

Patent Proprietor:
Bristol-Myers Squibb Company

Opponent:
Patentanwälte Isenbruck Bösl Hörschler PartG mbB

Headword:
CTLA4 production/BRISTOL-MYERS SQUIBB

Relevant legal provisions:
EPC Art. 113(1), 123(2), 123(3), 83, 54(3), 56, 104
RPBA Art. 12(4)

Keyword:
"Main Request - requirements of the EPC met (yes)"

Decisions cited:

Catchword:

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Beschwerdekammern

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Case Number: T 1255/13 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 2 July 2019

Appellant: (Opponent)	Patentanwälte Isenbruck Bösl Hörschler PartG mbB Prinzregentenstrasse 68 81675 München (DE)
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Representative:	Wolter, Thomas Reitstötter Kinzebach Patentanwälte Sternwartstrasse 4 81679 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 21 March 2013 concerning maintenance of the European Patent No. 1576182 in amended form.**

Composition of the Board:

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

Summary of Facts and Submissions

I. European patent No. 1 576 182 is based on European patent application No. 03808535.3 (published as International patent application WO 2004/058944; hereinafter "the patent application") and was opposed on the grounds of Articles 100(a), (b) and (c) EPC. The opposition division considered the main request to contravene Article 123(2) EPC and took the view that auxiliary request 1 complied with the requirements of the EPC.

II. The opponent (appellant) lodged an appeal against the decision of the opposition division.

III. The patent proprietor (respondent) replied to appellant's statement of grounds of appeal and submitted new auxiliary requests 1a, 1b, 2, 2a, 2b, 3, 3a, 3b, 4, 4a, and 4b, and filed new documents D22 to D26.

IV. As an auxiliary measure, both parties requested oral proceedings.

V. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion on some of the legal and substantive matters of the case.

VI. In reply thereto, the appellant, with a letter dated 14 May 2019, without making any substantive submissions, informed the board that it did no longer intend to be present at the oral proceedings. The respondent replied to the board's communication by filing further arguments, a new amended main request

and auxiliary requests 1a, 1b, 2, 2a, 2b, 3, 3a, 3b, 4, 4a, 4b.

VII. Oral proceedings were held on 2 July 2019 in the presence of the respondent only.

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

"1. A cell culture process for the production of a soluble CTLA4 molecule, comprising:

a) culturing CHO cells which produce a soluble CTLA4 molecule in cell culture under conditions that allow for CTLA4 production; and

b) feeding the cells with D-galactose."

IX. Dependent claims 2 to 9 define specific embodiments of the process of claim 1.

X. The following documents are cited in this decision:

D1: W02004/008100 (publication date 22 January 2004)

D1a: US60/396.221 (Priority document of D1);

D4: A.R. Flesher et al., Biotechnology and Bioengineering, 1995, Vol. 46, pages 399-407;

D5: D.R. Mack et al., Glycoconjugate Journal, 1998, 15, pages 1155-1163;

D7: EP 0481791 A2 (publication date 22 April 1992);

D9: S. Nahrgang, Thèse No. 2608, École Polytechnique Fédérale de Lausanne, 2002;

D10: C. Altamirano et al., Biotechnol. Prog, 2000, Vol. 16, pages 69-75;

D11: C. Altamirano et al., Animal Cell Technology: Products from Cells, Cells as Products, 1999, pages 95-97;

D12: M. Gawlitzek et al., Biotechnology and Bioengineering, 2000, Vol. 68, N°6, pages 637-646;

D16: WO 99/28455 (publication date 10 June 1999);

D19: M.R. Lifely et al. Glycobiology, 1995, vol.5, pages 813-822.

XI. Appellant's submissions, insofar as relevant to the present decision, may be summarized as follows:

Main request

Amendments (Article 123(2) EPC)

The subject matter of claims 5, 6 and 9 extended beyond the content of the patent application, because they referred back to several claims while the corresponding claims of the patent application referred back to a single claim only. These multiple back-references created combinations of features that were not disclosed in the patent application.

Claims 5 and 6

Claim 5 referring back to claim 4 defined a large scale cell culture in which D-galactose was sustained at a concentration of 0.1 to 10 g/L. Claim 6 referring back

to claims 5 and then claim 4 defined a large scale cell culture of 500L and greater in which D-galactose was sustained at a concentration of 0.1 to 10 g/L. Such combinations were not directly and unambiguously disclosed in the patent application, where the scale of the cell culture and the residual amounts of galactose were listed in different paragraphs (page 19, last paragraph to page 20, 2nd paragraph).

Claim 9

Claim 9 introduced subject-matter going beyond the patent application as filed, when it referred back to claims 5 or 6 for which no basis was found in the present application.

Article 83 EPC - Insufficiency of disclosure

The invention defined in claim 1 covered many variables, such as soluble CTLA4 molecules, amounts of galactose, time points of the addition of galactose, size of the reactor, time points of the addition of polyanionic compounds, time points of harvest, time points of temperature shifts, which rendered the limited number of experiments in the patent insufficient. Hence, a skilled person had to test all the amounts of feeding media and the different ratios between feeding medium and culture medium to determine whether any of these combinations achieved an enhanced sialylation of CTLA4. This amounted to an undue burden.

The effect of adding galactose to enhance sialylation was questionable. Indeed, Figure 3 of the patent showed that an enhanced sialylation was observed although no galactose was detectable in the culture medium when a concentration of 3 g/L galactose was used in the

feeding medium (see Table 3 of the patent; compare 7.8 SA (NANA) at 3.0 g/l galactose with 6.1 SA (NANA) with no addition of galactose).
The examples of the patent taught that feeding with D-galactose started one day post inoculation (page 29, lines 28-31), but did not show that another galactose feeding strategy solved the technical problem of the invention.
The examples of the patent did not render plausible that any of the compounds falling under the term "soluble CTLA4 molecule" were workable.

Therefore, the subject-matter of claim 1 was not disclosed in a manner sufficiently clear and complete to be carried out by a person skilled in the art.

Novelty (Article 54(3) EPC)

Document D1 was filed on 14 July 2003, claiming priority of US application No. 60/396,221 filed on 15 July 2002, and entered the European phase as European Patent application No. 03764511.6 on 11 February 2005. It was considered prior art under Article 54(3) EPC.

It referred to methods and media for controlling sialylation of proteins produced by mammalian cells (see document D1, page 1, last paragraph), and to methods for the production of secreted sialylated proteins to be used preferably in pharmacological applications (see document D1, page 2, line 2; page 1, lines 19-21). It disclosed medium containing galactose and cells which were preferably CHO cells (see document D1 claims 1 and 9; page 14, 3rd paragraph). Document D1 disclosed further the step of "adding" galactose to the medium, while the glycoprotein to be produced included a ligand of CD proteins, such as chimeric proteins

capable of recognizing CD80 (see page 8, line 10; page 11, lines 24-27 and page 12, lines 23, 25 and 28).

It was common general knowledge at the priority date that secreted proteins were generally soluble and that CTLA4 was a CD80 ligand. A skilled person reading document D1 would have immediately (and only) thought of CTLA4 and of CTLA4Ig, its chimeric form, as binding agents to CD80. Thus, it disclosed the production of a CTLA4 molecule and also of a process of producing a chimeric CTLA4, which deprived claim 1 of novelty.

For the same reasons, claims 3 and 4 lacked novelty over document D1 (see page 16, lines 1-2).

Inventive step (Article 56 EPC)

According to the established jurisprudence of the Boards of Appeal, the evaluation of inventive step under Article 56 EPC was a two-step process: (1) "Has the technical problem identified indeed been solved?" and (2) "Does the solution to the technical problem involve an inventive step?".

The process of claim 1 was allegedly capable to enhance the sialic acid content in glycostructures of the CTLA4 protein produced in CHO cells, influencing various properties of the protein, such as absorption, solubility, thermal stability, serum half life, clearance, physical and chemical structure/behaviour and immunogenicity, as indicated in items [0002] and [0004] of the patent. The enhanced amount of sialic acid in glycostructures imparted an increased quality to the final glycoprotein product (see item [0020] of the patent).

Document D4 was the closest prior art. It was directed to the carbohydrate analysis of the CTLA4 protein and studied the correlation of the carbohydrate composition with the in vivo clearance profile. Various batches of human CTLA4Ig (CTLA4 fused to IgG domains) produced in CHO cells had various clearance profiles. Batches with accelerated clearance (a slow clearance rate is regarded as advantageous in the art) (PBR12, PBR13 and VERI-asialo) had a lower content of Neu5Ac (a general abbreviation for N-acetylneuraminic acid, the predominant sialic acid found in mammalian cells) than those with a slower clearance rate (e.g. PBR10 or VERI) (see Figs. 3 and 4; section "High-Resolution Polyacrylamide Gel Electrophoresis of Oligosaccharides" on pages 403 and 404). The clearance rate of murine CTLA4Ig was highly dependent on the cell line used for producing it. The fusion protein produced from CHO cells cleared more slowly than that from NS-0 cells (see Fig.8). The CTLA4Ig derived from CHO cells released the sialic acid NeuAc5, whereas the CTLA4Ig derived from NS-0 cells did not release Neu5Ac but another sialic acid (Neu5Gc) (see section "Characterization of Murine CTLA4Ig" on pages 404 and 405). The chapter "DISCUSSION" stated that preparations with less Neu5Ac than control preparations had increased clearance rates, which was attributed to the absence of Neu5Ac, leading to increased amounts of terminal galactose or possibly N-acetylgalactosamine, recognized by asialoglycoprotein receptors found on hepatocytes (see page 406, lines 21-25). Thus, document D4 disclosed different culturing conditions using different components in the culture medium resulting in different degrees of sialylation and different clearance rates.

The difference between document D4 and claim 1 was that the method of claim 1 required the addition of D-galactose to the medium. Claim 1 did not demand the production of a sialylated CTLA4 molecule, which was only defined as embodiment in claim 12.

The problem of producing a sialylated soluble CTLA4 protein was not solved over the entire scope of claim 1, for the following reasons:

First claim 1 encompassed a method of discontinuous feeding for which a decrease of sialic acid levels on CTLA4 compared to the method using no galactose was shown (see Table 3 on page 31; item 10.3.1 of the decision under appeal). Second when a concentration of 20.0 g/L of galactose was used the sialic acid concentration and titer of protein were reduced compared to the sialic acid and titer obtained when a concentration of 12.5 g/L of galactose was used (see Table 3 of the patent). Since claim 1 embraced even higher concentrations than 20 g/L of galactose, the beneficial effect imparted by it's addition was expected to vanish. Therefore, the technical problem had to be reformulated as the provision of an alternative method of producing a sialylated soluble CTLA4 molecule.

The skilled person looking for an alternative process of culturing CHO cells to produce proteins without particular features would have looked at document D16.

Document D16 related to a method of preparing polypeptides with appropriate glycosylation from eukaryotic cells, whereby cells are cultured in the presence of at least two carbohydrates. Glucose, mannose and galactose were preferred (see Title, claim

1 and page 5, paragraph 2 and 3). The method produced a glycoprotein with an enhanced sialylation degree (see page 7, paragraph 4 to page 8, paragraph 1). CHO cells were equally used for producing different kinds of erythropoietin (see Examples 2 to 4). It followed from the results disclosed in Tables 1 to 3 that the use of galactose enhanced the sialic acid content of proteins produced in several cell lines, including CHO cells. In summary, the process for producing the glycosylated protein comprised a step of culturing the cells in the medium and of obtaining the protein from the medium, and was characterized by adding at least two carbohydrates to the medium (see claims 1 and 2, page 5, 2nd and 3rd paragraph). The controlled addition of nutrients, in particular with feeding (indicating feeding after inoculation), was disclosed too (see page 5, last paragraph; page 6, 2nd and last paragraph; page 7, 2nd paragraph; page 9, 1st and 2nd paragraphs).

It was known from documents D4 and D16 that the enhanced degree of sialylation imparted advantageous properties to proteins. Thus, a skilled person, faced with the technical problem identified above, had an incentive to use the method described in document D16 to arrive at the solution claimed. Examples 2 to 4 of document D16 provided sufficient information to the skilled person that the method had to be applicable to erythropoietin produced in CHO cells. Thus, the use of galactose in the media of document D4 was expected to produce CTLA4 with a comparable degree of sialylation.

The skilled person would have determined, at worst, by routine tests whether galactose in the culture medium induced the same sialylation effect in HeLa S3 as in CHO cells (see documents D4 and D16).

Document D5 described the altered expression of sialylated carbohydrate on Lewis antigens in HT29 colonic carcinoma cells. The switch from glucose-containing media to glucose-free, galactose-containing media resulted in an increased expression of sialylated antigens mediated by increased sialyl-transferase activities in HT29 cells (see abstract and page 1159, 2nd paragraph and page 1161, second paragraph). The prolonged culturing of CaCo-2 cells during in vitro maturation enhanced its α-2,6 sialyltransferase activity. Thus, the use of galactose increased the activity of sialyltransferases and the amount of sialic acid on expressed proteins.
The skilled person was motivated to combine the teaching of document D4, which described that sialic acid has a beneficial effect on soluble CTLA4, with that of document D5 to arrive at the claimed invention or, at worst, was in a try-and-see situtation to determine by routine tests whether the addition of galactose into the culture medium resulted in a higher sialylated recombinant protein in CHO cells, known to incorporate all the enzymes necessary to catalyse protein glycosylation.

Document D7 disclosed a culture medium for CHO cells and the use of galactose as an energy source (see page 3, line 31). The chemically defined medium, comprising inter alia galactose as an energy source, was developed to circumvent problems arising from the use of media comprising serum and animal components. Apart from being safe, the medium was stated to have other advantages (see page 3, 2nd paragraph).

Starting with document D4 the skilled person faced with the technical problem of finding an alternative method of producing soluble CTLA4 would have turned to D7 to

replace a routine medium for producing a soluble CTL4 protein in CHO cells with a chemically defined medium comprising galactose. This combination of features was obvious and the enhanced sialylation of CTLA4 was merely a bonus effect for which no inventive step could be acknowledged.

Documents D10 and D11 described the simultaneous replacement of glucose by galactose, known to be a slowly metabolized substitute of glucose, and glutamine by glutamate, as a nitrogen source, achieving altogether best results as regards the accumulation of lactate and ammonium, known to have inhibitory effects on cells compared to other feeding agents (including glucose). The use of galactose in a culture medium of CHO cells resulted "in an optimal situation" for the CHO cells (see abstract of D10, page 74, 1st paragraph and Figure 3). Consequently, the skilled person faced with the technical problem of providing an alternative method for producing soluble CTLA4 would have combined D4 with D10 and would have used galactose instead of glucose in the culture medium of CHO cells.

XII. Respondent's submissions, insofar as relevant to the present decision, may be summarized as follows:

Admission of the main request (Article 12(4) RPBA)

The main request and the auxiliary requests should be admitted into the appeal proceedings. Their submission was a direct and expedient reaction to the Board's communication under Article 15(1) RPBA. It involved merely the deletion of some dependent claims and a renumbering of the dependencies of the remaining claims.

Main request

Amendments (Article 123(2) EPC)

Independent claim 1 of the main request was the same as independent claim 1 underlying the decision under appeal. The sole difference was that dependent claims were deleted and dependencies renumbered.

Claims 5 and 6

The subject matters of claims 5 and 6 were derived from the last sentence of the first full paragraph on page 19 of the sentence bridging pages 19 and 20 read in combination with the last sentence of the first full paragraph on page 20 (lines 14-17) of the patent application reciting the claimed concentration and the sentence on page 18, lines 15 to 17, reciting numerical values for the reactor size.

Novelty (Article 54(3) EPC)

Document D1 claimed an earlier priority date than the patent. It had an earlier filing date than the patent, but was published later than the filing date of the patent. Thus, only content of D1 which was entitled to its priority date qualified as state of the art under Article 54(3) EPC.

Document D1 described a method of culturing mammalian cells to produce a huge number of diverse proteins in a medium comprising either (i) galactose and fructose, (ii) N-acetyl mannosamine and galactose or (iii) only acetyl-mannosamine (claims 1, 19, 33 respectively). Example 1 described a culture medium comprising sugars on day 0 (see page 20, lines 33 and 34; page 22, lines

19 and 20). The "adding" of sugar to the medium (see page 8, lines 9-11) occurred accordingly prior to inoculation, while in the patent the feeding with galactose was clearly defined as one or more additions of galactose to the cell culture after inoculation (see [0052] of the patent).

The protein to be expressed could be a secreted protein and be produced in CHO cells (see page 2, line 23). The method could produce in vitro selected chimeric proteins capable to bind a specific target protein and to modify its activity (see page 12, lines 11-12). Suitable antigens or combinations of antigens, including CD80 (B7.1), were disclosed. CTLA4 was only mentioned as a target antigen among others (see page 13, line 15), while CTLA4 chimeric protein was not mentioned. As numerous proteins besides CTLA4, e.g. CD28 or any anti-CD80 antibody were known to recognize CD80, document D1 did not anticipate the process defined in claim 1.

Inventive step (Article 56 EPC)

The patent provided ample evidence that the claimed process solved the objective technical problem of providing a method for the production of soluble CTLA4 molecules having an improved degree of sialylation. Example 2 showed that feeding with D-galactose versus not feeding with D-galactose enhanced sialic acid content and reversed a scale effect. Table 3 showed sialylation for 20.0 g/L to be slightly lower than for 12.5 g/L galactose but still higher than without galactose. Hence, these passages indicated that feeding the cells with galactose increased the product’s sialic acid content in all cases.

The process had to be carried out under conditions allowing CTLA4 production. This wording excluded any addition of galactose concentrations inducing a detrimental osmotic effect to the cell culture. Although discontinuous feeding resulted in the production of a soluble CTLA4 molecule with a lower sialic acid content than when continuously fed a medium containing 12.5 g/L galactose (see experimental Set IV and V in example 2), a slight increase of sialic acid content on soluble CTLA4 molecules was nonetheless observed when a discontinuous feeding strategy using a feeding medium comprising 3 g/L D-galactose was compared to a feeding strategy using a feeding medium lacking D-galactose (Experimental Set III vs Experimental Set IV).

The presence of a temperature shift was not essential for the process of claim 1, as the negative controls used in Example 2 included temperature shifts too. Thus, the technical effect was shown to be independent of temperature shifts and need not be mentioned in claim 1.

Document D4 disclosed that different culturing conditions using different components in the culture medium resulted in different degrees of sialylation and different clearance rates. The cause of less sialylation for some batches and not others was not determined. Document D4 confirmed that the choice of the production cell line usually had a strong influence on the glycosylation pattern whilst cultivation conditions provided only a limited possibility to change the glycosylation pattern of a given cell line (see D9, page 71, last sentence, and page 87, last sentence of first paragraph; D19 first two paragraphs of the discussion).

Since document D4 was missing the steps of feeding the cells with galactose, the skilled person could not arrive at the process of claim 1 in an obvious manner.

Human HeLa S3 cells were used for determining the effect of adding sugars to the culture medium (see D16 page 8, lines 15 and 16 and page 10, line 5). However, there was no disclosure of how CHO cells were cultured to produce glycosylated erythropoietin in examples 1 to 4. Hence, the skilled person could not plausibly assign the effect observed on erythropoietin expression in HeLa S3 cells to erythropoietin expression in CHO cells under the same culture conditions, as major differences in the host cells' ability to glycosylate recombinant proteins were known to exist.

Document D5 described the altered expression of sialylated carbohydrate antigens, such as sialyl Lewis A, sialyl Lewis C and Tn/sialyl Tn, in a carcinoma cell line (HT29) that was progressively transferred from glucose containing medium to glucose-free, galactose containing medium (see D5, page 1156, 1st paragraph). The sialyl transferase activities, both the α-2,6 and α-2,3 sialyltransferases, in HT29 cells grown in glucose-free medium containing galactose were higher than in HT29 cells grown in glucose containing media (see page 1162, Table 1). The process of progressively transferring cells to a glucose-free, galactose containing medium could not be considered as “feeding” which, as defined in claim 1, resulted in an increase in culture volume of approximately 30-60 % of the original culture volume (see [0048] and [0052] of the patent specification), even if this document used the word “fed” (see D5, page 1156, left-hand column, 1st paragraph).

The progressive transfer of the cell culture induced an enterocyte-like differentiation in colonic carcinoma cells associated with alterations in growth rate, morphological appearance, disaccharidase activity and mucin expression, which led to an alteration of the surface and secreted carbohydrate antigens (page 1160, left-hand column, 1st paragraph under "Discussion") and suggested that the absence of glucose and not the presence of galactose caused these cells to differentiate.
Thus, document D5 did not suggest to feed galactose to alter the expression of carbohydrate antigens in CHO cells. It described that the sialytransferases responsible for the synthesis of the sialylated epitopes were increased in HT29 cells, while in CaCo-2 cells, said activity was induced only after prolonged culturing (page 1161, right hand column, second full paragraph). The reaction of two human colon adenocarcinoma cell lines producing glycoproteins to a modification of the carbohydrate in the growth medium was not identical. This did not render plausible that a rodent CHO cell line producing glycoproteins would react in a predetermined manner, even more when this cell line was known to lack a functional α-2,6 sialyltransferase which was however present in the two human colon adenocarcinoma cell lines.

Document D7 described essentially serum-free media for CHO cells and disclosed the use of 1,000-10,000 mg/L monosaccharide as energy source. Galactose was one of many monosaccharides identified as useful energy sources. There was no indication how to increase a protein's sialylation and no disclosure of adding a feed medium comprising D-galactose to a cell culture. A skilled person had therefore no reason to combine document D7 with D4.

Documents D10 and D11 described the simultaneous substitution of glucose and glutamine to avoid accumulation of lactate and ammonium in CHO cell culture media. This effect was attributed to the use of glutamate, as the use of galactose increased the ammonium concentrations above that measured for glucose (see D10, page 74, Fig. 3f; D11, page 97, lines 2-5). An increase of ammonium from 1 to 15 mM was described to be concomitant to a decrease in the TNFR-IgG terminal galactosylation and sialylation (see D12 abstract, lines 14-16).

In view of documents D10 to D12, the skilled person aiming at producing highly sialylated protein, e.g. CTLA4, would not have used galactose in the medium.

Apportionment of costs (Article 104 EPC)

The appellant's letter indicating its intention not to attend the oral proceedings was ambiguous and left the respondent in the dark as to whether it would attend oral proceedings or not. A withdrawal of the request for oral proceedings would have been a clear signal of its true intentions. In view of this uncertainty, the respondent had to fully prepare the case. The apportionment of 50% of the costs incurred by the respondent for the preparation to the oral proceedings was therefore considered legitimate.

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

XIV. The respondent requested that the appeal be dismissed.

Reasons for the Decision

Article 113(1) EPC

1. The duly summoned appellant did not attend the oral proceedings.

1.1 By its decision not to attend the oral proceedings and not to file substantive arguments in reply to the issues raised in the board's communication pursuant to Article 15(1) RPBA, the appellant has chosen not to make use of the opportunity to comment on the board's provisional opinion, either in writing or at the oral proceedings, although this opinion was partially to the appellant's disadvantage. According to Rule 115 (2) EPC and Article 15(3) RPBA, the board is not obliged to delay any step in the proceedings, including its decision, by reason only of the absence at the oral proceedings of any party duly summoned who may then be treated as relying on its written case.

Main request (claims 1-9)
Admission of the main request (Article 12(4) RPBA)

2. The main request is identical to both the auxiliary request 1 of the decision under appeal and the main request filed with the respondent's reply to the statement of grounds of appeal, with the exception that dependent claims 3-5, 7, 8, 11, 13-16, 18-21, 23-26 were deleted and the dependencies renumbered.

3. In its communication in preparation of the oral proceedings, the board, contrary to the opposition division, took the view that some dependent claims of the main request extended to subject matter beyond the content of the patent application. In response to the

board's communication, the respondent submitted the current main request. Since the deletion of several dependent claims neither creates a fresh case nor affects procedural economy, the board, availing itself of its discretionary power, decided to admit the new main request into the appeal proceedings.

Amendments 123(2) EPC

4. Appellant argued that the subject matter of claims 5, 6 and 9 of the main request extended beyond the content of the patent application, because the claims referred back to several claims, while the corresponding claims of the patent application referred to a single claim only.

4.1 Claim 5 referring back to claim 4 defined a large scale cell culture in which D-galactose is sustained at a concentration of 0.1 to 10 g/L. This subject matter was not disclosed in the patent application, where the scale of the cell culture and the residual amounts of galactose were listed in different paragraphs (page 19, last paragraph to page 20, 2nd paragraph).

Claim 6 referring back to claim 4 defined a large scale cell culture of 500L and greater in which D-galactose is sustained at a concentration of 0.1 to 10 g/L for which there was neither a basis in the original set of claims nor in the patent application.

Claim 9 referred to the purification of the soluble CTLA4 molecule produced by claims 1 to 8. Any reference to a claim infringing Article 123(2) EPC, resulted in claim 9 also infringing Article 123(2) EPC.

4.2 In the board's view both claims 5 and 6 are derivable from the last sentence of the first full paragraph on page 19 or the sentence bridging pages 19 and 20, which states that "the methods of the invention are suitable for all reactor scales at which protein production occur, including, but not limited to large and small scale", when read in combination with both the last sentence of the first full paragraph on page 20 (lines 14-17) and page 20, lines 6-9, and lines 14-21 of the patent application, which disclose that

- "*[i]n accordance with the methods of this invention, the galactose concentration in the feeding medium is preferably provided in an amount which affords a sustained or maintained level of D-galactose in the culture, or reactor, during the culturing process*"

and that

- "*it is preferred that the residual galactose concentration in the culture medium used for culturing cells (e.g., in a reactor or culturing vessel) is maintained and sustained throughout the culturing run in an amount of about 0.1-10 g/L [...]. These residual concentrations of galactose in the culture medium apply whether the galactose is fed via a feeding medium or in some other way.*"

The addition of D-galactose to large scale cultures, in particular "500 L and greater", is disclosed on page 18 line 30 to page 19 line 1; page 18, line 16 and page 20, line 2.

4.3 The subject-matters of both, claims 5 and 6, do not present the skilled person with information not directly and unambiguously derivable from the patent application as originally filed.

5. Since claim 9 does not refer, via its back references, to subject-matter extending beyond the disclosure of the patent application, it complies with Article 123(2)EPC.

6. Hence, the main request does not contravene Article 123(2) EPC.

Sufficiency of disclosure (Article 83 EPC)

7. The appellant submitted in essence that the invention defined in claim 1 covered too many variables: the amounts of galactose, the time points of addition of galactose, the size of the reactor, time points of the addition of polyanionic compounds, time points of harvest, time points of temperature shifts, rendering the limited number of experiments in the patent insufficient to determine which of the combinations actually led to enhanced sialylation. Moroever, Figure 3 of the patent showed that no galactose was detectable in the culture medium when a concentration of 3 g/L galactose was used in the feeding medium, although an enhanced sialylation was observed. The addition of galactose to enhance sialylation was therefore questionable.

7.1 The board notes that a person skilled in the art, reading the patent and using its common general knowledge, is able to perform a cell culture process comprising steps (a) and (b) recited in claim 1 for producing a soluble CTLA4 molecule without undue

burden. The patent provides more than one example, with different concentrations of galactose in the feeding medium (paragraphs [0042], [0046] and example 2 of the patent).

7.2 There are no facts on file to substantiate appellant's argument that establishing the necessary parameters when modifying the procedure described in the examples indeed requires an undue amount of work.

In the absence of verifiable facts, the objection of insufficiency of disclosure on the basis that the claim encompasses too many undetermined parameters must fail.

7.3 Appellant's doubts that the addition of galactose may not enhance sialylation, as shown in Figure 3, are not persuasive. Although an enhanced sialylation is reported when both 3.0 g/l galactose and no galactose were added to the culture medium in Table 3 (i.e. 7.8 SA (NANA) and 6.1 SA (NANA) respectively), Figure 3 shows that when a feed with a concentration of 3 g/L of D-galactose is used, no residual D-galactose concentration is measured in the culture medium of the CHO cells. There is no contradiction between these results. First, Figure 3 does not describe any degree of sialylation but the concentration of residual D-galactose in the culture medium. Second, an undetectable concentration of residual D-galactose in the CHO cell culture medium may be explained by its entire consumption. Third, Table 3 reports that the addition of 3.0 g/L of D-galactose in the feed results in a higher sialylation on CTLA4 than when no D-galactose is used, irrespective of the fate of the culture medium's residual D-galactose concentration.

7.4 The board is therefore not convinced by appellant's arguments and considers the requirements of Article 83 EPC to be met.

Novelty (Article 54(3) EPC)

8. The cell culture process of claim 1 is for the production of a soluble CTLA4 molecule.

9. The appellant submitted that document D1 anticipated the method of claim 1.

10. Document D1, prior art according to Article 54(3) EPC, discloses methods and media for culturing mammalian cells and controlling the sialic acid content of proteins, optionally secreted proteins, produced by these cells (see page 1, last paragraph). Galactose is present in the medium and CHO cells are particularly preferred cells (see claims 1 and 9; page 14, 3rd paragraph). Proteins that can be produced include proteins comprising all or part of the amino acid sequences of differentiation antigens known as CD proteins or their ligands (page 11, lines 24-27). Chimeric proteins that recognize any of a long list of proteins disclosed on pages 12 and 13, including ligands to CD80 and CTLA4 can also be produced (page 12, line 28; page 13, line 15).

10.1 It is undisputed that soluble CTLA4 binds to CD80. However, numerous proteins besides CTLA4, e.g. CD28 or any anti-CD80 antibody are capable of binding CD80. Thus, the generic disclosure of a method for the production of chimeric proteins binding to CD80 in document D1 does not amount to the direct and unambiguous disclosure of a method for producing CTLA4, let alone of soluble CTLA4 as a CD80 ligand.

10.2 For this reason alone, the subject-matter of claim 1 is novel (Article 54(3) EPC). The same conclusion applies to claims dependent thereon.

Inventive step (Article 56 EPC)

11. It is uncontested that document D4 represents the closest prior art for the cell culture process according to claim 1.

11.1 Document D4 discloses various batches of human CTLA4Ig (CTLA4 fused to IgG domains) produced in CHO cells having various clearance profiles. Batches with accelerated clearance (PBR12, PBR13 and VERI-asialo) had a lower content of Neu5Ac than those with a slower clearance rate (e.g. PBR10 or VERI) (see Figs. 3 and 4; section "High-Resolution Polyacrylamide Gel Electrophoresis of Oligosaccharides" on pages 403 and 404). The clearance rate of murine CTLA4Ig was stated to be highly dependent on the cell line used for producing it, e.g. from CHO cells it cleared more slowly than from NS-0 cells (see Fig. 8). A sialic acid type analysis revealed that the CTLA4Ig from CHO cells had NeuAc5, whereas the CTLA4Ig from NS-0 cells did not release Neu5Ac. It was extrapolated that a reduced amount of Neu5Ac was associated with increased clearance rates and increased amounts of terminal galactose or possibly N-acetyl-galactosamine recognized by asialoglycoprotein receptors found on hepatocytes (see "Characterization of Murine CTLA4Ig" on pages 404 and 405 and page 406, lines 21-25).

11.2 The process of claim 1 differs from the method of document D4 in that it uses a step of feeding the cells with D-galactose resulting in different degrees of sialylation and clearance rates. In the context of the

culture process of claim 1, the step of feeding refers to supplementing the culture medium during the production phase according to any schedule to support continued protein production (see [0158] of the patent).

According to paragraphs [0171] and [0178] of the patent, the term soluble CTLA4 molecule refers to a non-cell-surface bound molecule comprising wild type CTLA4 or any portion that binds B7, a portion defined as preferably the extracellular domain of CTLA4 or any part or segment thereof that recognizes and binds to or interferes with a B7 so that it blocks binding to CD28 CTLA4. This definition does not exclude soluble CTLA4 fragments without a sialylation site.

11.3 As a consequence, the problem to be solved is defined as the provision of an alternative method of producing a soluble CTLA4 - excluding any detrimental effect. The proposed solution is the process of claim 1, comprising the step of feeding the cells with D-galactose.

11.4 The examples of the patent specification show that the process of claim 1 indeed solves this problem.

11.5 It remains to be established whether the claimed solution involves an inventive step.

11.5.1 An inventive step is independent of whether the skilled person could have altered features of the method described in the closest prior art document to arrive at the claimed invention, but depends upon whether the skilled person "would have done so" or in other words had a motivation to do so. To determine whether such a motivation exists, it is necessary to "identify conclusive reasons on the basis of tangible evidence

that would have prompted the skilled person to act in one way or another" (see decision T 1014/07 reason 8).

11.6 Document D4 neither pointed at nor suggested the feeding of CHO cells with D-galactose. Nor does it disclose D-galactose as a component of any medium.

11.7 In document D5 it was determined whether altered *in vitro* culture conditions caused altered expression of sialylated carbohydrate antigens on the surface of HT29 cells or on secreted mucins (see abstract). A gradual transfer of HT29 colonic carcinoma cells from a conventional glucose-containing to a glucose-free galactose containing medium and its effect on the cell's phenotype and the expression of surface and secreted sialylated carbohydrates was analysed (see for instance page 1160, left column). Switching the cells from a glucose- to a galactose-containing medium increased the activities of the GalNAc-peptide: α-2,6 sialyltransferase and the Galß1,3GlcNAc: α-2,3 sialyltransferase. It was also described that human colonic adenocarcinoma CaCo-2 cells spontaneously developed an enterocyte-like differentiation upon prolonged culturing in glucose containing medium and that only the activity of the α-2,6 sialyltransferase, but not the corresponding α-2,3 sialyltransferase increased (see page 1161, col.2 second full paragraph).

11.8 The skilled person could thus derive from document D5 that only HT29 cells require a transfer from a glucose-containing to a galactose-containing medium to increase sialyltransferase activities whereas the induction of these activities in CaCo-2 cells depends on the cell's maturation by prolonged incubation without any transfer to galactose containing medium.

11.9 Since document D5 is not concerned with the production of heterologous proteins, the board fails to see why, unless with hindsight knowledge of the claimed invention, the skilled person confronted with the afore mentioned technical problem would have turned its attention to this document in the first place.
Even if the skilled person turned to document D5, he could not determine whether the induction of sialyltransferase activities in the cultured HT29 cells was caused by the withdrawal of glucose or the addition of galactose in the culture medium, whether all or only some of the sialyltransferase activities were inducible in cells other than HT29 and CaCo-2 cells by culture medium transfer and finally whether proteins other than mucins would be sialylated.
Since the sialyltransferases in the two related human adenocarcinoma cell lines were induced differently, the skilled person could not deduce whether this effect could be obtained for a different protein, such as soluble CTLA4 protein, in another cell line, such as CHO cells, by feeding cells with galactose.

11.10 Document D7 disclosed essentially serum-free media for CHO cells and the use of 1,000-10,000 mg/L monosaccharide as energy source. Even if galactose was one example from among many monosaccharides, it does not disclose the addition of a feed medium comprising D-galactose to a cell culture.

11.11 Documents D10 and D11 described that the accumulation of lactate and ammonium in the CHO cell culture medium was avoided by the simultaneous substitution of glucose and glutamine. This effect was attributed to the use of glutamate, while the use of galactose increased the

ammonium concentrations above that measured for glucose (see D10, page 74, Fig. 3f; D11, page 97, lines 2-5).

11.12 It follows that a skilled person had no reason to turn to any of documents D5, D7, D10 or D11 and to combine their teaching with that of document D4. It would therefore not have arrived at the solution proposed in claim 1 in an obvious way.

Apportionment of costs (Article 104(1) EPC)

12. The respondent, referring to decision T 301/12 of 30 May 2018, requested an apportionment of 50% of its costs for preparing the case. It argued that appellant's letter informing the board that it did no longer intend to be present at the oral proceedings was ambiguous, leaving the respondent in the dark with regard to appellant's true intentions. Therefore it had to prepare for defending its positions also with regard to issues which the board, according to its preliminary communication, was likely to decide in its favour.

13. In the present case, both parties to the appeal proceedings had requested oral proceedings in the event the board did not follow their requests. Appellant's letter dated 14 May 2019, informing the board that it no longer intended to be present at the oral proceedings, was filed one and a half months before the scheduled oral proceedings. Even though this formulation is not unequivocal, it leaves little room for uncertainty and cannot be considered as an irresponsible or malicious intention that could justify, for reasons of equity, an apportionment of costs in favour of the respondent.

13.1 Before the oral proceedings, the respondent presented new sets of claims, i.e. a new main and new auxiliary requests, taking into account the board's preliminary and provisional opinion as expressed in its communication pursuant to Article 15(1) RPBA. Since the admission of such late filed requests is at the board's discretion, it was clear that the respondent intended to defend orally the newly filed requests.

13.2 Thus in the present circumstances, the respondent had to prepare for its case in full, irrespective of whether the appellant intended to attend the scheduled oral proceedings or not.

13.3 The request for an apportionment of costs is therefore rejected.

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 as amended in the following version:
Description:
Pages 3 - 42 of the patent specification.
Claims:
1 to 9 of the main request filed under cover of a letter dated 10 May 2019.
Drawings, Figures:
1 -19 of the patent specification.

3. The request for apportionment of costs is refused.

The Registrar: The Chairman:

T. Buschek B. Stolz

Decision electronically authenticated