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Datasheet for the decision of 24 July 2019

Case Number: T 0688/14 - 3.3.08

Application Number: 06725404.5

Publication Number: 1863920

IPC: C12P21/02, C07K14/755

Language of the proceedings: EN

Title of invention:
METHOD FOR ISOLATION OF RECOMBINANTLY PRODUCED PROTEINS

Patent Proprietor:
Octapharma AG

Opponent:
Novo Nordisk A/S

Headword:
Recombinant Factor VIII production/OCTAPHARMA

Relevant legal provisions:
EPC Art. 54, 56, 123(2)

Keyword:
Main request - novelty (no)
Auxiliary request - added subject-matter (no), novelty (yes), inventive step (no);

Decisions cited:
T 0002/81, T 0455/91, T 0123/97, T 0659/00, T 0214/01, T 0190/03, T 0769/03, T 1439/04, T 0516/08, T 1710/09, T 2487/12, T 0268/13, T 0633/13, T 1634/15

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 0688/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 24 July 2019

Appellant I: (Patent Proprietor)	Octapharma AG Seidenstrasse 2 8853 Lachen (CH)
Representative:	Diepholz, Meikel Patent- und Rechtsanwälte Ullrich & Naumann PartG mbB Schneidmühlstrasse 21 69115 Heidelberg (DE)
Appellant II: (Opponent)	Novo Nordisk A/S Novo Allé DK-2880 Bagsvaerd (DK)
Representative:	Goodfellow, Hugh Robin Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 15 January 2014 concerning maintenance of the European Patent No. 1863920 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	P. Julià
	J. Geschwind

Summary of Facts and Submissions

I. European patent no. 1 863 920 is based on European patent application no. 06 725 404.5 (published under the PCT as International patent application WO 2006/103258, hereinafter "the patent application") and it was granted with 14 claims.

II. An opposition was filed on the grounds as set forth in Articles 100(a) and 100(c) EPC. The opposition division considered the main request (claims as granted) not to comply with Article 100(c) EPC and the first auxiliary request to lack novelty (Article 54 EPC). The patent was maintained in amended form upon the basis of a second auxiliary request.

III. The patent proprietor and the opponent (appellants I and II, respectively) appealed the decision of the opposition division and submitted statements setting out their grounds of appeal. Appellant I maintained the first auxiliary request before the opposition division as its main request. As an auxiliary request, both appellants requested oral proceedings.

IV. The parties replied to the respective statements of grounds of appeal. Appellant I made the second auxiliary request upon the basis of which the opposition division maintained the patent its auxiliary request in appeal proceedings.

V. The appellants were summoned to oral proceedings and, in a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), they were informed of the board's provisional, non-binding opinion on some of the issues of the case.

VI. None of the parties replied in substance to the board's communication.

VII. Oral proceedings were held on 24 July 2019 in the presence of both appellants.

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

"1. A method for the recombinant production of at least one target protein, which is human factor VIII or a B-domain deleted mutein thereof, in mammalian cells, which comprises effecting cultivation of mammalian cells, being capable of expression of said at least one target protein, in suspension culture, under serum-free conditions and subjecting a suspension of said cells, prior to separation of the protein from the cells, to a non-physiologically increased concentration of at least one ionic substance selected from NH_4Acetate, $MgCl_2$, KH_2PO_4, Na_2SO_4, KCl, NaCl, $CaCl_2$, an amino acid with a charged side chain, and a peptone."

IX. Claim 1 of the auxiliary request is identical to claim 1 of the main request, except for the replacement of "an amino acid with a charged side chain" by "arginine, lysine". Claims 4, 9 and 11 of this request read as follows:

"4. The method according to any one of claims 1 to 3, wherein:
(i) KCl is added to raise its concentration ...
(ii) $CaCl_2$ is added to raise its concentration in the cell suspension to a concentration ranging from 0.01 to 0.5 M, preferably from 0.05 to 0.2 M, most preferably to a concentration of about 0.1 M; and/or
(iii) lysine is added to raise its concentration ...
(iv) arginine is added to raise its concentration ...

(v) a peptone is added to raise its concentration ...

9. The method of claim 8, wherein the release composition comprises:
(i) $CaCl_2$, preferably at a concentration ranging from 0.01 to 0.05 M; and/or
(ii) KCl, preferably at a concentration ranging from 0.1 to 0.2 M; and/or
(iii) arginine or lysine, preferably at a concentration ranging from 0.05 to 0.2 M.

11. The method according to any one of claims 1 to 10, wherein
(i) KCl is added to raise its concentration in the cell suspension to a concentration ranging from 0.4 to 2 M, preferably from 0.4 to 1 M, most preferably to a concentration of about 0.5 M; and/or ... "

X. The following documents are cited in this decision:

(3): B.G.D. Boedeker, Seminars in Thrombosis and Hemostasis, 2001, Vol. 27(4), pages 385 to 394;

(8): R. Adamson, Ann. Hematol., 1994, Vol. 68, pages S9 to S14;

(10): B. Alberts *et al.*, "Molecular Biology of the Cell", 2nd edition 1989, pages 284 and 285;

(11): L. Stryer, "Biochemistry", 3rd edition 1988, pages 292 and 293;

(12): H. Lodish *et al.*, "Molecular Cell Biology", 4th edition 2000, pages 82 to 85;

(13): B.D. Hames and N.M. Hooper, "Instant Notes in

Biochemistry", 2nd edition 2000, pages 124 to 129;

(14): K. Ohlendieck, in "Methods in Molecular Biology", 2nd edition 2004, Vol. 244, pages 283 to 293;

(18): P.C. Spiegel *et al.*, Blood, 2001, Vol. 98, pages 13 to 19;

(21): Invitrogen, "Technical Resources - Media Formulations; 11320 - DMEM/F-12";

(22): US 5,851,800 (publication date: 22 December 1998).

XI. The submissions of appellant I, insofar as relevant to this decision, may be summarised as follows:

Main request
Article 54 EPC

According to the case law, a method-claim was characterised not only by the physical steps of the method but also by its purpose. Thus, a method-claim directed to the production of a product was anticipated only by methods producing this product (cf. T 268/13 of 7 July 2017, point 2.8 of the Reasons). Example 1 of document (22) disclosed the production of human factor VIII in CHO cells by lowering the culture temperature and adding butyric acid. The efficiency of several protease inhibitors was reported in this example. L-histidine was used as a protease inhibitor in tests 6 and 7 and resulted in a lower production of factor VIII than in a control method without addition of any protease inhibitor (cf. Table II of document (22)). Thus, the methods of tests 6 and 7 were

not methods for the production of factor VIII but methods that inhibited this production. According to the case law, these methods could not anticipate the method of claim 1.

Moreover, the purpose and the effect of adding L-histidine in tests 6 and 7 were different and unrelated to the purpose and effect disclosed in the patent in suit. This was also reflected by the concentrations of L-histidine added into the culture media used in the methods reported in Example 1 (0.52 mM, 5.2 mM in document (22) *vs.* 0.25 M in Table 9 of the patent). In fact, the production of factor VIII disclosed in document (22) was not due to the effect of L-histidine as disclosed in the patent in suit (release of factor VIII from the membranes of CHO cells), but to the temperature shift and the addition of butyric acid.

Auxiliary request
Article 123(2) EPC

According to the case law, the shrinking of a generic group was allowed if it did not result in singling out a compound or a group of compounds (intermediate generalisation) or in the provision of a technical contribution not originally disclosed in the patent application. Claim 1 resulted from a shrinking of the more generic features present in claim 1 of the patent application. The limitations introduced into claim 1 neither resulted in a singling out nor provided a technical contribution that was not originally disclosed in the patent application; they were not taken from independent lists with equally weighted members but from those members which were disclosed as preferred embodiments of the patent application.

Claim 3(i) and page 11, lines 27 to 30 of the patent application disclosed factor VIII and B-domain deleted factor VIII as the more preferred target proteins; they were the sole proteins exemplified in the patent application. Claim 3(ii) and page 13, lines 8 to 10 of the patent application identified mammalian cells as the preferred eukaryotic cells; all examples were carried out in suspension cultures of mammalian cells. Claims 1 and 2 of the patent application referred to suspension cultures and this type of culture was one of only two alternatives in claim 6(i). This disclosure was found on page 18, lines 14 to 16 which referred to the method of embodiments (1) and (3); embodiment (1) corresponding to the generic method of claim 1. Claim 3(iv) and page 14, lines 20 to 25 of the patent application identified the ionic substances in claim 1 as the most preferred substances. The deletion of histidine from the preferred amino acids with charged side chains did not provide a technical contribution because histidine was originally disclosed as an equally weighted alternative to the other amino acids. Nor did this deletion single out any amino acid or create an intermediate generalisation, it merely shrunk the original list.

Article 56 EPC

The closest prior art document (8) disclosed the production of recombinant factor VIII with reference to both non-activated (native, intact) factor VIII and activated factor VIII (factor VIIIa). Whilst factor VIIIa was associated with the cell membrane (Figure 3), factor VIII was complexed with the von Willebrand factor (vWF). Neither from document (8) nor from any other prior art document on file was it derivable that, in a system mimicking the *in vivo*

situation (cell cultivation), factor VIII acted as a peripheral membrane protein. Starting from this prior art, the technical problem to be solved was the provision of an alternative method for the production of recombinant factor VIII. The claimed method solved this problem and, indeed, produced increased amounts of factor VIII as shown in the examples of the patent.

There was no hint in document (8) that could have led a skilled person to the claimed method. On the contrary, document (8) informed the skilled person that factor VIII was a complicated, very large molecule, highly sensitive to proteolytic degradation and that the production of this factor in a serum-free medium was achieved only by co-expression with several copies of vWF, a large molecule of high molecular weight acting as a blanket or chaperone that stabilised and protected factor VIII from proteolysis. Hindsight was required for moving away from the advantageous co-expression system disclosed in document (8) and designing a very simple system wherein factor VIII was neither stabilised nor protected from proteolytic degradation. The more so, since there were alternative systems available to the skilled person which were more similar to the co-expression system disclosed in document (8). In particular, document (18) disclosed the C2 domain of factor VIII to have a membrane-binding region that included or overlapped with the binding site of factor VIII for vWF and the antibody BO2C11, an antibody that competed with vWF for binding to factor VIII. The co-expression of factor VIII with this antibody would have resulted in the recombinant factor VIII being complexed with this antibody instead of vWF, reducing thereby the exposure of factor VIII to proteolytic degradation.

Hindsight was also required for moving away from the advantageous industrial, large-scale production of recombinant factor VIII disclosed in document (8) to a very simple system suitable only for small-scale production of recombinant factor VIII, if at all. The fact that the claimed method was simple did not deprive it of inventive step.

Although the disruption and release of peripheral membrane proteins from cell membranes by a treatment with high ionic strength solutions was well-known in the art long before the priority date of the patent, as shown by the textbooks documents (10) to (14), there was no prior art document on file suggesting, let alone disclosing, the method of claim 1.

XII. The submissions of appellant II, insofar as relevant to this decision, may be summarised as follows:

Main request
Article 54 EPC

Claim 1 was not directed to a method for improving the production of factor VIII; there was no requirement in claim 1 as regards the amount of factor VIII produced. Thus, the claimed method was a mere method for the production of factor VIII characterised by several features (serum-free conditions, mammalian host cells, suspension culture), which included an increase in the concentration of at least one of the ionic substances indicated in the claim, such as an amino acid with a charged side chain (L-histidine). The methods in tests 6 and 7 of Example 1 of document (22) comprised all features characterising the method of claim 1 and they were directed to the production of recombinant factor VIII, even though only for comparing them with

other methods disclosed in document (22). Thus, these methods anticipated the method of claim 1.

Auxiliary request
Article 123(2) EPC

According to the case law, the claimed subject-matter had to be directly and unambiguously disclosed in the patent application; the patent application could not be used as a reservoir from which features of separate embodiments could be combined to create new embodiments. In the absence of any pointer, it was not allowable to single out an alternative from a list of equally weighted alternatives and to combine it with other alternatives which were themselves selected from lists of other equally weighted alternatives. The less so when all these alternatives were disclosed at different levels of preference (more, even more, particularly preferred; T 1710/09 of 12 April 2011). The method of claim 1 resulted from a selection of alternatives from at least four lists of equally weighted alternatives: a) the target protein (human factor VIII, B-domain deleted mutein thereof), b) the eukaryotic host cells (mammalian cells), c) the mammalian host cells being cultivated in suspension, and d) the specific ionic substances listed in claim 1. The selection of these alternatives, originally disclosed at different levels of preference, resulted in intermediate generalisations with no basis in the patent application.

Claim 1 of the patent application was directed to a method for producing at least one target protein; claim 3(i) and page 11, lines 7 to 30 of the patent application identified this protein, at the broadest level of preference, as being from human, animal,

plants, insects, etc. It was further defined as (preferred) plasma proteins, peptide hormones, growth factors, cytokines and antibodies, wherein the (more preferable) human factor VIII protein and (even more preferable) B-domain deleted factor VIII were cited within a list of more than 22 plasma proteins and blood clotting factors. Claim 3(ii) and page 13, lines 4 to 17 of the patent application provided a list of eukaryotic cells, wherein at the broadest level of preference they were isolated from invertebrates or vertebrates, including (preferred) mammalian cells and (more preferred) HEK 293T cells within a list of more preferred cells. Claim 6(i) of the patent application defined cultivation of these cells either in suspension or adherent culture; however, this claim was not directly dependent on claim 1 but only on claim 5; not all features in claim 5 of the patent application were present in claim 1 of the auxiliary request. The disclosure concerning a suspension culture on page 18, lines 14 to 16 of the patent application referred back to the method of embodiments (1) to (3) disclosed on page 8, line 22 to page 9, line 15 of the patent application; none of these methods was identical to the method of claim 1 of the auxiliary request; the method of embodiment (1) was generic and did not contain any of the limitations present in claim 1 of the auxiliary request. The ionic substances listed in claim 1 of the auxiliary request were an arbitrary selection from the list of ionic substances disclosed in claim 3(iv) and page 14, lines 5 to 26 of the patent application.

The dependency of claim 4 had no basis in the corresponding claim of the patent application nor was it derivable from the patent application as a whole; in particular, there was no basis for the range 0.01 to 0.5 M $CaCl_2$. The particular combination of the specific

salts and preferred salt concentrations in claim 9 with the features of claim 8 was not derivable from the patent application. The choice of the specific concentration ranges given in claim 11, in particular the concentration range of at least 0.4 to 2 M KCl had no basis in the patent application.

Article 56 EPC

The closest prior art document (8) disclosed the production of factor VIII with co-expression of vWF in a serum-free medium using CHO host cells; the secreted recombinant factor VIII remained associated with the membrane of these cells and was subsequently degraded. The tendency of factor VIII to associate with this membrane was overcome by vWF because it displaced the factor VIII, stuck to the outside of the CHO cell, and bound it to its surface. The skilled person was made aware thereby that factor VIII was a peripheral membrane protein. Indeed, document (18) showed the C2 domain of factor VIII to contain a membrane-binding region; thus, binding/sticking to the cell membrane was known to be an intrinsic property of factor VIII. Although the method of document (8) was for large-scale production of factor VIII, claim 1 was not limited thereto; nor was it limited to any mammalian cell, a particular concentration for any of the ionic substances listed in the claim, nor a yield for the factor VIII produced. In view of the scope of claim 1 and the variability in the amount of factor VIII produced in the examples of the patent, the technical problem to be solved was the provision of an alternative method for production of factor VIII. The claimed method was an obvious solution to this problem.

The importance of factor VIII and methods for its industrial production were known before the priority date of the patent (document (3)). Thus, the skilled person had a strong motivation to look for alternative methods. Once the problem was identified, namely that factor VIII was a peripheral membrane protein, the solution lay close at hand for a skilled person and known to every undergraduate student as shown by excerpts from textbooks (10) to (14), which referred to the removal or release of such proteins from the cell membrane by high ionic strength solutions. Thus, it was obvious for a skilled person to try such a basic method and, in view of the positive results obtained with a large number of peripheral membrane proteins reported in these textbooks, a reasonable expectation of success was also given. None of the alleged difficulties would have prevented a skilled person from trying this method. Document (3) showed that, although factor VIII was a large, complex molecule, the skilled person knew how to deal with it even in industrial settings. Moreover, it further stated that vWF was not necessary for stabilising factor VIII. Indeed, a skilled person would have been motivated not to use vWF so as to avoid an additional costly purification step for obtaining pure factor VIII. Although factor VIII was a proteolytically-sensitive protein, measures were known to avoid proteolytic degradation, such as use of low proteolytic host cells (documents (3) and (8)), use of protease inhibitors (document (22)), etc. In any case, no yield was required in claim 1 and the patent reported variable results depending on the selected ionic substances, their concentration, etc. Thus, a skilled person would have tried the method described in documents (10) to (14) with a reasonable expectation of success.

XIII. Appellant I (patent proprietor) requested that the decision under appeal be set aside and that the patent be maintained in amended form upon the basis of the main request or, in the alternative, of the auxiliary request.

XIV. Appellant II (opponent) requested that the decision under appeal be set aside and that the patent be revoked.

Reasons for the Decision

Main request

1. The main request is identical to the first auxiliary request underlying the decision under appeal and thus, it already forms part of the proceedings.

Article 54 EPC

2. Document (22) is concerned with the production of recombinant polypeptides and proteins, in particular polypeptides and proteins secreted into the cell culture medium, which may be impaired by a variety of proteolytic enzymes (cf. column 2, lines 32 to 34). Document (22) refers to this problem in connection with factor VIII and further refers to various solutions that have been suggested for reducing the degradation by proteases of recombinant factor VIII (cf. column 3, lines 11 to 14). In order to increase the half-life of these polypeptides and proteins and, more particularly, that of the recombinant factor VIII, document (22) discloses that "certain protease inhibitors have a surprisingly positive impact on the activity of polypeptides during cultivation of host cells expressing recombinant polypeptides. The presence of

these inhibitors results in higher productivity" (cf. column 4, lines 1 to 11 and lines 29 to 33).

3. The efficiency of the protease inhibitors "according to the invention" is shown in Example 1 of document (22), wherein these inhibitors are compared to other protease inhibitors "not according to the invention" such as, for instance, the amino acid L-histidine with a charged side chain (cf. Table II, tests 6 and 7). Example 1 discloses the production of recombinant factor VIII by mammalian CHO cells "cultivated under growth conditions ... in a complete culture medium such as ASF or a mixture of DMEM and Ham's Medium F-12" at an initial temperature of 37°C. At the beginning of the production phase (day 0), the temperature was lowered to 34°C. "On day 3, the culture medium was placed by a fresh medium including 0.5 mM of butyric acid ... On day 4, a suspension of the cells in production was alequoted to polypropylene tubes for continuous cultivation and the protease inhibitors were added. On day 5, the medium was replaced and the protease inhibitors added. Replacement of medium was performed on day 6, day 7, day 10 (accumulated value after 72 hours). On day 11, the experiments were stopped" (cf. column 10, lines 10 to 36).

4. Document (21) shows that the commercial serum-free DMEM/F-12 medium used in Example 1 of document (22) contains a concentration of 0.15 mM L-histidine. In tests 6 and 7 of this example, the (physiological) concentration of L-histidine was increased to the (non-physiological) concentrations of 0.52 mM and 5.2 mM. In both tests, 6 and 7, recombinant factor VIII was produced (accumulated value from day 6 to day 11 of 43.9 IU/ml and 18.3 IU/ml, respectively; cf. Table II) albeit at a lower level than when produced in the

presence of the protease inhibitors "according to the invention" (cf. Table I). Nevertheless, when the experiments were stopped at day 11, the highest cell viability of all tests - with inhibitors "according to the invention" and "not according to the invention" - is reported to be this of test 7 (95.4%), and the cell viability of test 6 (92.8%) is not much different from that reported for the other inhibitors "according to the invention" (cf. Tables III and IV).

5. It is well-known in the field of recombinant protein production in mammalian host cells that a temperature shift (lowering the temperature at day 0) between cultivation and production conditions and the addition of butyric acid (at day 3) enhances the production of the recombinant protein. Whilst a temperature shift arrests the cell cycle and reduces the metabolic rates, butyric acid inhibits histone deacetylases and promotes histone hyperacetylation, resulting thereby in a higher or enhanced DNA transcription. Thus, the production of recombinant factor VIII as disclosed in document (22) is not due to the presence of L-histidine in the medium but to the temperature shift and the addition of butyric acid.

6. The purpose and effect of using increased concentrations of L-histidine in the method disclosed in document (22) are different from those disclosed in the patent. This is also reflected by the different amounts of histidine added into the culture media used in Example 1 of document (22) and into the media used in the examples of the patent.

However, claim 1 is not a use-claim directed to the use of an amino acid with a charged side chain (histidine), but a method-claim directed to the production of (at

least) one target protein (factor VIII or a B-domain deleted mutein thereof). Claim 1 does not describe all the specific steps of said method but requires the culturing of mammalian cells in suspension under serum-free conditions and to subject "a suspension of said cells, prior to separation of the protein from the cells, to a non-physiologically increased concentration of at least one ionic substance selected" from the list of ionic substances indicated in the claim. A temperature shift and/or the addition of butyric acid into the culture medium are not excluded from the claimed method (cf. paragraph [0164] of the patent).

7. There is no limitation in claim 1 to the amount of (at least one) ionic substance added into the culture medium nor any purpose-related limitation or intended-effect associated with the (non-physiologically) increased concentration of this substance. Moreover, claim 1 does not require any particular yield nor does it define any control or standard method for comparison. Therefore, according to the case law of the Boards of Appeal, none of these features limits the scope of claim 1 or is taken into account for delimiting the claimed method from another method disclosed in the art such as the method described in document (22) (cf. "Case Law of the Boards of Appeal of the EPO", 8th edition 2016, I.C.4.8, 110,; *inter alia*, T 2487/12 of 27 October 2015, point 1.13 of the Reasons; T 1634/15 of 14 October 2016, point 11 of the Reasons).

8. Appellant I further argues that, according to the established case law, the indication of a purpose in a method-claim must be taken into account when assessing novelty and, since the methods exemplified in tests 6 and 7 of document (22) resulted in a significantly

lower production of factor VIII as compared to a control method without histidine, these methods were not appropriate for the production of factor VIII; they were actually methods for inhibiting such a production (cf. point XI *supra*).

9. While the indication of a purpose in a method-claim for attaining a functional effect must be taken into account when assessing novelty, this is not the case for a method-claim aimed at the production of a specific product (cf. "Case Law", *supra*, I.C.8.1.3.b), 150, and I.C.8.1.3.c), 152; see, *inter alia*, T 633/13 of 17 October 2018, points 12 to 14 of the Reasons). In any case, the methods reported in tests 6 and 7 of Example 1 of document (22) produce factor VIII, i.e. the same product as the method of claim 1. Although the methods of tests 6 and 7 result in lower amounts of human factor VIII, particularly when compared with the methods "according to the invention" described in document (22), the sole purpose of these methods is the production of factor VIII, even though admittedly only for comparative purposes. The purpose of these methods is not to inhibit the production of factor VIII, certainly not for the method of test 6, which yields an accumulated amount of factor VIII similar (93%) to that achieved using a standard method.

10. In view of all the above considerations, the method of claim 1 lacks novelty over document (22).

Auxiliary request

11. The auxiliary request is identical to the second auxiliary request underlying the decision under appeal and thus, it already forms part of the proceedings.

Article 123(2) EPC

12. In essence, appellant II argued that the combination of features present in claim 1 had no basis in the patent application, even though there was a basis for each of these features (cf. point XII *supra*).

13. A first limitation introduced into claim 1 is the selection of "human factor VIII or a B-domain deleted mutein thereof" as the target proteins for recombinant production. It is not disputed that these proteins are disclosed as preferred target proteins in the patent application (cf. page 11, lines 27 to 30). Indeed, claim 3(i) of the patent application refers to human factor VIII and to a B-domain deleted mutein thereof as a more preferred and as an even more preferred target protein, respectively. The relevance of these proteins is directly derivable from the examples of the patent application, wherein the production of a recombinant B-domain deleted human factor VIII is exemplified (see page 26, lines 10 to 13 for the expression plasmids) and the absorption studies of target proteins to cell membranes are carried out by using human factor VIII and B-domain deleted human factor VIII (see Examples 16 to 19).

14. The examples of the patent application are all carried out in a serum-free medium (cf. paragraph bridging pages 26 and 27) using mammalian cell lines (cf. paragraph bridging pages 25 and 26) cultured in suspension (cf. page 27, lines 9 to 13). Mammalian cells adapted to serum-free culture conditions are disclosed as particularly preferred cells (cf. page 13, lines 8 to 10 and 17 and 18) and, whilst the methods for the production of the recombinant protein are all exemplified with several lines of HEK 293T (human

embryonic kidney) cells, in the exemplified absorption studies BHK (Baby hamster kidney) cells are also used. Claim 2 of the patent application refers to the addition of the ionic substance into the cell suspension during continuous cultivation and claim 6 refers to the cultivation in suspension culture or adherent culture. The same disclosure is found on page 18, lines 14 to 16 of the patent application referring to the methods of embodiments (1) to (3), wherein embodiment (1) corresponds to the method of claim 1 of the auxiliary request but in a more generic form.

15. The ionic substances listed in claim 1 of the auxiliary request are disclosed as the most preferred ionic substances in claim 3(iv) of the patent application. The particularly preferred ionic salts and preferred amino acids with charged side chains are also disclosed on page 14, lines 20 to 24 of the patent application. The deletion of L-histidine in claim 1 of the auxiliary request from the list of three specific amino acids with charged side chains present in the disclosures of the patent application is a mere limitation and it does not broaden the scope of the claim nor does it create new subject-matter or an intermediate generalisation, the skilled person is not presented with information not originally disclosed in the patent application (cf. "Case Law", *supra*, II.E.1.10, 448). Most but not all ionic substances and combinations listed in claim 1 of the auxiliary request are exemplified in the patent application.

16. In view thereof, the board considers that the combination of features in claim 1 of the auxiliary request is directly and unambiguously derivable from the patent application. In the board's view, it is not

the result of an arbitrary combination of features which, according to appellant II, are disclosed at different levels of preference in the patent application. On the contrary, these features are all disclosed as particularly preferred or more preferred alternatives in the patent application, and combinations of these features are directly exemplified in the patent application. Thus, the subject-matter of claim 1 of the auxiliary request does not contravene Article 123(2) EPC.

17. As regards the objection of added subject-matter raised against dependent claims 4, 9 and 11, the following issues are relevant:

17.1 Claim 4 of the auxiliary request corresponds to claim 4 of the patent application except for the range 0.01 to 0.5 M $CaCl_2$. Claim 4 of the patent application is dependent only on claim 3, which is itself dependent on claims 1 and 2. Although the dependency of claim 4 of the auxiliary request is different - since it is directly dependent on claims 1 to 3, the combination of the subject-matter of claim 4 of the auxiliary request with that of either claims 1 or 2 does not result in any specific new subject-matter. The less so in view of the disclosure on page 15, line 25 to page 17, line 3 of the patent application, wherein "the preferred mode of addition and the preferred concentration of the preferred ionic substances" are described in general terms for all embodiments of the invention. As regards the range 0.01 to 0.5 M $CaCl_2$, it is a combination of a lower limit of one - out of eight - preferred sub-ranges (0.01 to 0.1 M; cf. page 16, line 13 of the patent application) with the upper limit of the broadest preferred range (>0.002 to 0.5 M; cf. page 16, line 9 of the patent application). According to the

case law of the Boards of Appeal, such a combination of range limits does not contravene Article 123(2) EPC (cf. "Case Law", *supra*, II.E.1.3.1, 414; decision T 2/81, OJ EPO 1982, 394).

17.2 The subject-matter of claim 9 of the auxiliary request is disclosed on page 17, lines 8 to 18 of the patent application, in particular lines 13 to 18 for the range of concentrations mentioned in that claim (see also claim 8 of the patent application). The concentration ranges cited in claim 11 of the auxiliary request are disclosed on page 16, line 1 to page 17, line 3 of the patent application, wherein the range 0.4 to 2 M KCl results from a direct combination of lower and upper limits of preferred subranges. As for claim 4, such a combination does not result in new subject-matter.

18. Thus, the auxiliary request does not contravene Article 123(2) EPC.

Article 54 EPC

19. The findings of the opposition division on Article 54 EPC have not been contested in appeal proceedings. According thereto, the claimed subject-matter is novel (cf. page 11, point 21.12 of the decision under appeal).

Article 56 EPC
The closest prior art document (8)

20. In the board's view, the closest prior art document (8) is concerned with the production of recombinant non-activated (native, intact) human factor VIII and not with activated factor VIII (factor VIIIa). Moreover, the board considers that document (8) discloses that

the recombinant human factor VIII behaves as a peripheral membrane protein.

20.1 Document (8) discloses the production of recombinant human factor VIII in a suspension culture of CHO host cells and states that "when the cells are grown in a serum-free medium, factor VIII was not expressed". However, it further states that "[i]t was this phospholipid membrane-binding [intrinsic] characteristic of the factor VIII molecule that disallowed its production in serum-free medium. When secreted, it remained associated with the plasma membrane of the CHO cells and subsequently degraded" (cf. page S10, left-hand column, first full-paragraph). According to document (8), co-expression with vWF overcomes "the tendency of factor VIII to associate with the plasma membrane of the CHO cell (Fig. 5), and the protein which was stuck to the outside of the cell became displaced onto the surface of the vWF molecule, where it was subsequently stabilized and protected from proteolysis" (cf. page S10, left-hand column, last full paragraph). Document (8) reports the advantages of a CHO "cell that contained multiple copies of both the factor VIII and the vWF gene ... engineered to be completely serum-independent" (cf. page S10, right-hand column, last two sentences). The production CHO cell line produces only one unique transcript corresponding to the intact factor VIII (cf. page S9, right-hand column, second paragraph; page S10, Figure 1, and page S12, paragraph bridging left and right-hand columns); the production of "highly intact factor VIII" is stated to be "the aim in the production of factor VIII" (cf. page S9, right-hand column, last sentence of the first paragraph). There is no reference in document (8) to factor VIIIa, even though Figure 3 of

this document shows the position of factor VIIIa with the plasma membrane of the platelet.

20.2 Figure 5 of document (8) illustrates the synthesis and processing of factor VIII in mammalian cells in general. According thereto, the primary translation product is translocated into the lumen of the endoplasmic reticulum (ER) and a minor proportion (10%) thereof attains the proper conformation to be transported into the Golgi, wherein factor VIII is cleaved to its mature form with a heavy chain (A1-A2-B domains; about 210 kDa MW, with fragments ranging from 180 kDa to 90 kDa) and a light chain (A3-C1-C2 domains; 80 kDa MW). The mature factor VIII is held together by metal (Cu) ion bridges and Figure 5 shows it associated with the cell membrane by the C2 domain.

20.3 There is no mention in any of these paragraphs and in Figure 5 to the activation of factor VIII by thrombin cleavage and it is derivable from these disclosures that vWF and the membrane phospholipid compete for factor VIII binding in the light chain (through the C2 domain) so that vWF promotes the displacement of factor VIII (the C2 domain) from the membrane phospholipid surface of the cell. The activation of factor VIII (when associated with vWF) by thrombin cleavage releases vWF and allows the activated factor VIII (factor VIIIa) to bind again to the cell membrane.

20.4 This interpretation is not contradicted by any other document on file and is fully in line with the prior art acknowledged by the patent itself. In paragraph [0005] of the patent, it is stated that "when utilizing mammalian cells as production hosts ... the secretion of the produced proteins is rather low. It is apparent that secreted products often adhere to the

cell membrane and that this has an influence on the product release". This is also known to be the case for the CHO cells when used as mammalian host cells (cf. page 4, lines 3 to 6 and 10 to 12 of the patent). Indeed, according to the patent itself, both the technical problem addressed by the patent and the proposed solution are based on, and explained by, this background knowledge from the prior art (cf. paragraphs [0052] and [0053] of the patent).

The objective technical problem and the proposed solution

21. Starting from this prior art, the objective technical problem to be solved is the provision of an alternative method for the production of recombinant human factor VIII or a B-domain deleted mutein thereof. At the oral proceedings before the board, appellant I did not pursue the formulation proposed at an earlier stage of the appeal proceedings and before the opposition division, namely the provision of an improved method for the production of these proteins (cf. page 9, last full sentence in the last paragraph and page 14, lines 8 to 10 of appellant I's reply to the grounds of appeal of appellant II; page 11, point 22.3 of the decision under appeal).

21.1 Although none of the examples disclosed in the patent provides a direct comparison of the claimed method with that described in document (8), the reference methods used in this document and in the patent (cf. page 13, paragraph [0077]; see for instance "reference CS1" on page 13, Table 1 of the patent) are the same, namely a method for producing recombinant human factor VIII with neither co-expression of vWF nor an increased concentration of (at least) one ionic substance selected from those listed in claim 1. Whilst the

method of document (8) results in a four times increased production of recombinant human factor VIII when compared to the reference method (cf. page S10, left-hand column, second full paragraph, and page S11, Figure 4 of document (8)), the method disclosed in the examples of the patent may result in a 20 fold increased production of recombinant human factor VIII when compared to the reference method (cf. page 14, Table 2 of Example 2; page 19, Table 11 of Example 5A; and page 20, Table 12 of Example 5B). Thus, although through indirect comparison, there is evidence on file that the claimed method may provide an improvement over the method described in document (8).

21.2 However, according to the case law, the comparative tests have to demonstrate that the advantageous effect is attained over the whole area claimed (cf. "Case Law", *supra*, I.D.10.9, 251). In the board's view, the examples of the patent show that the results obtained are highly dependent on the nature of the ionic substance(s) selected as well as on their concentration. Claim 1 does not comprise any limitation as regards the mammalian host cell (cf. Figure 2 of document (8) showing the low proteolytic activity of CHO cells compared to that of BHK cells), the ionic substances selected and their concentrations. In view of the results shown in the examples of the patent and the scope of claim 1, the board considers that an advantageous effect cannot be achieved over the whole scope of claim 1 and therefore, the objective technical problem must be formulated in less ambitious terms (cf. "Case Law", *supra*, I.D.4.4, 177), namely in those formulated by the parties at the oral proceedings before the board.

22. It is common ground between the parties that the claimed subject-matter solves the technical problem formulated in the less ambitious terms and, indeed, over the whole scope of the claim.

Obviousness of the proposed solution

23. In view of the textbook documents (10) to (14), the board acknowledges that the removal of peripheral membrane proteins from a cell membrane by using high ionic strength solutions (such as 1 M NaCl or 1 M KCl in documents (13) and (14)) is part of the common general knowledge of a person skilled in the art and that, once the recombinant human factor VIII was identified as behaving as a peripheral membrane protein (document (8)), it was obvious for the skilled person to release the recombinant human factor VIII from the mammalian host cell membranes by using solutions of high ionic strength, i.e. the skilled person **could** have applied conditions of high ionic strength for dissociating the recombinant factor VIII "stuck to the outside of the [CHO host] cell" (cf. page S10, left-hand column, last full paragraph of document (8)).

24. However, according to the case law, the relevant question in such a situation is not whether the skilled person could have done it but whether it **would** have done it, i.e. whether the skilled person would have redesigned the co-expression system described in document (8) and modified it by applying conditions of high ionic strength, i.e. increasing the concentration of at least one of the ionic substances listed in claim 1 (cf. "Case Law", *supra*, I.D.5, 183). In the board's view, this question can only be answered in the positive. For the board to arrive at this conclusion, the following points are relevant:

25. The question whether a skilled person would do something may be answered by assessing first whether there was any motivation or incentive for the skilled person to do it and, if this question is answered in the positive, to assess then the expectations of the skilled person for doing it.

25.1 As regards the first question, although the case law defines the skilled person as being cautious and having a conservative attitude (cf. "Case Law", *supra*, I.D. 8.1.3, 191), it also acknowledges that furthering the existing state of art belongs to the normal tasks of the skilled person and that routine adaptations or trials as well as the use of known alternatives do not go beyond what may be normally expected from an average person skilled in the art (cf. *inter alia*, T 455/91, OJ EPO 1995, 684, point 5.1.3.3 of the Reasons; T 659/00 of 1 July 2003, points 8 and 9 of the Reasons; T 769/03 of 23 September 2004, point 6 of the Reasons; T 1439/04 of 22 June 2006, point 3 of the Reasons). In the present case, the skilled person was well aware of the commercial relevance of human factor VIII as reflected by the widespread interest for its industrial production (cf. document (3)). Thus, there is no doubt that a skilled person would be highly motivated to seek appropriate modifications, changes and alternatives to the methods for production described in the art. In the present case, the proposed solution is part of the common general knowledge of the skilled person; it does not require the skilled person to turn to a particular prior art document, the combination of teachings from different technical fields, etc. Moreover, it neither takes incalculable risks nor goes against any prejudice, on the contrary, it relies on a basic technique that was widespread in the relevant technical

field and, as put forward by appellant II, was known to every undergraduate student.

25.2 As regards the expectations of the person skilled in the art, the board considers that they are always directly associated with the intended purpose and thus, intrinsically linked to the formulated technical problem. When the intended purpose or the technical problem are formulated in very ambitious terms, such as the achievement of a significant improvement or a surprisingly advantageous effect over the prior art, the expectations of the skilled person to succeed are correspondingly more difficult to be fulfilled. The expectations to succeed are certainly much greater, when the intended purpose or the technical problem are formulated in less ambitious terms, such as in the present case, namely the provision of a simple alternative to the method known from the prior art.

25.3 It is also worth noting the case law dealing with the assessment of an expectation of success for what in this case law has been called a "try and see" situation. According thereto, there is a difference between the expectation to succeed and the certainty to success, the latter not being required for deciding on a lack of inventive step (cf. "Case Law", *supra*, I.D. 7.1, 185). Indeed, the case law acknowledges that, in certain cases, a skilled person may adopt a "try and see" attitude and, in these cases, there is not need to have any sort of expectation (cf. "Case Law", *supra*, I.D.7.2, 187). Whilst appellant II argues that this situation applies to the present case, appellant I argues that, in the present case, the skilled person would have adopted a sceptical attitude and not a "try-and-see" attitude.

25.4 The board agrees with appellant I that document (8) discloses that vWF not only overcomes the tendency of the recombinant factor VIII to associate with the CHO membrane but also to stabilize and protect it from proteolysis by acting, in the words of appellant I, as a blanket or chaperone (cf. page S10, left-hand column, last full paragraph of document (8)). However, contrary to appellant I, the board does not consider that, in view of the effects of vWF, there were reasons for a skilled person to adopt a sceptical attitude when looking for a method for the production of recombinant human factor VIII without co-expression of vWF. In the board's view, document (8) not only informs the skilled person of the presence of several problems when trying such a method but it also provides means and measures to overcome them, such as the selection of available host cell lines with low proteolytic activity. Indeed, these problems were already known in the art and means and measures to solve them were also provided therein, such as the use of protease inhibitors in document (22). None of these means and measures is excluded from the scope of claim 1 of the auxiliary requests. There was no reason for a person skilled in the art to ignore all the information at hand and to select the worst possible known system or method when facing a "try-and-see" situation. It is worth noting here that, in the present case, the skilled person was not looking for an improvement or a surprisingly advantageous effect, but only for an alternative method.

26. It follows from the above that the person skilled in the art could and, in the board's view, would have modified the system described in document (8) by applying conditions of high ionic strength, i.e.

increasing the concentration of at least one of the ionic substances listed in claim 1.

27. As a further argument to support inventive step, appellant I referred to document (18) as providing an alternative for replacing the vWF in the method disclosed in document (8) which was much closer to the co-expression system disclosed in document (8), namely a co-expression of human factor VIII with the antibody BO2C11 whose binding site for factor VIII includes or overlaps the factor VIII binding site for vWF. The board does not agree and considers that, as stated in the case law, "the mere fact that it is possible to imagine other, more or less obvious solutions does not necessarily imply that an invention involves an inventive step, or can be regarded as an inventive selection" (cf. T 214/01 of 7 March 2003, point 3.11 of the Reasons; T 190/03 of 29 March 2006, point 14 of the Reasons). In the present case, the alleged presence of another possible obvious solution is not relevant and does not change the findings of obviousness for the proposed solution, i.e. the method of claim 1.

28. Appellant I has also referred to what has been called in the case law "secondary indicia" for supporting an inventive step (cf. "Case Law", *supra*, I.D.10, 243). The board is however not able to follow this argumentation.

28.1 Although the case law acknowledges that, in certain situations, a simple solution may be indicative of inventive step (cf. "Case Law", *supra*, I.D.10.7, 249), this is not always the case. In the decisions dealing with this situation, the simple solution did not lower the yield of the method and/or the quality of a product known in the art; the product being of commercial

interest and produced in industrial settings. Moreover, in most cases, the simple solution brought about a surprising (functional) improvement and/or resulted in an improved effect. However, even though the commercial interest of human factor VIII was well-known (*inter alia*, document (3)) and reference is made in the patent to 10 L bioreactors (cf. paragraphs [0076] and [0150]; see also page 24, Example 11 of the patent), the claimed method is neither limited to the large-scale production in an industrial setting nor does it require to achieve any surprising effect or improvement at all.

28.2 Appellant I has also referred to what has been called in the case law the "time factor", namely the time lapsed between the publication of the closest prior art (document (8), 1994) and the filing date of the patent (2006), as an additional indication of inventive step. Again, even though the case law acknowledges that the time factor may in certain cases be indicative of inventive step (cf. "Case Law", *supra*, I.D.10.3, 246), this is not always the case. The failure to adopt an obvious solution has been associated with a variety of possible causes, in particular, those related to commercial reasons such as the avoidance of investment costs involved in the adoption of a new technique on an industrial scale (cf. T 123/97 of 10 September 1998, point 2.4.5 of the Reasons; T 516/08 of 16 December 2009, point 10 of the Reasons). Moreover, in the present case, the time lapsed between the publication date of the industrial production of recombinant human factor VIII in serum-free conditions (mid- or in the late nineties; cf. documents (3) and (8)) and the priority date of the patent (early 2005) does not compare with the time lapsed in most of the cases underlying the relevant decisions referred to in the case law (over 20, 60, up to 100 years).

29. In view of all these considerations, the board arrives at the conclusion that the auxiliary request does not fulfil the requirements of Article 56 EPC.

Conclusion

30. In the absence of a request that fulfils the requirements of the EPC, the patent must be revoked.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

C. Rodríguez Rodríguez B. Stolz

Decision electronically authenticated