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D E C I S I O N
of 26 November 2003

Case Number: T 1059/01 - 3.3.8

Application Number: 90901443.3

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Language of the proceedings: EN

Title of invention:
Process for the preparation of human DNase

Patentee:
Genentech Inc.

Opponent:
ANTISOMA PLC

Headword:
Human DNase/Genentech

Relevant legal provisions:
EPC Art. 56

Keyword:
"All requests - inventive step (no)"

Decisions cited:
T 0455/91

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1059/01 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 26 November 2003

Appellant: (Proprietor of the patent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 18 July 2001 revoking European patent No. 0449968 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: M. R. Vega Laso
M. B. Günzel

Summary of Facts and Submissions

I. The appeal lies from the decision of the opposition division issued on 18 July 2001 whereby European patent No. 0 449 968 with the title "Process for the preparation of human DNase" was revoked pursuant to Article 102(1) EPC. The patent had been opposed on the grounds of Article 100(a) and (b) EPC, in particular lack of inventive step (Article 56 EPC) and lack of sufficient disclosure (Article 83 EPC).

II. The opposition division decided that neither the subject-matter of the claims of the main request (claims as granted) nor that of the three auxiliary requests then on file involved an inventive step. With respect to the opposition ground of Article 100(b) EPC the opposition division considered that the requirements of Rule 55(c) EPC were not met, because the alleged objection that the claimed subject-matter was not sufficiently disclosed in the patent had not been substantiated within the time limit of Article 99(1) EPC.

III. On 24 September 2001, the patentee (appellant) filed a notice of appeal and requested that the decision of the opposition division be set aside. On 27 November 2001, the appellant submitted a statement of grounds of appeal accompanied by six new auxiliary requests that replaced the auxiliary requests filed during the opposition proceedings. The main request remained the maintenance of the patent as granted. To support its line of argumentation, the appellant submitted three new documents. Oral proceedings were requested in the

event that the board decided not to follow any of the appellant's requests.

IV. Claim 1 as granted (**main request**) read as follows:

"1. A process for producing a polypeptide having DNase activity comprising (i) transforming a host cell with nucleic acid encoding a polypeptide which comprises the mature human DNase amino acid sequence shown in Figure 1 or a substitutional, insertional or deletional variant thereof having DNase activity, which variant is not immunogenic in humans, (ii) growing in culture host cells that express the polypeptide having DNase activity, and (iii) recovering the polypeptide from the culture."

Independent claim 2 was directed to a process for producing variants of the DNase polypeptide having a single amino acid substitution at one residue of the mature human DNase amino acid sequence shown in Figure 1 of the patent, the variants not being subject to any limitation with regard to their immunogenicity. Claim 3 was directed to a process according to claim 1, the produced polypeptide being defined by means of the nucleotide sequence encoding it. Claims 4 to 17 were dependent claims directed to further embodiments of the process. Finally, independent claims 18 and 19 were directed to compositions in which the transformed host cells are growing in culture and expressing the polypeptide as defined in claims 1 and 2 respectively.

V. Claim 1 of the **first auxiliary request** as filed on appeal differed from claim 1 of the main request in that the host cells producing the polypeptide having

DNase activity were limited to eukaryotic cells or *E. coli*, in the latter case the polypeptide being expressed as an intracellular protein and being recovered from within the cell. Independent claims 2, 18 and 19 included the same limitation. The wording of dependent claims 3 to 17 remained unchanged.

VI. Claim 1 of the **second auxiliary request** differed from that of the first auxiliary request in that it contained an additional limitation in respect of the production of the polypeptide in eukaryotic cells. For the production of a polypeptide having DNase activity said host cells were transformed with a nucleic acid that encoded a preprotein comprising the polypeptide, and the transformed host cells expressed the preprotein and secreted the polypeptide into the culture medium. Claims 4, 5, 7 and 8 of the first auxiliary request were omitted and the remaining claims renumbered. The same limitation as in claim 1 was found in claim 2 and in renumbered claims 14 and 15.

VII. Claims 1, 2, 14 and 15 of the **third auxiliary request** differed from the corresponding claims in the second auxiliary request in that the preprotein comprised the polypeptide and the native human DNase signal, the amino acid sequence of which was shown underlined in Figure 1 of the patent.

VIII. The **fourth and fifth auxiliary requests** were concerned exclusively with processes for producing a polypeptide having DNase activity in eukaryotic cells. Claims 1, 2, 14 and 15 of the fourth auxiliary request included the same limiting features specified in the second auxiliary request (preprotein comprising the

polypeptide was expressed in host cells which secrete the polypeptide into the culture medium). In the fifth auxiliary request, claims 1, 2, 14 and 15 included the same limitations as the corresponding claims in the third auxiliary request (native human DNase signal).

IX. The **sixth auxiliary request** was exclusively concerned with processes for producing a polypeptide having DNase activity in *E. coli*, the limiting features being the same as in the corresponding embodiment in the first, second and third auxiliary requests.

X. Claims 3 to 13 of the third, fourth, fifth and sixth auxiliary requests essentially corresponded to those of the second auxiliary request but referred to the specific processes defined above.

XI. The respondent (opponent) submitted his comments on the statement of grounds of appeal together with copies of six newly-cited documents. In its submission, the respondent discussed comprehensively the issue of inventive step (Article 56 EPC) with regard to the requests on file, and referred to the issue of sufficiency (Article 83 EPC). The appellant submitted counterarguments and requested remittal of the case to the opposition division should the new material filed by the respondent be considered relevant.

XII. The parties were summoned to oral proceedings. In a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal sent with the summons, the board expressed the provisional opinion that the essential issue to be discussed at oral proceedings in relation to the main request was that of inventive step,

and that the reference by the respondent to the issue of sufficiency was understood by the board merely to emphasise the need to apply the same criteria when evaluating the disclosure content of the prior art and that of the contested patent within the framework of the judgment on inventive step.

XIII. In response to the question raised by the board in its communication as to the basis under Article 123(2) EPC or Rule 57a EPC for amendments introduced in some of the new auxiliary requests, the appellant sent further comments. Oral proceedings were held on 26 November 2003.

XIV. The documents referred to in the present decision are the following:

(3) M. Takahara et al., J. Biol. Chem., 1985, Vol. 260, pages 2670 to 2674;

(4) R. Quaas et al., Eur. J. Biochem., 1988, Vol. 173, pages 617 to 622;

(5) D. Shields et al., Biochemical Society Transactions, 1988, Vol. 16, pages 195 to 196;

(6) R. W. Old and S. B. Primrose, Principles of Gene Manipulation, 3rd Edition, 1985, Blackwell Scientific Publications, pages 99 to 101 and 199;

(7) M. M. Bendig, Genetic Engineering, 1988, Vol. 7, pages 91 to 127; and

(8) R. Sasada et al., Cell Structure and Function, 1988, Vol. 13, pages 129 to 141.

XV. The appellant's submissions in writing and during oral proceedings may be summarised as follows:

With regard to the main request, the appellant argued that it would not have been obvious to the skilled person at the priority date of the patent to try to use a cell culture for the production of human DNase, and the prior art did not provide any technical basis for a reasonable expectation of success. For the first time in the patent in suit was it shown to be possible to produce human DNase in cell culture in sufficiently large quantities for commercial use.

As to the production of human DNase as an intracellular protein in *E. coli* claimed in the first auxiliary request, the appellant argued that the fact that the production of human DNase as intracellular protein in recombinant *E. coli* cells does not give rise to the toxicity and cell death reported in document (5) for bovine DNase was entirely unexpected and unpredictable at the priority date. Documents (3) and (4) were irrelevant in this regard because the embodiment did not relate to prokaryotic secretion.

With respect to the alternative production of human DNase in eukaryotic cells, the appellant argued that there could be no expectation that a recombinant host cell forced by genetic engineering to produce DNase would necessarily be able to apply the mechanisms of sequestering and containment used by cells in vivo. Since cells growing and dividing lacked nuclear

membranes, the produced DNase could come into contact with the DNA of the cell and degrade it.

As to the use of the native human DNase signal for directing secretion in eukaryotes, the appellant contended that it was not obvious before presentation of the disclosure in the patent that human DNase had a signal in the first place. But even if the sequence of the native signal had been available, there was nothing in the prior art that made it obvious to the ordinary person skilled in the art to employ the human DNase signal as opposed to a heterologous signal.

XVI. The respondent's submissions in writing and at oral proceedings may be summarised as follows:

Any of documents (3), (4) or (5) could be considered as the closest prior art in respect of the subject-matter of claim 1 of the main request (production of human DNase in host cells growing in culture). Starting from document (5) and seeking to avoid the toxicity problems described in this document in connection with the expression of bovine DNase in *E. coli*, the skilled person would have applied the method disclosed in document (4), thus arriving at a process falling under the terms of claim 1 of the patent in suit. Further, the production of human DNase in eukaryotic cells would have been obvious in view of documents (3) or (4) combined with general knowledge as exemplified in documents (6), (7) and/or (8). In the light of documents (3) or (4) also the expression of the human DNase as a preprotein did not involve an inventive step.

As regards the use of the native signal sequence of the human DNase, once the gene encoding human DNase had been isolated the skilled person would readily have been able to identify such a signal, since the characteristic structural properties of signal sequences were well known in the art at the priority date of the contested patent.

Finally, with respect to the production of human DNase as intracellular protein in *E. coli* the respondent argued that, in view of document (5), the problem to be solved was to avoid leakiness in the expression system that was utilised. The obvious solution was the use of more tightly controlled expression vectors as disclosed in the prior art.

XVII. The appellant requested that the decision under appeal be set aside and that the patent be maintained as granted or, auxiliarly, on the basis of one of the auxiliary requests 1 to 6 filed on 27 November 2001.

The respondent requested that the appeal be dismissed.

Reasons for the Decision

Admission of new documents

1. In the present case the parties relied on new documents filed either at a late stage of the opposition proceedings or on appeal, these documents not having been considered by the opposition division when reaching its decision. In the board's judgment, none of the new documents introduces into the proceedings any

facts, evidence or arguments that might be more relevant to the case than those presented in the notice of opposition. However, after hearing the parties and in view of the fact that the appellant did not strongly oppose to it, the board has decided to admit all new documents submitted by the parties.

Main request

2. In the contested decision the opposition division came to the conclusion that the subject-matter of claim 1 as granted did not involve an inventive step in view of a combination of documents (5) and (4). Thus, the question at issue is whether, having regard to the state of the art at the priority date, the subject-matter of claim 1 was not obvious to a person skilled in the art (Article 56 EPC).

3. The closest prior art is represented by document (5) which describes the cloning of part of the gene for bovine DNase I and the production of a fusion protein having DNase activity in *E. coli*. In an attempt to clone the bovine DNase gene, a cDNA library was prepared from mRNA extracted from bovine parotid gland, the cDNA fragments being inserted at the 3'-terminus of the *lacZ* gene under the control of a temperature-sensitive promoter. When *E. coli* cells transformed with a construct comprising a putative fragment of the bovine DNase gene were grown at 30°C, no fusion product could be detected, whereas at 42°C the cells were non-viable. Thus, it was concluded that the cloned insert must direct the synthesis of a toxic product.

4. It can be derived from document (5) that the production of a protein with DNase activity in *E. coli* host cells could arrest cell growth in consequence of its toxic effect. In the light of document (5), the objective technical problem underlying the patent in suit can then be defined as the provision of a method to produce **human** DNase in host cells **growing** in culture.

5. Although the production of **human** DNase is not suggested in document (5), it was nonetheless obvious in view of the known drawbacks associated with a commercial bovine DNase preparation (Dornavac, Merck; see background art referred to in paragraph [0004] of the contested patent) that the human protein was more desirable for therapeutic purposes. The person skilled in the art was well aware of the advantages of constitutive expression in a continuous cell culture for the production of a given protein. Thus, to think of providing such a method for the production of human DNase was *per se* not inventive.

6. The problem stated above is solved according to claim 1 of the patent in suit by transforming a host cell with a nucleic acid that encodes a polypeptide comprising the mature human DNase amino acid sequence set forth in Figure 1 or a variant thereof having DNase activity, and growing the transformed host cells that express human DNase in culture. The proposed approach is exemplified in Examples 3 and 4 of the patent, where the production of human DNase is shown both in eukaryotic (human embryonic kidney cells and CHO cells) and prokaryotic cells (*E. coli*).

7. Thus, the question arises whether the skilled person faced with the stated technical problem would have arrived at a process falling under the terms of claim 1 by combining the teaching of document (5) with other relevant prior art and/or the general common knowledge at the priority date.

8. Claim 1 of the contested patent requires that, in a first step of the process for the production of DNase a host cell be transformed with a nucleic acid which encodes a polypeptide comprising the amino acid sequence of the human DNase protein. However, at the priority date of the patent in suit the human DNase gene had not yet been isolated. In the contested decision the opposition division came to the conclusion that, on the basis of the information available at the priority date (see paragraph [0002] of the contested patent for a review of the prior art), the skilled person could reasonably have expected to clone the human DNase gene by means of routine experimentation. The board notes that in the light of the prior art on file there are *prima facie* no technical problems or uncertainties that would either deter the skilled person from applying standard cloning strategies in order to clone the human DNase gene, or put in jeopardy any expectation of success. The appellant, neither during the proceedings before the opposition division nor in appeal proceedings, put forward any evidence or arguments that could support an inventive step with respect to the cloning of the human DNase gene. Thus, the board sees no reason to question the findings of the opposition division.

9. The board cannot follow the appellant's argument that the disclosure of document (5) would have motivated the skilled person to use a cell-free system in order to overcome possible cell toxicity problems associated with the DNase activity. The use of a rabbit reticulocyte lysate as disclosed in document (5) with the aim of confirming the presence of DNase mRNA in RNA isolated from bovine parotid gland, would not be considered by the skilled person to be relevant in the context of producing DNase in host cells growing in culture, because a **cell-free** system could not solve the problem of producing a protein in a host cell **growing in culture**. Furthermore, an average skilled person would have been aware of the fact that by translating mRNA in a cell-free system only small quantities of the desired product can be obtained, such quantities being perhaps suitable for a preliminary analysis (as performed in document (5)) but certainly not for pharmaceutical purposes.

It is therefore concluded that, having regard to the disclosure of document (5), a skilled person seeking to produce human DNase in host cells would have regarded the expression of the human DNase gene in host cells using recombinant DNA techniques as the most feasible method to solve the stated problem.

10. The question which remains to be answered in relation to the subject-matter of claim 1 is whether an inventive step can be acknowledged with respect to the provision of transformed host cells **growing** in culture **while** producing human DNase protein.

11. The board concurs with the opposition division in that the term "host cells" in claim 1 encompasses both prokaryotic and eukaryotic cells. At the priority date of the patent in suit it was common general knowledge that for the production of proteins in cell culture different types of eukaryotic and prokaryotic host cells could be used, the choice of a particular type of host cell being mainly dependent upon the properties of the desired protein. Since prokaryotic cells do not carry out post-translational modifications frequently found in human proteins such as glycosylation or phosphorylation, mammalian cells, for instance Chinese hamster ovary (CHO) cells, had been used routinely to produce high levels of various human proteins, eg tPA or interferon-ã (see document (7), page 95, first full paragraph).

Thus, having regard to the common general knowledge exemplified by document (7) as a representative of a whole body of prior art documents, the skilled person seeking to solve the problem of producing human DNase in host cells growing in culture would have thought of eukaryotic cells, in particular CHO cells as a suitable host. Contrary to the appellant's argument, the difficulties reported in document (5) in connection with the expression of the bovine DNase gene in *E. coli* would not have prevented the skilled person from trying to produce human DNase in host cells, but rather motivated him/her to look for an alternative type of host cell.

12. Furthermore, when trying to produce human DNase in eukaryotic cells, in particular CHO cells, the skilled person would have had more than a reasonable

expectation of success because, even in the case that the protein could not be secreted into the medium, toxicity problems were not to be expected on account of the strong compartmentalisation of eukaryotic cells. This was supported by the fact that potentially toxic mammalian proteins, for instance interferon-ã, could be successfully produced in high levels by CHO cells growing in culture (see document (7), page 95, first sentence of the first full paragraph).

13. In sum, the board judges that in view of document (5) in combination with the common general knowledge at the priority date as exemplified by document (7), the production of human DNase in eukaryotic cells growing in culture would have been obvious to the skilled person. Thus, since the subject-matter of claim 1 lacks an inventive step, the main request fails to meet the requirements of Article 56 EPC.

First auxiliary request

14. Claim 1 of the first auxiliary request is directed to a method for the production of human DNase in two alternative host cells, namely eukaryotic cells or *E. coli*. As explained above, the use of eukaryotic cells growing in culture for the production of human DNase is considered not to involve an inventive step. For this reason alone the request at issue is not allowable under Article 56 EPC. However, the question of inventive step will be examined below in respect also of the second embodiment claimed, namely the production of human DNase as an **intracellular** protein in *E. coli* (see Section V *supra*).

15. Prior to any considerations in regard to inventive step of this embodiment, the board considers it necessary to assess objectively the scope of the claims at issue in accordance with the well-established case law of the boards of appeal (see Case Law of the Boards of Appeal of the European Patent Office, 4th edition 2001, chapter II.B.4), as the interpretation of the term "intracellular protein" in claim 1 is relevant in the context of Article 56 EPC.

16. The precise meaning of the term "intracellular protein" is not expressly disclosed in the patent specification, and Example 3.4 of the patent, which according to the appellant constitutes the basis for the claimed embodiment, does not allow a clear and technically sensible interpretation of this term. It is noted that in the appellant's argument in support of an inventive step for this embodiment the term "intracellular" has been construed to mean "cytoplasmatic". However, nothing in the patent specification suggests such a narrow interpretation. The fact that in Example 3.4 the protein is recovered from the cells by SDS solubilisation rather indicates that the term "intracellular protein" has to be construed according to its general meaning in the art, ie as a reference not only to proteins located in the cytoplasm, but also to proteins located outside the cytoplasmatic membrane (ie in the periplasmic space) or in the outer membrane.

17. On this account, documents (3) and (4) become relevant for the assessment of inventive step with respect to the subject-matter of claim 1. Document (3) discloses the production of staphylococcal nuclease A in *E. coli* as a periplasmic protein, by expressing a chimeric gene

that encodes nuclease A fused to the signal peptide of the *E. coli* OmpA protein. The fusion protein is translocated across the cytoplasmatic membrane of *E. coli* with concomitant cleavage of the signal peptide (see document (3), abstract). In document (4) the same strategy is employed to produce ribonuclease T1 from *Aspergillus oryzae* in *E. coli*, an active ribonuclease that contains four additional amino acids at the N-terminus being isolated from the periplasmic fraction of the host (see document (4), abstract).

18. Starting from document (5) and in view of documents (3) and (4), the skilled person would have considered producing human DNase as a periplasmic (ie intracellular) protein in *E. coli* using the approach disclosed therein, ie by fusing the human DNase gene to a sequence encoding a suitable signal sequence, eg the signal peptide of the *E. coli* OmpA protein. It was also reasonable for the skilled person to expect that *E. coli* host cells expressing the human DNase protein would continue growing in culture, as the translocation of the protein into the periplasmic space would circumvent any possible toxic effects due to its nuclease activity. The fact that the proteins being produced in the prior art documents (3) and (4) were not mammalian but bacterial and fungal nucleases, respectively, would not have deterred the skilled person from applying the approach disclosed in said documents to the production of human DNase, as the nature of the signal peptide and not the origin of the protein would have been the relevant issue to be considered when aiming at exporting a protein into the periplasmic space of *E. coli*.

19. For these reasons, the subject-matter of claim 1 of the first auxiliary request is considered to be obvious. Thus, the requirements of Article 56 EPC are not fulfilled.

Second and third auxiliary requests

20. In claim 1 of the second and third auxiliary requests two alternative embodiments are claimed, the second embodiment being identical to that in claim 1 of the first auxiliary request (ie production of human DNase as an intracellular protein in *E. coli*). Since this subject-matter has been considered to be obvious in the light of the prior art (see points 15 to 19 *supra*), the requirements of Article 56 EPC are not met by these requests.

Fourth auxiliary request

21. Claim 1 of the fourth auxiliary request differs from claim 1 of the first auxiliary request in that, when the host cell is eukaryotic, the human DNase is expressed as a preprotein and secreted into the culture medium as mature protein (see Section VIII *supra*).

22. These additional features do not change the objective technical problem as defined starting from document (5) (see point 4 *supra*).

23. The suggested solution is now to provide an additional peptide or protein sequence that, when fused to the DNase protein, acts as a secretory signal in eukaryotic cells directing the DNase outside the cell. Upon

secretion the secretory signal is removed and the mature DNase protein appears in the culture medium.

24. As stated above (see points 8 to 9 *supra*), it would have been obvious to the skilled person to produce human DNase in eukaryotic host cells, in particular mammalian cells using recombinant DNA techniques. Being aware from document (5) of a toxic effect of the DNase possibly due to the degradation of the genetic material (see document (5), page 195, right column, last paragraph, first sentence), the skilled person would have looked for a way to target the DNase protein outside the cells, in order to prevent contact with the cell's genetic material. Document (8) would have suggested to him/her a method of general applicability. This document discloses a method for inducing mammalian cells to secrete human EGF and IgE by fusing these proteins to the secretory signal of the human IL-2 protein. Upon secretion the signal sequence was removed proteolytically, and biologically active EGF and IgE were found in the culture medium. Referring to the method of fusing the desired protein to a secretory signal document (8) states that "these methods are applicable elsewhere, as in the production and secretion of useful proteins whose signal sequences are not known, by using known leader sequences of other secretory protein genes" (see page 129, second paragraph under the abstract).

Thus, in view of document (8), the person skilled in the art would have regarded it as obvious to fuse the human DNase protein to a secretory signal in order to direct this protein through the cell membrane out of the eukaryotic cell where it has been produced, the

secretory signal being removed before the protein is secreted into the culture medium.

25. During the oral proceedings the appellant argued that the skilled person would not have considered using a foreign secretory signal to direct the human DNase protein to the culture medium on account of possible toxicity problems. Referring to Figure 7, panel B of document (8), the appellant observed that, in clone number 6, one third of the protein produced remained within the cell (see black bar). If a protein with DNase activity was to be produced, the accumulation of the protein within the cell would have led to a degradation of the genetic material which, as the cells are dividing (see document (8), Figure 4), would become exposed during mitosis.

26. The respondent admitted that a fraction of the DNase protein produced might be present, at a certain stage in the secretion process, inside the cell. However, since the protein would not be free in the cytoplasm but strictly confined to the vesicles of the secretory system, it could never come into contact with the cell's genetic material, not even during mitosis.

27. In the board's judgment, the skilled person was aware of the exquisite compartmentalisation characteristic of eukaryotic cells; thus, the possibility that some DNase protein remained within the cell would not have deterred him/her from trying the approach suggested in document (8) with a reasonable expectation of success.

28. On this account, it is considered that, by combining the teachings of documents (5) and (8), the skilled person would have arrived at a process falling within the scope of claim 1, which thus lacks an inventive step. Consequently, the fourth auxiliary request of which claim 1 is part is not allowable under Article 56 EPC.

Fifth auxiliary request

29. Claim 1 of the fifth auxiliary request differs from claim 1 of the previous request in that, when the host cell is eukaryotic, the preprotein comprises the native human DNase signal having the amino acid sequence as shown underlined in Figure 1 of the patent (see Section VIII *supra*).

30. As stated in point 8 *supra*, the appellant has not provided any evidence or arguments in support of the non-obviousness of the cloned human DNase gene as shown in Figure 1. Thus, the board must assume that the skilled person would have been able to clone the **complete** coding sequence of the human DNase gene, including the sequence encoding the secretory signal shown underlined in Figure 1, without exercising any inventive skills.

31. The appellant argued that at the priority date of the patent it was not known that the human DNase had a secretory signal and that, even once it was available, it would not have been obvious to the person skilled in the art to employ the native DNase signal, as opposed to a heterologous signal, eg as taught in document (8).

32. The board does not concur with the appellant in this respect. Once the complete coding sequence of the human DNase gene as shown in Figure 1 had been cloned and sequenced by routine experimentation, the encoded amino acid sequence could have been derived therefrom without difficulty. A cursory glance at the derived sequence would have sufficed for a skilled person to notice that the first amino acid after the methionine is a lysine, whereas the analysis of urinary mature DNase had revealed a leucine at the N-terminus of the protein (see paragraph [0002] of the patent in suit with reference to the prior art). This observation would have provided a skilled person working in the field of expression of mammalian proteins with the necessary motivation to examine more carefully the N-terminal amino acid sequence of the protein derived from the cloned gene. As the features of signal sequences in prokaryotic and eukaryotic proteins were well known in the art at the priority date (see, for instance, document (8), first paragraph under the abstract), the skilled person would have readily recognised that the N-terminal domain of the DNase protein encoded by the cloned gene corresponded to a secretory signal. Also, given that the human DNase protein is secreted by pancreas cells, the skilled person would reasonably have expected to find some kind of mechanism, eg a signal sequence that directs the protein outside the cells.

33. The appellant has provided no convincing evidence in support of his allegation that the skilled person would not contemplate using the native signal but would rather use a heterologous one instead. It follows from the passage in document (8) cited above (see point 24

supra) that foreign signals were considered to be useful mainly in cases where the signal sequence of the protein to be produced was not known. Thus, the board has no doubt that, once it had become obvious that the human DNase gene encoded a preprotein with a secretory signal, the skilled person, whose attitude is cautious and conservative (see T 455/91, OJ 1995, 684), would have thought - as a first option - of using said native signal to direct the DNase protein outside the host cells.

34. In view of the foregoing, the board concludes that the process according to claim 1 was obvious to the skilled person. Thus, an inventive step within the meaning of Article 56 EPC cannot be acknowledged for the subject-matter of this claim.

Sixth auxiliary request

35. Claim 1 is directed exclusively to a process for the production of human DNase as an intracellular protein in *E. coli*, the particular features of this process being identical to those of the second embodiment of claim 1 of the first auxiliary request. For the reasons given above (see points 15 to 19 *supra*), the subject-matter of claim 1 does not fulfil the requirements of Article 56 EPC.

Conclusions

36. None of the requests on file meets the requirements of Article 56 EPC. Thus, the contested decision cannot be set aside.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani