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Datasheet for the decision of 6 July 2021

Case Number: T 0148/18 - 3.3.08

Application Number: 08731084.3

Publication Number: 2115126

IPC: C12N5/00, C07K14/435, C12P21/02

Language of the proceedings: EN

Title of invention:
USE OF COPPER AND GLUTAMATE IN CELL CULTURE FOR PRODUCTION OF POLYPEPTIDES

Patent Proprietor:
Wyeth LLC

Opponent:
Rogers, Alex Lee

Headword:
Mammalian cell culture medium for TNFR-Ig production/WYETH

Relevant legal provisions:
EPC Art. 100(a), 100(b), 56, 83

Keyword:
Main request (claims as granted) - sufficiency of disclosure (yes); inventive step (yes)

Decisions cited:
G 0001/03, T 0708/05, T 1186/05, T 0871/08

Catchword:

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Case Number: T 0148/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 6 July 2021

Appellant: (Opponent)	Rogers, Alex Lee Haseltine Lake Kempner LLP Redcliff Quay 120 Redcliff Street Bristol BS1 6HU (GB)
Representative:	Dr. Werner-Jones, Leonard Hoffmann Eitle Patent- und Rechtsanwälte PartmbB Arabellastraße 30 81925 München (DE)
Respondent: (Patent Proprietor)	Wyeth LLC 235 East 42nd Street New York, NY 10017-5755 (US)
Representative:	Mr. Pringot, Thomas Dr. Markus, Marc Pfizer European Patent Department 23-25 avenue du Docteur Lannelongue 75668 Paris Cedex 14 (FR)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 27 October 2017 rejecting the opposition filed against European patent No. 2115126 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	P. Julià
	R. Winkelhofer

Summary of Facts and Submissions

I. European patent no. 2 115 126 is based on European patent application no. 08 731 084.3, originally filed under the PCT and published as WO 2008/109410 (hereinafter "the patent application"). The patent was granted with 29 claims.

II. Independent claim 1 reads as follows:

"1. A method of producing a polypeptide in a cell culture comprising steps of:

culturing mammalian cells that contain a gene encoding a polypeptide of interest in a cell culture medium comprising between 0.5 and 5 µM copper and between 1.7 and 33 mM glutamate;
maintaining the culture at a first temperature range for a first period of time sufficient to allow the cells to reproduce to a viable cell density within a range of 20%-80% of the maximal possible viable cell density if the culture were maintained at the first temperature range;
shifting the culture to a second temperature range, wherein at least one temperature of the second temperature range is lower than the lowest temperature of the first temperature range;
maintaining the culture for a second period of time under conditions and for a time sufficient to permit expression of the polypeptide, wherein the fraction of misfolded and/or aggregated polypeptide, relative to the total polypeptide produced, is decreased compared to the fraction of misfolded and/or aggregated polypeptide that would be observed under otherwise

identical conditions in an otherwise identical medium that lacks copper and glutamate;
wherein the polypeptide is TNFR-Ig."

Independent claim 18 reads as claim 1 except for the maintenance of the culture for the second period of time which reads as follows:

"18. A method of producing a polypeptide in a cell culture comprising steps of:

... [as in claim 1] ...

maintaining the culture for a second period of time under conditions and for a time sufficient to permit expression of the polypeptide, wherein the glycosylation pattern of the expressed polypeptide is increased relative to the glycosylation pattern that would be observed on the expressed polypeptide under otherwise identical conditions in an otherwise identical medium that lacks copper and glutamate;

wherein the polypeptide is TNFR-Ig."

III. An opposition was filed on the grounds set forth in Articles 100(a) and 100(b) EPC. A new ground of opposition under Article 100(c) EPC, raised after the filing of the notice of opposition, was not admitted into the proceedings by the opposition division. The main request (claims as granted) was considered to fulfil the requirements of the EPC and thus, the opposition division rejected the opposition (Article 101(2) EPC).

IV. An appeal was lodged by the opponent (appellant). In the statement setting out the grounds of appeal, the

appellant maintained the objections raised under Articles 100(a), 100(b) and 100(c) EPC against the granted claims and filed new evidence (document (15)).

V. In reply thereto, the patent proprietor (respondent) filed new evidence (document (16)), Annexes 1 to 3 (copies of the submissions made at the first instance and an updated list of the documents cited in these proceedings), and auxiliary requests 1 to 3. The respondent requested neither to admit appellant's new evidence nor the ground for opposition under Article 100(c) EPC into the appeal proceedings.

VI. With further submissions, the appellant filed additional evidence (documents (17) to (22)).

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

VIII. The board summoned the parties to oral proceeding. In a communication pursuant to Article 17 of the Rules of Procedure of the Boards of Appeal (RPBA 2020), they were informed of the board's provisional opinion on the issues of the appeal.

IX. Oral proceedings were held on 6 July 2021. At these proceedings, the appellant withdrew the request to introduce Article 100(c) EPC as a further ground for opposition, and to introduce documents (15) and (17) to (22) into the proceedings. The appellant did not raise any objection to consider document (16) but requested that auxiliary request 3 not to be admitted into the proceedings.

X. The following documents are cited in this decision:

(1): WO 2006/026447 (publication date: 9 March 2006);

(2): US 6,048,728 (publication date: 11 April 2000);

(3): C.T. Ling *et al.*, Experimental Cell Research, 1968, Vol. 52, pages 469 to 489;

(4): C. Altamirano *et al.*, Biotechnol. Prog., 2000, Vol. 16, pages 69 to 75;

(5): M. Gawlitzek *et al.*, Biotechnology and Bioengineering, 20 June 2000, Vol. 68, pages 637 to 646;

(7): US 7,294,481 (publication date: 13 November 2007);

(8): US 2003/0087372 (publication date: 8 May 2003);

(16): "Biochromatography. Theory and practice", edited by M.A. Vijayalakshmi, 2002, Taylor & Francis, London and New York, Chapter 4, pages 46 and 47.

XI. The arguments of the appellant, insofar as relevant to the present decision, may be summarised as follows:

Main request (Claims as granted)
Article 100(b) EPC (Article 83 EPC)

According to the case law, the structural features present in the claims had to provide the effects or functional requirements cited in the claims. However, in the present case, the technical effects mentioned in the claims were not supported by the patent; not even by the examples of the patent since they had a large number of gaps or incomplete teachings that required the skilled person to carry out further research for

arriving at the alleged effects and claimed subject-matter. In particular, there was no information on the actual mammalian cells used in the examples. Although the patent provided a list of cell lines, it was known in the art that there was considerable variability between these cells and no criteria and/or guidance were indicated in the patent for a skilled person to select a suitable cell line. Likewise, the specific culture medium used was not provided in the examples since none of the media disclosed in Table 1 of the patent met the requirements of the claims. The feature related to the percentage of maximal viable cell density cited in the claims was not addressed in the examples; indeed, it was not even possible to know whether this feature was achieved since the information provided in the examples did not allow for its calculation. If this feature was dependent on the (appropriate) starting or seeding density of the cell culture, the patent did not provide any guidance thereon; the information provided on viable cell density was only after the temperature shift had been carried out (Figures 6 and 11). Thus, undue burden was required for a skilled person to arrive at the claimed subject-matter over the entire scope of protection. Three specific issues were also relevant, namely (i) the control medium, (ii) the method for measuring the fraction of TNFR-Ig misfolded and/or aggregated, and (iii) the increased TNFR-Ig glycosylation pattern:

(i) a skilled person was not in a position to arrive at a control medium as defined in claims 1 and 18. According to the claims, the mammalian cells in the control culture were cultured under conditions identical to those of a non-control culture and in an otherwise identical medium that lacked copper and glutamate. There was no limitation on the type of

culture which could be a batch culture (claim 28), a fed-batch culture or a continuous, (feeding) perfusion culture (paragraphs [0014], [0047], [0065], [0157] and [0160] of the patent), in all of them the control medium had to lack copper and glutamate. Thus, this requirement could not be understood as being limited only to the beginning of the culture but had to be present during/throughout the whole culture. However, Figures 3, 8 and 13 of the patent showed that none of the controls used in the perfusion cultures described in Example 2 of the patent lacked glutamate (see also paragraph [0165]); for all of them, the glutamate concentration at the beginning of the culture was about 0.3 mM and, during the culture, it was within the concentration range defined in claims 1 and 18 for the other non-control culture. Indeed, all cell cultures described in Example 2 followed standard perfusion methods (with multiple feeds on 3, 6, 8 and 10 days; Tables 3, 4 and 5), and all feeding media (Table 2) contained glutamic acid which contributed to the presence of glutamate in the medium (paragraph [0160]). Thus, the presence of glutamate in the controls was inevitable. There was no guidance in the patent for the skilled person to arrive at a control medium lacking glutamate during/throughout the whole culture as required by the methods of claims 1 and 18.

(ii) there was no disclosure in the patent of any method for measuring the fraction of TNFR-Ig misfolded and/or aggregated, nor whether TNFR-Ig misfolding and aggregation could be measured by different methods. Figure 5, 10 and 15 referred to "% HIC Peak 3" but with no further guidance. Although hydrophobic interaction chromatography (HIC) was known in the art, the patent disclosed neither how to use HIC for TNFR-Ig (salt and salt concentration, hydrophobic resin, etc.) nor the

meaning and composition of "Peak 3". Different HIC conditions (wash/elution, salt, resin) could provide different results (elution patterns, peaks), and there was neither a standard HIC protocol for TNFR-Ig in the art nor was any disclosed in the patent. Document (7), published after the claimed priority date, reported the discovery of misfolded TNFR-Ig (column 3, last sentence of first paragraph) and the development of a HIC protocol for measuring misfolded TNFR-Ig. "HIC Peak 3" was shown to be non homogeneous, consisting of multiple species, and resolved into multiple HIC peaks (column 22, lines 24-28; Figure 5). Thus, document (7) showed that a research program was needed for a skilled person to arrive at a meaningful technical understanding of the abbreviation "% HIC Peak 3" and that this abbreviation was not even correct because "HIC Peak 3" consisted of multiple peaks. Thus, the patent provided neither a standard HIC protocol for TNFR-Ig, nor a meaningful information on "Peak 3", and this information was not derivable from the prior art.

(iii) Figure 18 showed the TNFR-Ig sialylation for the cell cultures described in experiment 2 of Example 2 (Table 4). The control culture had higher TNFR-Ig sialylation than any of the other cultures at 8 and 10 days; no results were provided for 12 days and no reasons were given in the patent for not providing them. Thus, nothing could be said on whether TNFR-Ig sialylation in these other cultures on that day 12 would have been better or worse than in the control culture. It was a mere speculation to rely on the decrease (in the controls) and increase (in the other cultures) of TNFR-Ig sialylation between 8 and 10 days and extrapolate therefrom what would have happened at 12 days. Indeed, Figure 19 showed that, in experiment 3 of Example 2 (Table 5), there was no correlation

between 8, 10 and 12 days and the increase of TNFR-Ig sialylation; the level of TNFR-Ig sialylation at 8 and/or 10 days for some cultures with copper and glutamate was higher than at 12 days. Moreover, contrary to the results of experiment 2, TNFR-Ig sialylation in the control culture of experiment 3 was lower than in the other cultures. There was no reason to rely on the results obtained in experiment 3 and not on those of experiment 2; the latter showing that not all culture conditions resulted in increased TNFR-Ig sialylation. Thus, the alleged technical effect was not achieved across the whole scope of the claims and the patent did not provide the skilled person with a complete guidance for the claimed methods (selection of cell line, culture medium, etc.), nor any guarantee of success. The failure in one of the only two experiments disclosed in the patent could not be considered an occasional failure and the patent failed to provide adequate information towards success and so overcome this failure. Thus, undue burden was required for a skilled person to find out under which conditions the alleged technical effect could be achieved.

Article 100(a) EPC (Article 56 EPC)

The closest prior art document (1) disclosed a method of producing TNFR-Ig identical to the methods of claims 1 and 18 (paragraphs [0008], [00123], [00167], [00168] and claims). Example 16 disclosed the use of Medium 9 for TNFR-Ig production and Table 14 showed the components of this medium. The concentration of copper was 464 nM which, when the normal rounding for comparing nM and µM values was taken into account (Guidelines for Examination, G-VI, 8.1 "Error margins in numerical values"; T 871/08 of 8 December 2011), corresponded to 0.5 µM and thus, within the range of

copper concentration indicated in claims 1 and 18. The sole technical difference between the method disclosed in document (1) and those of claims 1 and 18 was the concentration of glutamate in the medium. Since the technical effects cited in claims 1 and 18, if present (see Article 83 EPC), could not be attributed to this difference, the objective technical problem was the provision of an alternative method of culture, and in particular an alternative culture medium, for TNFR-Ig production. The solution proposed by the patent, the methods of claims 1 and 18 with a culture medium having a glutamate concentration within the range indicated in these claims, was obvious to the skilled person when considering the combination of document (1) with any of documents (2) to (4) or (8). Although these documents were not related to TNFR-Ig production, the skilled person when looking for alternative methods, in particular an alternative culture medium, would have consulted prior art relating to the production of other proteins, in particular those mentioned in document (1) and those related to TNFR-Ig, such as mAbs.

The purpose of the method disclosed in document (1) was not only to have a high TNFR-Ig titer but also TNFR-Ig of good quality, which was known to be affected by the conditions of the cell culture, in particular the levels of waste products, such as ammonium and lactate (paragraphs [0003] and [0006]). These products were known to have a direct effect on TNFR-Ig folding and glycosylation, i.e. the quality of the TNFR-Ig (paragraph [00102]). Thus, the levels of these waste products were minimised in the methods disclosed in document (1), resulting in a delicate balance between the amount and the quality of TNFR-Ig produced (paragraph [00110]). These methods of culture relied on culture media having one, some or all of five essential

features defined in paragraphs [00150] to [00156] of document (1), preferably all five features as in the claimed methods and exemplified by Medium 9 used in Example 16. Document (1) explicitly indicated that, using culture media having these essential features, the methods of culture could be further optimised (paragraph [00157]). Thus, the skilled person would have been motivated to modify the exemplified Medium 9, whilst retaining/maintaining the five essential features disclosed in document (1), in order to optimise the TNFR-Ig production disclosed in Example 16. Example 16 referred to the levels of lactate and ammonium (paragraphs [00303] and [00304]) and Figures 71 and 72 showed intermediate ammonium levels in (high-seed) cultures producing high TNFR-Ig amounts. Thus, there was room for the skilled person to further diminish the ammonium levels and improve the quality of TNFR-Ig.

The role of glutamine in ammonium production and the substitution of glutamine by glutamate in the culture medium was known in the art and disclosed also in document (1), such as in Examples 5 and 6. Document (8) referred also to the relevance of glutamine and glutamate in the medium and the presence of the waste by-product ammonium (paragraph [0131]). Document (8) disclosed a method of production of high titer/amounts of proteins in mammalian cell cultures using culture conditions similar to those disclosed in document (1), such as a high glucose concentration (paragraphs [0017] and [0057]) present also in Medium 9 of Example 16 of document (1) (15 g/L D-glucose). This was one of the four discoveries disclosed in document (8) which, according to this document, led to the advantages of the disclosed method (paragraphs [0124] to [0127]). A further discovery was an increased glutamate/glutamine

ratio (paragraph [0125]), wherein a low level of glutamine (0 to 15 mM) - substituted by glutamate (0.5 to 15 mM) - was preferred (paragraphs [0131] to [0133]). Thus, it would have been obvious for a skilled person to modify the composition of Medium 9 (decrease glutamine and/or increase glutamate) following the teachings of document (8) so as to achieve the advantageous glutamate/glutamine disclosed in document (8) in the modified Medium 9 (the preferred glutamate concentration according to paragraph [0132] of document (8) fell within the glutamate range of claims 1 and 18), whilst maintaining the advantageous five essential features disclosed in document (1). In doing so, the skilled person would have arrived at the methods of claims 1 and 18 in an obvious manner.

Although several feeds were performed in the perfusion cultures exemplified in the patent, no advantageous effect could be derived therefrom since no comparison was carried out and none was on file for claiming an improvement based on the manner in which the glutamate was added into the culture. Furthermore, although document (8) referred to the addition of glutamate during culturing (*inter alia*, paragraphs [0020], [0036], and [0132]), the addition of glutamate at the beginning of the culture was not excluded.

XII. The arguments of the respondent, insofar as relevant to the present decision, may be summarised as follows:

Main request (Claims as granted)

Article 100(b) EPC (Article 83 EPC)

The disclosure of the patent was sufficient for the skilled person to carry out the methods of claims 1 and

18 without undue burden. The features in these claims represented actions that a skilled person working in the field of protein production would have routinely performed, they could have been done by simple routine experimentation without involving undue burden. The examples of the patent supported the effects mentioned in the claims and were useful for the skilled person to put the invention into practice.

(i) a skilled person would not have understood claims 1 and 18 to require the control culture to lack copper and glutamate during/throughout the whole culture but only that the medium used for said control lacked copper and glutamate, as stated in paragraph [0031] and shown in the medium formulations of Example 1 (Table 1) of the patent, regardless of the type of culture used (perfusion, batch, etc.; paragraphs [0054] and [0055] of the patent). Since the mammalian cells were cultured and grown, the level of glutamate could not have been expected to remain constant during/throughout the whole process. The methods of claims 1 and 18 required the control culture to be performed under conditions identical to those of a cell culture in a medium with copper and glutamate concentrations within the ranges defined in these claims. This was also in line with the appellant's interpretation in the notice of opposition (page 3, point 4.1.1). No undue burden was required from a skilled person to prepare the (control) media disclosed in the patent and use them as known in the art and disclosed in the patent.

(ii) according to the case law, the skilled person in biotechnology was a team of specialists in the relevant field. For the production of a pharmaceutical product such as TNFR-Ig, the team had a specialist on product quality familiar with analytical (chromatographic)

methods used for assessing the quality of the product, such as the presence of misfolded and/or aggregated product. Figures 5, 10 and 15 of the patent referred to HIC for measuring the level of TNFR-Ig misfolded and/or aggregated. HIC was a standard method known in the art as shown by document (16). No undue burden was required from a skilled person to set up a protocol and carry out a HIC for identifying/isolating TNFR-Ig misfolded and/or aggregated, such as the Peak 3 indicated in these Figures. Indeed, as shown in document (7), the HIC results for TNFR-Ig were independent of the salt and HIC resin used. Moreover, claims 1 and 18 were not limited by any specific method and other alternative methods, such as size exclusion chromatography, were available to the skilled person.

(iii) Examples 1, 2 and 3 of the patent provided different information. The findings of Example 1 were used to designing the assays of Example 2. Likewise, those of Example 2 were used to designing the assays and conditions used in Example 3. These examples were progressive, not independent. Example 2 showed that, whilst the levels of sialylation decreased in the control culture, sialylation increased in the cultures with a medium containing copper and glutamate within the ranges indicated in claims 1 and 18 (Tables 4 and 6, Figure 18). In this sense, the results shown in Example 2 were not a failure (sialylation was greater in the control culture than in the other cultures) but provided a teaching/guidance for a skilled person to achieve the desired technical effect shown in Example 3 (Table 5 and 6, Figure 19), wherein the levels of sialylation of the control culture were always lower than in the other cultures with copper and glutamate in the medium. There was no evidence on file showing that the results in Example 2, if measured at 12 day of

culture, would not have been as those reported in Example 3 and in line with the decrease/increase levels of sialylation already shown in Example 2. The methods of claims 1 and 18 were not limited to any time/period of culture. The situations underlying the case law mentioned by the appellant were different from, and did not apply to, the present case.

Article 100(a) EPC (Article 56 EPC)

The difference between the methods of claims 1 and 18 and the method disclosed in the closest prior art document (1), in particular the production of TNFR-Ig using Medium 9 (page 67, Table 14, and Example 16), was the concentration of copper and glutamate. The copper concentration of 464 nM in Medium 9 was different from 0.5 µM, the lower-end of the range for copper indicated in claims 1 and 18, and there was neither a reason nor an indication in document (1) to increase the copper concentration. The technical effects of this difference were those cited in claims 1 and 18. Therefore, the objective technical problem was the provision of an improved method for TNFR-Ig production, wherein TNFR-Ig misfolding/aggregation and glycosylation/sialylation were decreased and increased, respectively. As shown by the examples of the patent (summary in Table 6), this problem was solved by the methods of claims 1 and 18. These methods were not obvious; there was no indication in document (1) to replace glutamine for glutamate in Medium 9, nor a suggestion of any effect of glutamate and copper on TNFR-Ig folding/aggregation and/or glycosylation. A skilled person would not have reviewed documents not related to the production of TNFR-Ig and thus, the combination of document (1) with any of documents (2) to (4) or (8) was not obvious because none of them related to TNFR-Ig production.

The Medium 9 used in Example 16 was a medium optimised by, and resulting from, a series of experiments disclosed in Examples 2, 5 and 6 of document (1). Thus, a skilled person would have been reluctant to modify the concentration of glutamine and glutamate of this medium in absence of any reason for doing so. The less so, since glutamine and glutamate levels had already been optimised to have low levels of the waste product ammonium. Studies on the levels of glutamine required in the culture medium and on the substitution of glutamine by glutamate (with a medium containing no glutamine) were reported in Examples 3, 5 and 6 of document (1). Indeed, the concentration of glutamine in Medium 9 was already very low (4 mM), within the preferred levels (0.5 to 5 mM) disclosed in paragraph [0131] and in line with the levels reported in paragraphs [0020] and [0171] of document (8). Moreover, according to the results shown in Figure 71 of document (1), the level of ammonium of the (high-seed) cultures producing the highest TNFR-Ig titer was around 1.4 mM, the lower ammonium value cited in paragraph [0170] of document (8) referring to the studies of document (5). Figure 3 of document (5) showed no disadvantageous effect when the concentration of ammonium was 1.4 mM but only at concentrations of 5 mM or higher. Thus, the skilled person had no reason or motivation to modify the composition of Medium 9, let alone the concentrations of glutamine and/or glutamate, in the light of document (8). The less so, since the addition of glutamate in the culture medium was only optional in document (8) and, if at all, only in a feed medium. In fact, there was no reason for a skilled person to combine the teachings of document (1) with those of document (8) because there was no reference at all to TNFR-Ig in this latter document.

XIII. The appellant (opponent) requests that the decision under appeal be set aside and that the patent be revoked.

XIV. The respondent (respondent) requests that the appeal be dismissed (main request) or, in the alternative, that the decision under appeal be set aside and the patent be maintained on the basis of any of auxiliary requests 1 to 3.

Reasons for the Decision

Main request (Claims as granted)

1. The methods of claims 1 and 18 are not limited to any specific mammalian cell and culture medium, the first and second temperature ranges are not specifically defined in these claims which refer also to a large range of viable cell density and to a glycosylation pattern in general, and there is no limitation to any specific method for measuring the technical effects mentioned in claims 1 and 18 (cf. point II *supra*).

2. The objections raised by the appellant under Article 83 EPC concern the breadth of the claims and are related to those raised under Article 56 EPC, in particular to objections related to the technical effects whereupon the objective technical problem is formulated and those concerning the question of whether the objective technical problem is solved across the whole scope of the claims.

3. According to decision G 1/03 (OJ EPO 2004, 413, point 2.5.2 of the Reasons), a lack of reproducibility of the claimed invention represents, in the case of an

effect not expressed in a claim but being part of the problem to be solved, a problem of inventive step. If the effect is expressed in the claim, there is lack of sufficient disclosure (cf. "Case Law of the Boards of Appeal of the EPO", 9th edition 2019, II.C.6.1, 362). In the present case, the technical effect is mentioned in claims 1 and 18, namely "wherein the fraction of misfolded and/or aggregated [TNFR-Ig] polypeptide, relative to the total polypeptide produced, is decreased compared to ..." (claim 1), and "wherein the glycosylation pattern of the expressed [TNFR-Ig] polypeptide is increased relative to the glycosylation pattern that ..." (claim 18). Thus, the alleged lack of reproducibility must be dealt with under Article 83 EPC.

Article 100(b) EPC (Article 83 EPC)

4. For the purpose of Article 83 EPC, the board will subsequently refer to the disclosure of the patent application.

5. In the communication pursuant to Article 17 RPBA 2020, the parties' were informed of the board's provisional opinion on the objections raised under Article 83 EPC. For some of these objections, no further arguments were put forward by any of the parties, notably not at the oral proceedings before the board. The board's provisional opinion was neither questioned nor did other aspects come up that would require its reconsideration. Therefore, for these objections, the board does not see any reason to deviate from its provisional opinion.

6. As regards the mammalian cells and the culture medium, it is thus to be noted that:

6.1 The methods of claims 1 and 18 rely on the culture of mammalian cells. Paragraphs [0060] to [0064] of the patent application provide general information on these cells as well as a list of several mammalian cells normally used in the field. Reference is made to the selection of cells producing high levels of protein and to the fact that different cell types may result in different glycosylation patterns. The skilled person is also made aware that the production of certain proteins and polypeptides may have detrimental effects on the characteristics of the cell (growth, viability, etc.) and that the selection of the cells may be based on considerations related to the large-scale production (cell growth, final cell density, etc.). This disclosure reflects the common general knowledge of a skilled person working in the technical field under consideration, i.e. large-scale mammalian host cell culture for production of recombinant proteins.

6.2 Indeed, this is also the case for the cell culture medium. Paragraphs [0077] to [0085] of the patent application refer to culture media in general and to several specific culture media known in the art. In this context, the role of copper and glutamate in the culture media for reducing misfolding/aggregation or for influencing the glycosylation pattern of the recombinant protein, is explicitly outlined. Moreover, exemplary culture and feed media are described in Example 1 (Tables 1 and 2). According thereto, "copper and/or glutamate may be added to within any of the inventive concentrations" (cf. paragraph [00174]). The selection of the culture medium would be made by the skilled person in accordance with the selected mammalian (host) cell, bearing in mind and taking into account the prior art describing the specific culture

characteristics and properties of said cell. In other words, the selection of both, mammalian host cell and cell culture medium, are not independent but, in a certain way, linked; a fact, well-known to a skilled person working in the technical field under consideration.

6.3 Moreover, regardless of the specific mammalian cells and medium selected, claims 1 and 18 require the claimed methods to provide a technical effect when compared to the same methods carried out using the same cells in an otherwise identical culture medium lacking copper and glutamate. These claims are further limited to TNFR-Ig production and thus, the skilled person does not face a method for the production of any polypeptide/protein which, as acknowledged in paragraph [0064] of the patent application, may have detrimental effects and require particular selections of host cell, culture medium and conditions. Since claims 1 and 18 are limited to TNFR-Ig production, all prior art concerned with the production of this specific protein is available to the skilled person and may also be taken into account for the selection of the mammalian host cell and culture medium (cf. paragraph [00119]). Thus, the appellant's objection in this regard is not persuasive.

7. As regards the maximal possible viable cell density under the first temperature range, it is to be noted that:

7.1 Claims 1 and 18 require to maintain the culture at a first temperature range for a period of time which is sufficient to reach 20% - 80% of the maximal possible viable cell density. This range falls within the values given in paragraph [0070] of the patent application.

Controlling cell viability and measuring viable cell density are routine tasks in the technical field.

7.2 The appellant's objections in this regard relate more to alleged deficiencies in the information provided in the examples of the patent than to an inability of the skilled person to measure and carry out the method step as indicated in the claims. The maintenance of the culture at the first temperature range is defined by the time required to achieve the viable cell density indicated in the claims which directly depends on several factors such as, *inter alia*, cell seeding density, specific mammalian host cell, culture medium and conditions, etc. It may be very short or very long depending on the specific fermentation set up selected (high/low cell density seeding, fast/slow cell growth, etc.), a fact well-known to the skilled person. Thus, the appellant's objection in this regard is likewise not persuasive.

8. As regards the maintenance of the cell culture at the second temperature range, it is to be noted that:

8.1 Claims 1 and 18 require to maintain the culture for a second period of time under certain conditions and for a time sufficient to permit TNFR-Ig expression. There is no limitation to this period of time; neither the period nor the amount/yield of recombinant TNFR-Ig are defined in the claims. Thus, the time period may be as long as to have a high yield of TNFR-Ig or as short as to have a low yield. The appellant's reference to Figures 7 and 12 of the patent application, wherein the cumulative specific productivity is measured beginning at day 7, and respondent's reference to days 10 and 12, only exemplify this fact. All these periods fall within the possible ranges specified in paragraph [0071]; a

paragraph, wherein a further possible criterion is indicated for deciding when the cell culture may be terminated (percentage of relative misfolded/aggregated protein), even though explicit reference is made therein to the flexibility of the skilled person for determining appropriate or optimal times. The appellant's objection on this issue relates neither to the non-reproducibility of the claimed methods nor to an insufficiency of the patent application, and it is thus also not persuasive.

9. Three objections raised under Article 83 EPC and addressed in the board's communication were further discussed at the oral proceedings before the board.

Control medium lacking copper and glutamate

10. The patent application provides the definitions of "medium", "cell culture medium" and "culture medium", in paragraph [0049], wherein reference is also made to a "feed medium" defined in paragraph [0040]. Paragraph [0049] further states that "[i]n certain embodiments, the cell culture medium is a mixture of a starting nutrient solution and any feed medium that is added after the beginning of the cell culture". And, in the same paragraph, the skilled reader is informed that exemplary culture media are shown in Table 1. This Table in Example 1 of the patent application discloses five culture media (Medium A to Medium E), wherein Medium D is a medium lacking copper and glutamate. Table 2 in Example 1 provides also five exemplary feed media (Medium F to Medium J), all of them lacking glutamate but containing different amounts of copper. Paragraph [00175] refers to these media and states that "the concentrations of one or more components of such feed media may be increased or decreased to achieve a

desired concentration of such components". The preparation of these media, including that of a medium lacking copper and glutamate, does not require undue burden for a skilled person.

11. The first step of the methods of claims 1 and 18 is "culturing mammalian cells ... in a cell culture medium comprising between 0.5 and 5 µM copper and between 1.7 and 33 mM glutamate". Example 2 of the patent application describes experiments 1, 2 and 3 carried out for the production of TNFR-Ig. The specific conditions of these experiments are disclosed in Tables 3, 4 and 5 and the concentration of copper and glutamate for both, the control and the other cultures, are indicated. Whilst all control cultures lack copper and glutamate, the concentrations of copper and glutamate in all other cultures are within the ranges indicated in claims 1 and 18. Paragraph [00178] refers to the materials and methods of these experiments and states that standard perfusion methods were used, wherein the "[c]opper and glutamate were administered to the initial cell culture on Day 0 of the production run". In paragraph [00183], the skilled reader, with reference to Figures 3, 8 and 13, is informed that "[g]lutamate accumulation was measured over the course of each production run" and reference is made to glutamate being "administered upfront (glutamate-only and combination conditions)". Indeed, Figures 3, 8 and 13 show the glutamate levels of cell cultures grown under the conditions of experiments 1, 2 and 3 (Tables 3, 4 and 5).

12. In all these figures, the levels of glutamate - both at the beginning of the culture and throughout the whole culture or production run (10 days) - in the cell cultures, other than the control cultures, are within

the concentration range indicated in claims 1 and 18. In the control cultures, the levels of glutamate are lower than 0.5 mM at the beginning of the culture but increase significantly throughout the culture to levels falling within the concentration range of claims 1 and 18, even though always remaining lower than those of the other cultures. This is acknowledged in paragraph [00183] of the patent application, wherein it is stated that, for these other cultures, "the final glutamate concentration was consistently about 1.5 mM higher than the control", explicitly acknowledging thereby the production of glutamate in the control medium during the culture or production run.

13. For determining or measuring the technical effect referred to in claims 1 and 18, these claims require to carry out a cell culture of the mammalian cells "under otherwise identical conditions in an otherwise identical medium that lacks copper and glutamate" (*supra*). As stated above, the preparation of a medium lacking copper and glutamate poses no undue burden on a skilled person. Nor are special skills required for culturing mammalian cells in such a cell culture medium under conditions otherwise identical to those used for culturing such cells in a culture medium as defined in claims 1 and 18. The skilled reader would be aware that this difference in the medium (lack of glutamate and copper) may have certain impacts on the dynamics and progression of the culture, in particular on the levels of glutamate produced during or throughout the cell culture, as shown in Figures 3, 8 and 13 and explicitly stated in paragraph [00183] of the patent application. Thus, it could not be reasonably expected that culture conditions remain identical throughout, and at every moment, for both the control culture and the other cultures. Moreover, the claims do not require the

control culture to lack glutamate and copper during or throughout the whole process.

14. If there is any ambiguity in the wording of claims 1 and 18, this ambiguity does not hinder or prevent a skilled person from carrying out the teachings of the patent application, i.e. the methods of these claims, and it is more a question of Article 84 EPC than Article 83 EPC. Likewise, the appellant's observation that Figures 3, 8 and 13 show the control cultures at the beginning of the culture not to lack glutamate but to have levels of glutamate of about 0.3 mM, may raise questions on the actual conditions of the control cultures described in the patent application (Article 84 EPC). However, whilst claim 1 requires the control medium to lack glutamate and copper, the method of claim 1 does not exclude glutamate and copper carried over from the medium used for initial growth and expansion of the mammalian cells before seeding the culture at the first temperature range. This may well result in the presence of certain amounts of glutamate and copper at the beginning of the culture at the first temperature range in the control cultures as well. This neither contradicts nor belies the teachings of the patent, nor prevents the skilled person from carrying out the comparison without undue burden.

Method for measuring TNFR-Ig misfolded and/or aggregated

15. Claim 1 requires to measure "the fraction of misfolded and/or aggregated [TNFR-Ig] polypeptide, relative to the total [TNFR-Ig] polypeptide" of both, the control and the other culture. There is no limitation or restriction as regards the method and the conditions used for carrying out said measurement and thus, the

skilled person may use any method or technique available from the art.

16. According to paragraphs [0015], [0020] and [0025] of the patent application, Figures 5, 10 and 15 show "the Day 10 relative level of misfolded and/or aggregated produced TNFR-Ig" and reference is made in these Figures to "% HIC Peak 3" as containing such TNFR-Ig. Thus, the skilled person is informed that HIC (hydrophobic interaction chromatography) is a technique appropriate for carrying out the measurement required by claim 1. It is not contested that HIC is a technique known in the art and available to the skilled person at the relevant date (document (16)). Claim 1 requires to compare the TNFR-Ig fraction of the control culture with that of the other culture and thus, it requires to carry out a comparison. Therefore, the specific conditions used for carrying out the HIC must be identical for the fractions of both cultures.

17. It belongs to the normal abilities of a skilled person - as defined in the case law in the field of biotechnology - to find HIC conditions that result in the separation of misfolded and/or aggregated TNFR-Ig from native (normally folded, non-aggregated) TNFR-Ig. These HIC conditions do not need, and are not required, to be identical to those exemplified in Figures 5, 10 and 15 of the patent application. Any HIC conditions resulting in the separation of both TNFR-Ig forms will be enough for a skilled person; regardless of whether the misfolded and/or aggregated TNFR-Ig is present in the third peak - as stated in the Figures of the patent application - or in another peak of the elution pattern resulting from the specific HIC conditions used.

18. Document (7) confirms the view that HIC is a technique appropriate for separating misfolded and/or aggregated TNFR-Ig from native (normally folded, non-aggregated) TNFR-Ig. Although this document is post-published and thus, the specific HIC conditions disclosed therein could not have been used by the skilled person at the relevant date for isolating the "Peak 3" referred to in the patent application, it nevertheless states that the HIC results are independent of the particular HIC resin and salt used (cf. column 20, lines 27 to 30 and 35 to 38). Document (7) also refers to SDS-PAGE as a technique appropriate for characterising different TNFR-Ig fractions (cf. column 19, lines 56 to 61; column 21, lines 8 to 11; and column 22, lines 34 and 35) and, as argued by the respondent, other techniques for isolating these fractions (such as molecular sieve chromatography) were also available to the skilled person at the relevant date. Thus, no undue effort is required from a skilled person to measure the fraction of misfolded and/or aggregated TNFR-Ig.

Increased TNFR-Ig glycosylation pattern

19. Claim 18 requires that the glycosylation pattern of the TNFR-Ig produced under the culture conditions mentioned is increased relative to the glycosylation pattern of the TNFR-Ig produced under otherwise identical conditions in an otherwise identical medium that lacks copper and glutamate. The effect of the claimed method on TNFR-Ig sialylation is disclosed in experiments 1, 2 and 3 of Example 2 and summarised in paragraph [00187]. With reference to Table 6, it is stated therein that "the results of Experiment 1 indicated that both the copper and glutamate conditions had higher total sialylation than the control condition". For experiments 2 and 3, reference is made to Tables 4 and

5 and to the relevance of the **time course data** from these experiments as described in these Tables, namely that there is "a stabilization or slight increase in total sialylation over time for those conditions containing copper and/or glutamate (see Figures 18 and 19)".

20. In fact, Figure 18 shows that, whilst the sialylation of TNFR-Ig increases (from 8 to 10 days) when using culture media containing either copper, glutamate, or a combination of copper and glutamate (Combo) in concentrations falling within the ranges cited in claim 18, the sialylation of TNFR-Ig decreases when using the control medium, even though it is always higher - at 8 and 10 days - than that of the other cultures. The increase and decrease (from 8, 10 and 12 days) of the TNFR-Ig sialylation in the Combo medium and the control medium, respectively, is also shown in Figure 19 of the patent application, wherein in this case however the TNFR-Ig sialylation in the Combo medium is always higher than in the control medium.

21. It is not contested that the results of experiment 3 shown in Figure 19 support the method of claim 18 since TNFR-Ig sialylation in the Combo medium is increased relative to that obtained in the control medium. Indeed, this is the case for all days assayed (8, 10 and 12 days), even though claim 18 does not require that the levels of TNFR-Ig sialylation during or throughout the whole culture in the non-control medium must always be higher than those of the control medium. Claim 18 requires only that this must be the case, and must so necessarily happen, at some time when the mammalian cells are cultured - under the conditions of the second temperature range - for a sufficient time, be it 8, 10, 12 days or even longer.

22. The results of experiment 2 shown in Figure 18 neither support nor represent a failure because, as stated above, the method of claim 18 requires to "culture for a second period of time under conditions and for **a time sufficient** to permit expression of the [TNFR-Ig] polypeptide, wherein the glycosylation pattern of the expressed [TNFR-Ig] polypeptide is increased" (emphasis added by the board). Thus, although no increase is observed at 8 and 10 days, nothing can be said about the level of TNFR-Ig sialylation at 12 days. If at all, Figure 18 shows a decrease and increase of the TNFR-Ig sialylation in the control medium and in the other medium, respectively, as indicated in paragraph [00187]. In line therewith, it can be expected that at day 12 the TNFR-Ig sialylation further decreases in the control culture medium and increases in the other medium. However, in view of the results shown in Figure 19 for other media containing copper and glutamate (Combo with extra copper, Combo with low copper), this must not necessarily be the case. Therefore, the question remains whether the culture of experiment 2 would have provided an increased TNFR-Ig sialylation if the mammalian cells would have been cultured in the second period of time for a sufficient time. On the basis of the evidence on file, this question cannot be answered, neither in the positive nor in the negative and thus, no conclusions can be drawn.

23. The fact that, in experiment 2 and Figure 18, the TNFR-Ig sialylation in the control culture medium at 8 and 10 days is higher than that of the other medium and that, in experiment 3 and Figure 19, the TNFR-Ig sialylation in the control culture medium at 8, 10, and 12 days is lower than that of the other medium, does

not have any relevance as regards Article 83 EPC. The conditions of both experiments are not identical, at least the second temperature range in experiment 2 (30°C) is different from that in experiment 3 (29.5°C). Since most of the features in claim 18 are defined by ranges and/or are left undefined (mammalian cell line, cell culture medium except for copper and glutamate concentrations), it is not surprising and it should be expected that, in light of the known variability of mammalian cells and their sensitivity to culture conditions, the dynamics and progression of these cultures are not always identical. Indeed, the patent application itself acknowledges that "one uniform effect among all the additions [of copper and glutamate] was a slight reduction in specific productivity compared to the control, a difference observed in all three experiments" (cf. paragraph [00182]). This effect might well be greater in the conditions of experiment 2 than in those of experiment 3. However, as stated above, whilst nothing can be derived from the results of experiment 2, those of experiment 3 clearly and unambiguously support the method of claim 18.

Conclusion on Article 100(b) EPC

24. It follows from these considerations that Article 100(c) EPC does not prejudice maintenance of the patent.

Article 100(a) EPC (Article 56 EPC)

The closest prior art and distinguishing technical features

25. The closest prior art document (1) discloses a method for producing TNFR-Ig in a large-scale cell culture

(cf. paragraph [00123] and claims) which comprises the steps defined in claims 1 and 18, namely an initial growth phase (cf. paragraphs [00164] to [00170]), shifting culture conditions - in particular a temperature shift (cf. paragraphs [00171] to [00175]), a subsequent production phase (cf. paragraphs [00176] to [00183]), and the monitoring of culture conditions - including glycosylation of the expressed (TNFR-Ig) protein (cf. paragraphs [00184] and [00185]).

26. The method, method steps and, in particular the culture media disclosed in document (1), are stated to allow high levels of (TNFR-Ig) protein production and to lessen the accumulation of undesirable factors, such as ammonium and/or lactate (cf. paragraph [0006]). These factors are described as "metabolic waste products" that are detrimental, *inter alia*, to the amount of recombinant (TNFR-Ig) protein produced, and alter "the folding, stability, glycosylation or other post-translational modification of the expressed polypeptide or protein". Lactate and ammonium, resulting from glucose and glutamine metabolism respectively, are identified as relevant waste products (cf. paragraph [00102]). The culture media disclosed in document (1) minimise detrimental effects of lactate and ammonium and even reverse their accumulation (cf. paragraphs [00148] and [00150]). Several parameters of the culture media are particularly relevant, namely the (cumulative) total amino acid concentration, the molar ratio of (cumulative) glutamine to (cumulative) asparagine and to the (cumulative) total amino acid concentration, and the molar ratio of (cumulative) inorganic ions to the (cumulative) total amino acid concentration (cf. paragraphs [00151] to [00156], claims).

27. Example 16 describes the production of TNFR-Ig in a CHO cell culture using Medium 9 (cf. paragraphs [00302] to [00304]). The composition of Medium 9 is disclosed in Table 14, wherein the concentrations of glutamate and copper are "monosodium glutamate 33.80 mg/L, 0.20 mM", "$CuSO_4$ 10.24 µg/L, 64.00 nM ; $CuSO_4.5H_20$ 99.88 µg/L, 400 nM" (cf. page 67).

28. It is not disputed that the glutamate concentration in Medium 9 (0.20 mM) does not fall within the concentration range of glutamate defined in claims 1 and 18 (between 1.7 and 33 mM). However, it is disputed whether the concentration of copper in Medium 9 (464 nM) falls within the concentration range of copper defined in claims 1 and 18 (between 0.5 and 5 µM). With reference to the Guidelines for Examination (G-VI, 8.1) and the case law (T 871/08 of 8 December 2011), the appellant argues that, when the normal rounding for comparing nM and µM values is taken into account, the copper concentration in Medium 9 is identical to the lower limit of the concentration range defined in claims 1 and 18.

29. The board disagrees with the appellant, as the copper concentration in Medium 9 does not fall within the concentration range defined in claims 1 and 18. There is no reason for expressing the concentration of copper in either µM or nM units. When comparing these values using nM units, there is no need to round the copper concentration of 464 nM in Medium 9, which is different from a concentration of 500 nM, the lower limit of the copper range in claims 1 and 18. In fact, even if expressed in µM units, the value 0.5 µM is different from 0.464 µM, and the board fails to see any compelling reason for rounding up this latter value, let alone in the manner done by the appellant. These

are different concentrations of copper in the culture medium which may be significant and technically relevant for a skilled person in the field of biotechnology.

30. In decision T 871/08 of 8 December 2011, the board stated that, when comparing a value of the prior art ("2.996:1") with those claimed ("from 3:1 to 9:1"), the state of the art has to be given the same accuracy as the one claimed and, since the values in the claims had been quoted without any digit after the comma, for comparison purposes the value 2.996 was rounded up to 3 (cf. point 2.3 of the Reasons). In the case underlying this decision, the value in the prior art and those in the claims were expressed in the same units and thus, contrary to the present case, there was no need to provide any conversion of units for carrying out a meaningful comparison. This was also the situation for the cases underlying decisions T 1186/05 of 6 December 2007 and T 708/05 of 14 February 2007, both cited in the decision T 871/08.

31. Whilst decision T 1186/05 refers to the importance of standard mathematical rules for rounding values (cf. points 3.6.1 to 3.6.5 of the Reasons), decision T 708/05 refers to the relevance of the parameter measured and the error range of the method used for measuring said parameter (cf. point 3 of the Reasons). Likewise, Part G, Chapter VI, point 8.1 of the Guidelines for Examination refers to error margins in numerical values relating to measurements subject to measurement errors and to the general convention in the scientific and technical literature for interpreting ranges of values in patent specifications. However, in none of the cases underlying these decisions, a conversion of units was required for a meaningful

comparison of the value disclosed in the prior art and those in the patent. In the present case, a conversion of units is necessary and, neither the application of the general convention or standard mathematical rules for rounding values nor the nature of the parameter measured and the error margins of said measurement, support the appellant's argumentation. The value of the copper concentration in Medium 9 disclosed in document (1) is different from the lower limit of the concentration range of copper defined in claims 1 and 18.

32. Thus, the concentration of both, glutamate and copper, in the culture medium are technical features that distinguish the method of producing TNFR-Ig disclosed in Example 16 of document (1) from the claimed methods.

The objective technical problem and its solution

33. Starting from this prior art, the objective technical problem may be formulated as the provision of an improved method of producing TNFR-Ig, wherein the improvement consists of a decreased TNFR-Ig misfolding and/or aggregation, and/or an increased glycosylation/ sialylation pattern. In view of the results disclosed in the patent, the methods of claims 1 and 18 solve this problem. Moreover, since the improvement is mentioned in the claims (cf. point 2 *supra*, reference to decision G 1/03), the scope of claims 1 and 18 embraces only these methods (and conditions) that provide this improvement and effects.

Obviousness and expectation of success

34. Paragraph [00150] of document (1) refers to the media formulations disclosed and states that "when used in

accordance with other culturing steps described herein, minimize and even reverse accumulation of lactate and ammonium", and that these media formulations "have been shown to have beneficial effects on cell growth and/or viability or on expression of polypeptide or protein". Immediately thereafter, there is a reference to the five technical features that characterise these formulations and provide said beneficial effects. Whilst features (i), (iii) and (iv) refer to the "cumulative amino acid amount" / "cumulative total amino acid" of these formulations, features (ii), (iii) and (v) refer to the "molar cumulative glutamine"; wherein feature (iii) reads "a molar cumulative glutamine to cumulative total amino acid of less than about 0.2".

35. These five features are described in more detail in paragraphs [00151] to [00156], and in paragraph [00157] it is stated that "[b]y utilizing media formulation which exhibit **one**, **some or all** of the above characteristics, one of ordinary skill in the art will be able to optimize cell growth and/or viability and to maximize the production of the expressed polypeptide" (emphasis by the board). As argued by the appellant, this might be seen as an incentive for a skilled person to optimise and improve media formulations based on the teachings of document (1), even though this statement is very general and does not lead the skilled person in any manner to the technical features distinguishing the claimed methods from those disclosed in document (1). In the board's view, this statement does not go beyond a description of what in the case law has been considered the normal task of a skilled person (cf. "Case Law", *supra*, I.D.9.11, 254).

36. Indeed, document (1) itself already indicates how to carry out such optimisation and reports, *inter alia*, on the effects of substituting glutamate for glutamine, substituting glucose and glutamine, and using glutamine starved media (cf. page 57 to 66, Examples 5, 6 and 7). The composition of Medium 9 disclosed in Example 8 of document (1) results from such optimisation process, it takes into account the results of the studies described in Examples 5, 6 and 7, and further intends to achieve a high cell density and recombinant protein titer, and low levels of lactate and ammonium (cf. *inter alia*, page 70, Example 10; page 75, Example 13; and page 82, Example 16).

37. A skilled person would not disregard this information; the series of experiments carried out and reported in document (1) cannot be simply ignored. The less so since, according to the case law, the skilled person has a conservative attitude and would neither try to enter unpredictable areas nor take incalculable risks (cf. "Case Law", *supra*, I.D.8.1.3, 205). The attitude of the average skilled person approaching an optimised medium would be different, for the purpose of (further) modification and improvement, from the attitude towards a non-optimised, arbitrary medium. A skilled person would be much more careful and cautious for the former than for the latter and, if anything, would not be prompted, in the absence of a clear suggestion or hint thereto, to change or alter those components, factors and parameters that had been optimised.

38. It is worth noting here that, although reference is made *en passant* to "folding, stability [and] glycosylation" (cf. sentence bridging pages 11 and 12), as well as to the monitoring of, *inter alia*, glycosylation (cf. page 38, last sentence first

paragraph), document (1) is silent on the effect of the optimised media, in particular of Medium 9 used in Example 16, on the folding/aggregation and glycosylation of the recombinant (TNFR-Ig) protein produced. There is no suggestion of an effect of glutamate concentrations, let alone of copper concentrations, on said properties.

39. In view thereof, the modification or alteration of the glutamate concentration in Medium 9 (increase by at least 10-fold), bearing in mind that such a modification alters also the total amino acid concentration of said medium (which is a parameter defining features (i), (iii) and (iv), *supra*), is neither straightforward nor obvious. The same conclusion applies even more to the modification or alteration of the concentration of copper in Medium 9.

39.1 Documents (2) to (4) have been cited under Article 56 EPC in combination with document (1). These documents are all concerned with mammalian cell culture media and, most of them, with the use of such media for the production of recombinant proteins which are mostly related to TNFR or Ig. The passages referred to by the appellant (*inter alia*, column 6, lines 51 to 54; column 6, line 66 to column 7, line 4 of document (2); page 74, left-hand column, first paragraph of document (4)), all deal with the substitution of glutamine by glutamate which, as explained above, is also a subject of document (1). In this sense, none of these documents goes beyond the information provided in document (1), and none of them suggest any link between copper concentration and misfolding/aggregation and/or glycosylation/sialylation of the recombinant protein produced.

39.2 In view of the large number of components, parameters and conditions of the culture media disclosed in document (1) which are open to alteration or modification, and bearing also in mind the (optimisation) studies and the data disclosed therein, the selection of documents (2) to (4) for a combination with document (1), so as to arrive at the subject-matter of claims 1 and 18, requires hindsight and results from an *ex-post facto* analysis (cf. "Case Law",*supra*, I.D.6, 199). None of these documents would have led the skilled person to the specific concentration range of glutamate defined in claims 1 and 18, let alone to the concentration range of copper. In the present case, the situation is far removed from what has been called in the case law a "one-way street" situation (cf. "Case Law",*supra*, I.D.10.8, 270), wherein a skilled person, starting from document (1), would have been directed in a straightforward manner to modify the glutamate and copper concentrations without having any other choice available. Moreover, in view of the disclosure of document (1) (cf. point 38 *supra*), it cannot be seen that the skilled person would have made such modifications with a reasonable expectation of achieving any of the improvements or advantageous effects referred to in the methods of claims 1 and 18 (cf. "Case Law", *supra*, I.D.7.1, 200).

40. The appellant has also relied on the combination of documents (1) and (8). According to the appellant, the reference in paragraph [00157] of document (1) (cf. point 35 *supra*) would have motivated the skilled person to modify or alter the composition of the culture media, in particular that of Medium 9, whilst retaining or maintaining the five essential technical features (i) to (v) identified in document (1), i.e. the composition of the modified culture media would have

remained within the framework defined by the constraints imposed by these five essential features. According to the appellant, such a modification of Medium 9, when following the teachings of document (8), would have led the skilled person to the claimed methods in an obvious manner.

40.1 This is not persuasive. Document (8) relates to methods of producing recombinant polypeptides/proteins in mammalian cell cultures, particularly the large-scale production of antibodies, antibody fragments and chimeric antibodies (cf. *inter alia*, paragraphs [0001], [0008], [0054] and [0071]). According to document (8), the method relies on four discoveries, namely the use of (i) high concentrations of glucose in the culturing phase (cf. *inter alia*, paragraphs [0011], [0016], [0017], [0033] and [0124]), (ii) an increased ratio of glutamate to glutamine (cf. *inter alia*, paragraphs [0020], [0036] and [0125]), (iii) temperature shifts during the culturing process (cf. *inter alia*, paragraphs [0023], [0039] and [0126]), and (iv) one or more additions of concentrated nutrient mixtures ("batch feed") to the (viable cell) culture during early and mid-production culturing phases (cf. *inter alia*, paragraphs [0022], [0032], [0038] and [0127]).

40.2 There is no doubt that some of the discoveries referred to in document (8) are common to, and overlap with, some of the parameters and conditions of the method disclosed in document (1), such as the use of a temperature shift. The concentration of D-glucose in Medium 9 (15 g/L; Table 14 on page 67 of document (1)) falls also within the high concentrations of glucose referred to in document (8). Moreover, according to document (8), "[t]he reduced glutamine content, alone

or in relation to glutamate concentration, is particularly useful in reducing creation of unwanted by-products", one of which is ammonium (cf. paragraphs [0125], [0158] and [0170]). The relevance of reducing these by-products (lactate and ammonium) and the role of glucose and glutamine in their production, is also disclosed in document (1) (cf. point 26 *supra*).

40.3 As a preferred embodiment, document (8) refers to a glutamine concentration throughout the culturing in a range of 0 to 15 mM, preferably 0.5 to 5 mM (cf. paragraph [0131]). Low glutamine concentrations, such as 7.5 mM or less, are preferred since they lead to lower levels of ammonium and lactate (cf. paragraph [0133]). It further states that "[i]n place of or in addition to glutamine, glutamate can be added. For example, from 0.5 to about 15 mM, e.g. from about 1 to about 10 mM of glutamate can be added during culturing. A ratio of glutamine to glutamate of from 0:2 to 2:0, preferably less than about 0.5:2 can be used" (cf. paragraph [0132]). Example 4 reports the use of glutamate (5 mM) as an alternative to glutamine (0 mM) in the cell culture and, with reference to Figure 4, states that these concentrations led to the lowest level of by-product (ammonium) accumulation (cf. paragraph [0168]).

40.4 It is not contested that some values for the glutamate concentration resulting from the ratio of glutamine to glutamate and/or (within) the preferred concentration ranges of glutamate disclosed in document (8), fall within the concentration range of glutamate cited in claims 1 and 18. Thus, a skilled person could have modified Medium 9 used in Example 16 of document (1) following the teachings of document (8) and arrived at a concentration of glutamate within the concentration

range cited in these claims. However, according to the case law, the question to be answered is not whether a skilled person **could** have modified Medium 9 in this manner but whether (s)he **would** have modified it so and, if this was the case, whether (s)he would have done this modification with an expectation of achieving any advantageous effect (cf. Case Law, *supra*, I.D.5, 197).

40.5 The relevance and the effects of glutamine in a fed-batch process are extensively dealt with in document (1), and studies on glutamine starvation in fed-batch process are described in Example 3. In this example, culture media with low starting levels of glutamine and elimination of glutamine from the feed are shown to result in low levels of ammonium and lactate, leading to increased cell viability and recombinant protein expression (cf. paragraph [00225]). Table 9 summarises the conditions used in Example 3 and shows that the concentration of the starting media in the low glutamine process is 4 mM (with no glutamine feed) (cf. page 55). According to the composition of Medium 9 shown in Table 14 of document (1), the concentrations of glutamine and glutamate are 4.00 mM and 0.2 mM, respectively. Thus, the concentration of glutamine and glutamate in Medium 9 disclosed in document (1), a medium which had been already optimised by taking into account the results and data obtained in the other examples of this document, clearly fall within the lower limit of the "more preferred" ranges of glutamine and glutamate disclosed in document (8). Therefore, a cautious skilled person would be rather reluctant to further modify the concentrations of glutamine and glutamate in Medium 9, all the more so because the concentrations of glutamine and total amino acid are highly relevant for the determination and

definition of the five features identified in document (1) as essential (cf. point 34 *supra*).

40.6 Document (8) not only refers to the correlation of increased glutamine concentration and ammonia levels but it also refers to the disadvantageous effect of ammonium on the sialylation of the recombinant protein produced (cf. paragraph [0170]). With reference to document (5), it states that a "40% decrease in terminal galactosylation was observed as ammonium increased from 1 mM to 15 mM post growth phase with CHO cells". Table 8 shows that increasing concentrations of glutamine (from 5 mM to 15 mM) in the culture medium result in increasing ammonium concentrations (from 4 mM to 13 mM) (cf. page 18, left-hand column). Immediately thereafter, paragraph [0171] refers to "less than 5 mM" as the advantageous reduced levels of glutamine of the invention "to be used in a cell culturing medium, thereby reducing lactate and ammonium ion accumulation".

40.7 As stated above, the concentration of glutamine in Medium 9 (4 mM) is "less than 5 mM". Figure 71 of document (1) shows the ammonium concentration obtained under the cell culture conditions of Example 16 and, for those (high-seed) culture conditions that result in the highest levels of recombinant protein (Figure 72), the concentration of ammonium is of about 1.5 mM. This concentration of ammonium is close to the lower level of the range cited in document (8) with reference to document (5) (cf. page 18, left-hand column of document (8)). Indeed, Figure 3 of document (5) shows that the highest content of galactose and sialic acid of TNFR-Ig is obtained at these low concentrations of ammonium (0 to 2 mM) and is significantly reduced at higher concentrations of ammonium (4 to 15 mM), such as

those in Table 8 of document (8). Therefore, in view of the low ammonium concentration shown in Figure 71 of document (1), a cautious skilled person would have been rather reluctant to further reduce the (already optimised) concentration of glutamine in Medium 9 - and correspondingly increase the concentration of glutamate in said medium - with the expectation of obtaining yet a further reduction in the levels of ammonium and thereby increased glycosylation and/or sialylation of TNFR-Ig.

40.8 In the light of these considerations, a skilled person, starting from the disclosure of document (1) and following the teachings of document (8), could have modified Medium 9 and achieve concentrations of glutamate falling with the range cited in claims 1 and 18. However, it cannot be said that the skilled person would have modified Medium 9 in such a manner, let alone with the expectation of having any of the advantageous effects cited in claims 1 and 18.

40.9 It is also worth noting that both, documents (1) and (8), are completely silent about possible effects of copper on folding and sialylation. Therefore, there was neither a motivation nor a reason for the skilled person to modify the concentration of copper in Medium 9 (464 nM) so as to achieve a concentration of copper falling within the range defined in these claims 1 and 18 (0.5 to 5 µM).

40.10 In conclusion, the skilled person would not have arrived at the methods of claims 1 and 18 in an obvious manner from the combination of documents (1) and (8).

Conclusion on Article 100(a) EPC (Article 56 EPC)

41. Thus, the main request fulfils the requirements of Article 56 EPC and Article 100(a) EPC does not prejudice maintenance of the patent.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Voyé B. Stolz

Decision electronically authenticated