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D E C I S I O N
of 25 November 2003

Case Number: T 0356/00 - 3.3.8

Application Number: 89912273.3

Publication Number: 0396699

IPC: C12N 15/85

Language of the proceedings: EN

Title of invention:
A genetic construct of which protein-coding DNA comprises introns and is designed for protein production in transgenic animals

Patentee:
PPL (Holdings) Limited

Opponent:
Pharming Holding NV

Headword:
Mammary gland FIX intron construct/PPL

Relevant legal provisions:
EPC Art. 54, 56, 83, 87, 88, 89

Keyword:
"Main request - novelty (yes)"
"Inventive step (yes)"
"Sufficiency of disclosure (yes)"

Decisions cited:
G 0002/98, T 0939/92

Catchword:
-

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Case Number: T 0356/00 - 3.3.8

DECISION
of the Technical Board of Appeal 3.3.8
of 25 November 2003

Appellant: (Opponent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 10 February 2000 rejecting the opposition filed against European patent No. 0396699 pursuant to Article 102(2) EPC.

Composition of the Board:

Chairman: S. C. Perryman
Members: P. Julia
T. J. H. Mennessier

Summary of Facts and Submissions

I. The European patent No. 0 396 699 with the title "A genetic construct of which protein-coding DNA comprises introns and is designed for protein production in transgenic animals" was granted with 14 claims based on European patent application No. 89 912 273.3, published as WO 90/05188, and claiming priority from GB 88 26446 of 11 November 1988.

II. The patent had been opposed on the grounds that independent claim 3 - as well as claims 4 to 14 insofar as directly or indirectly dependent on claim 3 - did not comply with Article 100(a) (lack of novelty and inventive step) and 100(b) EPC (insufficiency of disclosure). It had also been argued that none of the opposed claims enjoyed the claimed priority (Articles 87 to 89 EPC). The opposition division found that the grounds of opposition did not prejudice the maintenance of the patent and the opposition was rejected.

III. Claims 3, 4 and 13 as granted read as follows:

"3. A genetic construct comprising a 5' flanking sequence from a mammalian milk protein gene and DNA coding for factor IX or a protein having at least 90% homology therewith, wherein the protein-coding DNA comprises at least one intron, or part thereof, but not all of the introns, naturally occurring in a gene coding for factor IX and wherein the 5'-flanking sequence is sufficient to drive expression of the factor IX."

"4. A genetic construct as claimed in claim 3, wherein the factor IX-coding DNA comprises only the first intron naturally occurring in a gene coding for factor IX."

"13. A method of producing a substance comprising a polypeptide, the method comprising harvesting the substance from a transgenic non-human animal which comprises a genetic construct as claimed in any one of claims 1 to 8 integrated into its genome."

Claim 1, which had not been opposed, was identical to granted claim 3 but for α_1-antitrypsin instead of factor IX. Claim 2 defined the specific introns present in the genetic construct of claim 1. Claims 5 to 8, which were dependent on any one of the preceding claims, defined the milk protein gene, upstream region and the presence of a 3'-sequence. Claim 9 concerned the use of the genetic constructs in producing a transgenic non-human animal, wherein the recombinant protein was expressed in a secretory gland of the animal. Claim 10, dependent on claim 9, defined the non-human animal as a mammal and the secretory gland as a mammary gland. Claims 11 and 12 were directed, respectively, to a vector comprising the genetic constructs and to a cell containing said vector, whereas claim 14 related to a further specific embodiment of claim 13.

IV. An appeal was lodged by the opponent (appellant) against the decision of the opposition division. All objections originally raised in the opposition proceedings were maintained and several further prior art documents were submitted by the appellant.

V. The patentee (respondent) filed comments on the statement of grounds of appeal.

VI. A communication pursuant to Article 11(2) of the rules of procedure of the Boards of Appeal was issued by the board. The parties were informed therein of the board's preliminary opinion, namely that the subject-matter related to granted claim 3 was sufficiently disclosed, novel and entitled to the claimed priority. For the assessment of inventive step, the board outlined the points for discussion at oral proceedings, in particular the question of how the technical problem underlying the opposed patent should be formulated.

VII. In reply to the board's communication, both parties announced their intention of not attending the oral proceedings. None of the parties made further submissions or comments on the board's communication.

VIII. Oral proceedings took place on 25 November 2003. As announced, they were not attended by the parties.

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

(1): K.H. Choo et al., Nucleic Acids Res., 1987, Vol. 15, pages 871 to 884;

(2): WO-A-88/00239 (publication date: 14 January 1988);

(4): R.L. Brinster et al., Proc. Natl. Acad. Sci. USA, February 1988, Vol. 85, pages 836 to 840;

(5): A.J. Clark et al., Bio/Technology, May 1989, Vol. 7, pages 487 to 492;

(14): F. Yull et al., Proc. Natl. Acad. Sci. USA, November 1995, Vol. 92, pages 10899 to 10903;

(19): R. Lathe et al., in "Exploiting New Technologies in Animal Breeding: Genetic Developments", Eds C. Smith et al, Oxford University Press, 1986, pages 91 to 102;

(20): WO-A-88/10118 (publication date: 29 December 1988).

X. The appellant's arguments, insofar as they are relevant to the present decision, may be summarized as follows:

Articles 87 to 89 EPC (Entitlement to priority rights)

Example 4 of the opposed patent referred to the complicated construction route necessary for obtaining the genetic constructs of claim 3 as well as to the difficulties caused by the size of factor IX (FIX) genomic DNA fragments which required the development of new shuttle vectors for their manipulation. Example 4 was not, however, present in the priority document and thus, in the absence of this information, the priority document was not enabling for claim 3. Document D14 disclosed a cryptic splice site in the FIX cDNA which resulted in aberrant splicing and low expression of FIX in mammary glands. All genetic FIX constructs referred to in the priority document had this cryptic splice site and thus, no FIX could be obtained following the teachings of the priority document.

Article 83 EPC (Sufficiency of disclosure)

As shown by document D14, the only genetic FIX construct disclosed in the opposed patent - J FIXA 1 (example 4E) which corresponded to JFIXAi (document D14) - produced an aberrant splicing associated with low or undetectable levels of FIX expression. These levels of expression in the milk of transgenic animals pointed to a failure of the relevant genetic constructs to drive expression of FIX. There were more difficulties for expressing FIX than the ones admitted in the opposed patent, which was thus not enabling.

Article 54 EPC (Novelty)

Document D14 disclosed that the FIX cDNA contained a cryptic 3' splice site that, when expressed in mammary gland cells, resulted in the splicing of a 462 bp sequence of the mRNA near the 3' end. This 3' end sequence was an intron in the context of mammary gland expression. Document D1 disclosed an expression plasmid comprising a FIX cDNA sequence, which thus inherently contained the 3' end intron. This expression plasmid further comprised a 5' flanking sequence from the metallothionein gene, which - as shown by prior art documents reporting the presence of metallothionein in milk - was inherently a milk protein gene in the terms of the opposed claims. Similarly, document D2 disclosed the construction of an expression vector with FIX cDNA for use in the production of transgenic animals. The FIX cDNA contained the cryptic splice site and thus, inherently, the 3' end intron. The expression vector also comprised a 5' flanking sequence from a mammalian

milk protein gene (sheep α-lactoglobulin) as defined in claim 3 of the opposed patent. Therefore, both documents D1 and D2 inherently anticipated the subject-matter of claim 3 of the opposed patent.

Document D19 referred to the production of biological proteins (FIX) in transgenic mammals and to the use of mammary gland systems with milk protein gene promoters for driving the expression of the genes encoding those biological proteins. The presence of regulatory and enhancer sequences within structural genes, in particular within introns, was also referred to. The inclusion of such regulatory elements from FIX would inevitably mean including at least part of an FIX intron as required in claim 3 as granted.

Article 56 EPC (Inventive step)

According to the opposed patent, the technical problem to be solved was to improve the yields of FIX produced in the milk of transgenic animals. However, this problem could not be solved by the opposed patent, which was completely silent on the presence of a cryptic splice site in the FIX cDNA and its aberrant splicing in mammary glands. Document D14 showed that, without removing the cryptic 3' splice site, only very low or undetectable amounts of FIX could be obtained. Thus, the opposed patent did not provide any technical contribution over the known prior art. The production of FIX in the milk of transgenic animals was already known in the prior art as well as the importance of introns - in particular the regulatory and enhancer sequences therein - in connection with expression in transgenic systems. It was obvious for the skilled

person to combine the teachings of this prior art and enhance the expression of FIX in transgenic milk by employing one or more introns. In particular, if the opposed patent was not entitled to the claimed priority, the combination of documents D5 or D20 with D4 was considered to be relevant, whereas if priority was acknowledged, the combination of documents D1 or D2 with D4 was then relevant.

Document D5, using a construct comprising FIX cDNA and sequences derived from the milk protein gene β-lactoglobulin (BLG), disclosed the production of FIX in the milk of transgenic sheep and referred to the low level of FIX mRNA, efficiency of transcription and expression obtained. In this context, there was an explicit reference to document D4 emphasizing the importance of natural introns for an efficient transcription of genes in transgenic animals. In order to improve the expression of FIX in transgenic ewes, it was obvious to modify the constructs of document D5 in accordance with document D4, ie to use some of - but not all - the introns from FIX and thus, inevitably achieved the opposed subject-matter.

Document D20 disclosed the production of recombinant protein products (FIX) in the milk of transgenic mammals using milk gene promoters. Example 2 showed an expression construct for human tPA, which had been prepared with a genomic tPA clone that contained an intron. Following this example, the skilled person would have taken a genomic FIX clone and included one or more FIX introns for constructing an expression vector and thus, inevitably achieving the opposed subject-matter too.

If the opposed patent was entitled to the claimed priority, document D1 was then considered to be the closest prior art. The genetic construct of this document comprised a FIX cDNA and a 5' flanking sequence from metallothionein which was shown by prior art documents to be a mammalian milk protein gene. In order to enhance the level of expression of FIX in the milk of transgenic animals, the combination with document D4, which disclosed the importance of introns for an efficient transcription of genes in transgenic animals, was obvious to the skilled person and thus, the opposed subject-matter would inevitably be achieved. A similar approach followed from the combination of documents D2 and D4.

The opposed patent did not teach which introns, part of introns or combination thereof, were needed in order to obtain an enhanced expression of FIX in milk. Claim 3 as granted embraced many constructs which did not produce the technical effect required for solving the problem underlying the opposed patent and thus, according to the established case law of the Boards of Appeal, and in particular decision T 939/92 (OJ EPO 1996, 309), the nature and scope of granted claim 3 could not be reasonably predicted, in the sense that it was nothing more than a mere hope of success, and thus, no inventive contribution could be acknowledged.

XI. The respondent's arguments, insofar as they are relevant to the present decision, may be summarized as follows:

Articles 87 to 89 EPC (Entitlement to priority rights) and Article 83 EPC (Sufficiency of disclosure)

No specific reply or comments were received from the respondent on the arguments presented by the appellant under these Articles.

Article 54 EPC (Novelty)

An intron was an intervening sequence that had to be excised from a primary transcript so as to form a functional mRNA which translated into a functional protein. The aberrant splicing of FIX expressed in mammary glands was not an excision of an intron because removal of that sequence was not required for formation of functional mRNA. On the contrary, the aberrant splicing resulted in loss of function since coding sequences were lost. The FIX cDNA was a copy of functional FIX mRNA and, since the difference between a primary transcript and a mRNA was that the latter had all introns removed, all FIX cDNA sequences were exonic. The inclusion or exclusion of alternative exons - with alternative splicing of mature mRNA - always referred to exons but never to introns. The cryptic splice site of the FIX cDNA sequence did not result in excision of an intron since the excised sequence was part of a structural sequence and not interrupting it.

The presence of metallothionein in milk did not demonstrate that it was made in mammary epithelial cells. Mammary milk contained many proteins which originated in a variety of tissues, such as serum albumin made in liver. Serum albumin promoter did not work in mammary epithelial cells. Most of the

metallothionein present in milk arrived there via a similar process to that for serum albumin. Even if some metallothionein originated in the mammary gland, there was no evidence that it was actually synthesised in mammary epithelial cells. In fact, the mammary gland was known to be a complex organ containing many cell types and it could well be that metallothionein was produced by myoepithelial cells, which were in the outer layer of mammary tissue and not implicated in the synthesis and secretion of milk proteins. Thus, there was no evidence that metallothionein was a mammalian milk protein as defined in the opposed patent.

Article 56 EPC (Inventive step)

Document D14 disclosed transgenic animals expressing FIX cDNA constructs. Even if the level of FIX in the milk was low, the document referred to a transgenic sheep (10022) with the JFIXAi construct and which secreted 1 µg/ml of fully active FIX. Whilst this was not a high level of expression and it was subsequently improved upon by excision of the aberrant splice site, it was a commercially useful level of FIX, the therapeutic dose required being typically 0.25-0.5 µg/ml of patient blood. The opposed patent thus provided an identifiable technical contribution over the prior art.

XII. The appellant (opponent) had requested in writing that the decision under appeal be set aside and that the patent be revoked to the extent of the opposed claims in their entirety.

XIII. The respondent (patentee) had requested in writing that the appeal be dismissed and the patent be maintained as granted.

XIV. At the end of the oral proceedings the board announced its decision.

Reasons for the Decision

Articles 87 to 89 EPC (Entitlement to priority rights)

1. According to decision G 2/98 (OJ EPO 2001, 413), "*the requirement for claiming priority of "the same invention", referred to in Article 87(1) EPC, means that priority of a previous application in respect of a claim in a European patent application in accordance with Article 88 EPC is to be acknowledged only if the skilled person can derive the subject-matter of the claim directly and unambiguously, using common general knowledge, from the previous application as a whole*" (cf Headnote of decision G 2/98).

2. The genetic constructs disclosed in the priority document comprise a general "*DNA coding for a heterologous protein*" (cf *inter alia* page 6, first paragraph), wherein this heterologous protein is defined as "*any desired protein of interest*", including "*blood factors*", and in particular factor IX (FIX) (cf page 7, second full paragraph and page 5, second paragraph). Thus, the priority document explicitly refers to FIX and to the use of the disclosed expression systems in mammary glands of transgenic animals for the production of FIX. Example 3 discloses

the presence of FIX in the milk of transgenic sheep resulting from the expression in their mammary glands of a β-lactoglobulin (BLG)-FIX construct (pSS1tgXS-FIX) with a FIX cDNA sequence without introns. This genetic construct, in the absence of introns, is not efficiently expressed and results in a low level of FIX in the milk of transgenic sheep (cf page 46, first paragraph) or in a complete absence of FIX in the milk of transgenic mice (cf page 50, second paragraph). Thus, the relevance of a FIX construct as taught in the priority document - comprising at least one, but not all, of the introns naturally occurring in the gene of FIX - is acknowledged in the priority document.

3. The appellant has argued, however, that the priority document is not enabling for subject-matter related to FIX due, on the one hand, to the difficulties encountered in the manipulation of the large size fragments of human FIX genomic DNA and, on the other hand, to the presence of a cryptic splice site in FIX cDNA, which results in aberrant splicing in mammary glands and low expression of FIX, as shown by document D14 (to be taken as expert opinion) (cf point 10 *infra*).

4. The large size of the human FIX gene (about 34 kb) and the presence of seven FIX introns within this gene were known at the priority date (cf page 5, second paragraph). The priority document itself acknowledges the difficulties associated with the manipulation of large size genomic DNA and refers to suitable cloning vectors (cosmids) and restriction enzymes (cf page 4). The combination of particular fragments of human FIX genomic DNA - by digestion and subcloning - with the available FIX cDNA so as to produce genetic constructs

as taught in the priority document did not represent an undue burden for the skilled person at the priority date. Both the information and the technical means for putting into practice those teachings were available to the skilled person. A complicated route (cf page 23, lines 4 to 6 of the opposed patent) cannot be equated to an unknown route or a route insufficiently disclosed in the sense that it lacks an essential step for its successful performance. Thus, the genetic constructs of granted claim 3 are considered to be enabled by the priority document.

5. Example 3, using a FIX cDNA with the cryptic splice site referred to in document D14 (to be taken as expert opinion), discloses the detection of human FIX in the milk of transgenic sheep (by Northern and Western blotting, radioimmunoassays and FIX biological activity) (cf page 45 to page 46). Document D14 itself shows that a genetic construct comprising a FIX cDNA with the first FIX intron - and thus, with the cryptic splice site - is also able to produce FIX in the milk of transgenic sheep (1 µg/ml) (cf document D14, page 10899, right-hand column, last full paragraph). There is no evidence on file to suggest that similar results cannot be obtained with other genetic constructs as taught in the priority document. Thus, given that neither the use claims 9 or 10 nor the method claims 13 and 14 as granted require the production of any specific amount of FIX, the priority document is considered to be enabling for the subject-matter of these claims too.

6. The priority document indicates, as a preferred embodiment, that "*all but one*" intron may be present in the protein-coding DNA, in particular "*the first intron*

may be missing" (cf page 8, first full paragraph). This embodiment is exemplified by a α_1-antitrypsin (ATT) genetic construct with genomic ATT DNA having all ATT introns except for the large first one (cf page 40, example 2). There is, however, no suggestion of a genetic construct comprising the protein-coding region and the first intron alone. Such a specific genetic construct cannot be directly and unambiguously - neither explicit nor implicitly - derived from the priority document and thus, there is no basis in the priority document for the subject-matter of claim 4 as granted.

7. Thus, the board considers that claim 3 and other claims when dependent thereon - except for claim 4 - enjoy the claimed priority.

Article 83 EPC (Sufficiency of disclosure)

8. The teachings of the opposed patent are essentially identical to the ones of the priority document. The opposed patent further comprises an additional example, ie example 4, which exemplifies the actual production of a genetic FIX construct as defined in claim 3 and more particularly in claim 4 as granted. Thus, in view of the conclusions reached above for the priority document, the objections raised under Article 83 EPC are not considered to be relevant and the opposed patent is considered to fulfil the requirements of this Article.

Article 54 EPC (Novelty)

9. The board notes that there is no definition of intron in the opposed patent. However, explicitly reference is made to 5' flanking and 3'-sequences and these sequences are clearly not referred to as introns or parts thereof (cf page 3, lines 39 to 41 and lines 53 to 56). On the contrary, introns are always identified as non-coding sequences in-between coding sequences (exons), ie non-coding intervening sequences (cf *inter alia* page 5, lines 35 to 36 and Figure 6). This implicit definition agrees with the definition of intron found in the prior art, such as *inter alia* in document D4 (cited in the opposed patent in the context of introns), wherein an intron is defined as a non-coding sequence interrupting mRNA and separating functional or structural domains of the protein encoded by the exons (cf page 836, left-hand column, second paragraph). This is also the generally accepted definition of intron. There is nothing in the opposed patent that suggests that anything other than this standard definition of intron is meant.

10. Document D14 (to be taken as expert opinion) identifies a cryptic 3' RNA splice site in the FIX cDNA that produces a specific aberrant FIX splicing in the mammary glands of transgenic mammals. This cryptic splice site is located within exon 8 of FIX and results in a 462-bp deletion that encompasses the sequences encoding the C-terminal 109 amino acids, the stop codon, and 141 nucleotides of the 3' UTR of the known FIX mRNA sequence. The above definition of intron (cf point 9 *supra*) clearly excludes the technical features characterizing the aberrant spliced fragment, namely

the presence of a coding sequence and the 3'-sequences. It is also noted that document D14 only refers to a "*cryptic RNA splice site*" but nowhere is it suggested that the resulting aberrant spliced fragment is an intron.

11. Thus, the board cannot follow appellant's argumentation that human FIX cDNA inherently comprises an intron - the aberrant spliced fragment or 3' (end) intron - and that all prior art using human FIX cDNA for the production of FIX in the milk of transgenic animals implicitly comprise subject-matter falling within claim 3 as granted. In particular, the genetic construct pSS1tgXS-FIX disclosed in document D2 - having a 5' flanking sequence from a mammalian milk protein gene (BLG) and human FIX cDNA - does not comprise "*at least one intron, or part thereof, but not all of the introns naturally occurring in the gene coding for factor IX*" as required by granted claim 3.

12. Similarly the genetic construct of document D1 - having a metallothionein promoter and human FIX cDNA - does not comprise any intron. Moreover, in the context of the opposed patent, a "*5' flanking sequence from a mammalian milk protein gene*" is defined as a sequence that directs the expression of the associated protein gene in a tissue-specific manner to the mammary gland (cf *inter alia* page 11, lines 48 to 49 and page 19, lines 28 to 30 of the opposed patent). There is no information in document D1 concerning the presence of FIX in the milk of the transgenic mice. However, FIX is detected in both the liver and in plasma samples of these transgenic mice. From these results, it can already be derived that the metallothionein promoter

does not direct the expression of the associated FIX gene in a tissue-specific manner to the mammary gland. Moreover, this specific feature is not found in any of the documents on file disclosing the presence of metallothionein in milk, and which refer to said milk metallothionein as being transferred from blood serum and found in nearly all human tissues. Thus, the genetic constructs of document D1 do not anticipate the subject-matter of claim 3 as granted.

13. Document D19, a general review concerning the use of farm animals for the production of human proteins (such as FIX), refers to the specific advantages of the mammary system for expressing these proteins. In this context, document D19 states that "*...regulatory sequences, particularly those mediating tissue-specific expression, may often reside within the structural gene or even 3' to it...*" (cf page 98). In view of the fact that human FIX is not specifically expressed in mammary glands, the skilled person would not be prompted to retain naturally occurring FIX introns for expression in mammary glands of transgenic animals by this statement in document D19. On the contrary, if anything, the skilled person is taught to use structural genes or 3' sequences thereof - containing tissue-specific regulatory sequences - from proteins specifically expressed in mammary glands. This disclosure actually teaches away from the subject-matter of claim 3 as granted and the document is not relevant to novelty.

14. In conclusion, none of the documents on file affect the novelty of claim 3 as granted and claims dependent thereon. Thus, the requirements of Article 54 EPC are considered to be fulfilled.

Article 56 EPC (Inventive step)
Subject-matter entitled to priority

15. The closest prior art for subject-matter entitled to priority rights - claim 3 as granted and claims dependent thereon, except for granted claim 4 - is considered to be document D2. This document discloses the production of recombinant proteins in the milk of transgenic animals using flanking sequences of milk protein genes. These teachings are exemplified by a genetic construct - pSS1tgXS-FIX - comprising a fusion of the milk β-lactoglobulin (BLG) gene with the FIX cDNA. Example 8 shows the presence of FIX in the milk of two transgenic ewes - 6LL231 and 6LL240 - carrying this BLG-FIX construct. The levels of FIX are said to be low but detectable and they establish *"a basis for the production of human proteins in this manner"* (cf page 43).

16. Starting from this closest prior art, the technical problem underlying the opposed patent must be seen in the provision of alternative genetic FIX constructs. The claimed solution is provided by the genetic constructs comprising FIX cDNA and at least one, but not all, naturally occurring introns of the FIX gene. As discussed above in points 4 and 5, it is plausible that the genetic constructs will work, so the technical problem can be regarded as solved by the opposed patent.

17. Document D2 explicitly refers to the importance of the 5' and 3' sequences of a structural gene for the stability of the corresponding mRNA as well as to the possible presence of regulatory sequences - in

particular those mediating tissue-specific expression - within the structural gene or in the 3' sequences thereto (cf page 14, lines 8 to 25). This observation directly leads to the fusion constructs disclosed in document D2 itself, namely a cDNA sequence coding for the peptide of interest inserted into a milk protein gene with 5' flanking sequence, structural milk protein gene sequences and 3' sequences flanking the milk protein gene (cf page 19 and point 13 *supra* too). The appellant, however, has argued that reading these passages and, in light of the known prior art - in particular document D4 - disclosing the advantages of introns in the expression of cDNA, the skilled person would be prompted to use the naturally occurring FIX introns. Even if, for the sake of argument, such a straight link were accepted by the board, the claimed subject-matter cannot, however, be derived from the combination of these documents.

18. Document D4 discloses the positive effect of introns on the expression of two genes (mouse metallothionein gene and human β-globin) in different tissues (fetal liver and pancreas) of transgenic mice. No effect is found, however, on cultured cells. A possible general mechanism for explaining these effects is mentioned in this document, namely the stabilization of nuclear mRNA precursors and/or more efficient transport into cytoplasm. However, the results obtained do not support this general mechanism and therefore, other possible mechanisms are indicated. In particular, reference is made to the possible importance of tissue-specific factors or to the specific gene used and the presence of particular sequences within its introns, such as enhancers, sequences for maintaining the

transcriptional activity during certain stages of development, etc...(cf pages 839 and 840 under "Discussion"). In this context, it is worth noting that there is no reference in document D4 to the specific FIX gene or to the possible effect of introns in mammary glands of transgenic mice. Moreover, although document D4 explicitly acknowledges the difficulties of manipulating large size genes (cf page 836, right-hand column, full paragraph), all genetic constructs comprise a full-length genomic DNA with all naturally occurring introns and there is no suggestion that some - or all introns except for one - could be omitted from these constructs. Thus, the claimed genetic constructs are - neither from document D2 alone nor in combination with document D4 - derivable in an obvious manner.

Article 56 EPC (Inventive step)
Subject-matter not entitled to priority

19. Document D5 is considered to be the closest prior art for subject-matter not entitled to priority rights, ie claim 4 and other claims when dependent thereon. This document corresponds to the scientific publication of document D2 and discloses the same genetic construct (BLG-FIX with milk β-lactoglobulin gene and FIX cDNA) and transgenic ewes (6LL231 and 6LL240, approximately 25 ng FIX/ml) as the ones of document D2 (cf page 488, Figure 1 and left-hand column, second full paragraph). Even if more detailed information is disclosed, the general teachings of document D5 do not go much beyond the ones of document D2. In order to explain the low levels of FIX, document D5 gives several possible reasons (rearrangement of the 5' end, FIX sequestering in casein micelles, etc..) and it refers to document D4

stating that "*recent studies suggest that genes lacking their natural introns may not be transcribed efficiently in transgenic animals*" (cf page 490, left-hand column, last sentence of first full paragraph). However, as stated in point 18 *supra*, document D4 only discloses genetic constructs with genomic DNA comprising all the naturally occurring introns and there is no suggestion that some - or all introns except for one - could be omitted, let alone an indication to delete all introns except for the first naturally occurring one. Thus, a specific genetic construct comprising the genomic FIX DNA with only the first FIX intron - the subject-matter of granted claim 4 - cannot be derived from the combination of documents D5 and D4 in an obvious manner.

20. It has also been argued that document D20 alone or in combination with other prior art is relevant for the assessment of inventive step of subject-matter not entitled to priority rights. This document discloses the production of recombinant proteins in the milk of mammals and explicitly refers, among other proteins, to FIX. The document is exemplified by the production of tissue plasminogen activator (TPA) using the milk protein casein (CAS). In order to use the CAS signal peptide for directing the secretion of TPA from the mammary glands, example 2 refers to the presence of a BamHI site in intron II which separates the signal peptide from the mature sequence in both the TAP and CAS genes. This common restriction site is used for producing a fusion construct comprising the CAS signal peptide and the TPA cDNA with part of intron II. However, no particular advantage or effect is associated with this (partial) intron and there is no

evident motivation to introduce introns (or parts thereof) in other cDNAs. Moreover, there is no evidence on file showing the presence of a restriction site shared with the CAS gene separating the signal and the mature sequences of FIX, let alone that, using such a hypothetical restriction site, a fusion construct with only the first (complete) intron naturally occurring in the FIX gene could be directly obtained as required by granted claim 4. In the absence of any effect or evident motivation, the board does not consider that the skilled person even if he or she should consider this document and other documents of the prior art, in particular document D4, at the same time, would arrive at the subject-matter of claim 4.

21. The board considers that the factual situation underlying the decision T 939/92 of 12 September 1995 (OJ EPO 1996, 309) differs essentially from the one of the opposed patent. Whereas that decision concerned a large number of possible chemical compounds which were required to have a significant technical effect (herbicidal activity) over the prior art, the present case only concerns a limited number of specific combinations of known elements (seven FIX introns) and a relatively unambitious technical effect - the (very) low levels of FIX disclosed in document D2 (cf point 16 *supra*). There is technical evidence on file that at least one of the claimed combinations - the construct of granted claim 4 (1 µg FIX/ml, cf point 5 *supra*) - achieves the desired result and there is nothing on file suggesting, let alone demonstrating, that the presence of introns - all introns or at least one intron or part thereof - would not at least achieve the

low levels of FIX expression disclosed in the closest prior art document D2 (25 ng FIX/ml, cf point 19 *supra*).

22. In conclusion, the board considers that the subject-matter of claims 3 and 4 as granted - and claims dependent thereon - fulfils the requirements of Article 56 EPC. Thus, the opposed patent satisfies the requirements of the EPC.

Order

For these reasons it is decided that:

1. The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski

S. Perryman