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D E C I S I O N
of 27 June 2001

Case Number: T 0032/97 - 3.3.4

Application Number: 84113424.0

Publication Number: 0149040

IPC: A61K 39/245

Language of the proceedings: EN

Title of invention:
Modified live pseudorabies viruses, vaccines for pseudorabies disease containing same, methods for production of same and methods for use of same.

Patentee:
NOVAGENE, INC., et al

Opponent:
American Home Product Corporation
AKZO NOBEL PHARMA B.V

Headword:
Deleted tk-PRV vaccines/NOVAGENE

Relevant legal provisions:
EPC Art. 54, 56, 69, 84, 114, 123(2), 123(3)
EPC R. 88

Keyword:
"Late filed documents - admitted"
"Correction of obvious errors in the claims - allowed"
"Added subject-matter - (no)"
"Extension of scope of protection - (no)"
"Claims clear and supported - (yes)"
"Sufficiency of disclosure - (yes)"
"Late raised objection to novelty - not allowed"
"Inventive step - (yes)"

Decisions cited:
G 0009/91, G 0010/91, G 0007/95, T 0435/91

Catchword:
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Case Number: T 0032/97 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 27 June 2001

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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 4 November 1996 concerning maintenance of European patent No. 0 149 040 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: A. L. L. Marie
S. C. Perryman

Summary of Facts and Submissions

I. European Patent No. 0 149 040, with the title "Modified live pseudorabies viruses, vaccines for pseudorabies disease containing same, methods for production of same and methods for use of same" was granted on the basis of a set of 37 claims. Claims 1 to 4, 6, 16 and 18 to 21 read as follows:

"1. A pseudorabies virus obtainable by recombinant DNA techniques which fails to produce any functional TK as a result of a deletion in the tk-gene, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5."

"2. Pseudorabies virus according to claim 1, wherein said pseudorabies virus is temperature-resistant."

"3. A temperature-resistant pseudorabies virus which fails to produce any functional TK as a result of a deletion in the tk-gene, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig.5 obtainable by the following process steps:

(1) constructing a hybrid plasmid comprising a cloning vector and a DNA fragment of PRV containing substantially all of the PRV tk-gene;

(2) co-transfecting, in tk^+ host cells, the hybrid plasmid of step (1) with DNA from a temperature-resistant PRV tk^- mutagen induced mutant;

(3) selecting, in tk^- host cells, for PRV tk^+ from the virus produced in step (2);

(4) deleting DNA sequences from the hybrid plasmid of step (1) such that less than substantially all of the PRV tk-gene is present, while retaining PRV DNA sequences adjacent to each side of the deletion;

(5) co-transfecting, in tk^+ host cells, PRV tk^+ DNA derived from the PRV tk^+ obtained in step (3) with the resulting hybrid plasmid of step (4); and

(6) selecting, in tk^- host cells, for PRV tk^- from the virus produced in step (5) to produce temperature-resistant PRV tk^- deletion mutants."

"4. Pseudorabies virus according to any of claims 1 to 3, wherein said deletion is about 10 to 1,500 bp in size."

"6. Pseudorabies virus according to claim 1, wherein said virus has the identifying characteristics of PRV-(BUK-dl 3) (ATCC No. VR-2074)."

"16. A modified live virus vaccine for pseudorabies disease comprising:

(1) a pharmaceutically acceptable amount of a pseudorabies virus obtainable by recombinant DNA techniques which fails to produce a functional TK as a result of a deletion of the tk-gene, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5; and

(2) a pharmaceutically acceptable carrier or diluent."

"18. A modified live virus vaccine for pseudorabies disease comprising:

(1) a pharmaceutically acceptable amount of a temperature-resistant virus which fails to produce any functional TK as a result of a deletion in the tk-gene, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5, obtainable by the following process steps:

(a) constructing a hybrid plasmid comprising a cloning vector and a DNA fragment of PRV containing substantially all of the PRV tk-gene;

(b) co-transfecting, in tk^+ host cells, the hybrid plasmid of step (a) with DNA from a temperature-resistant PRV tk^- mutagen-induced mutant;

(c) selecting, in tk^- host cells, for PRV tk^+ from the virus produce in step (b);

(d) deleting DNA sequences from the hybrid plasmid of step (a) such that less than substantially all of the PRV tk-gene is present, while retaining PRV DNA sequences adjacent to each side of the deletion;

(e) co-transfecting, in tk^+ host cells, PRV tk^+ DNA derived from the PRV tk^+ obtained in step (c) with the resulting plasmid of step (d); and

(f) selecting in tk^- host cells, for PRV tk^- from

the virus produced in step (e) to produce temperature-resistant PRV tk^- deletion mutants; and

(2) a pharmaceutically acceptable carrier or diluent."

"19. Vaccine according to any of claims 16 to 18, wherein said deletion is about 10 to 1,500 bp in size."

"20. Vaccine according to any of claims 16 to 18, wherein said deletion is about 75 to 750 bp in size."

"21. Vaccine according to claim 16, wherein said virus has the identifying characteristics of PRV-(BUK-dl 3) (ATCC No. VR-2074)."

II. Patentability was challenged in an opposition procedure and resulted in the maintenance of the patent in an amended form on the basis of a set of 23 claims submitted during the oral proceedings, which was found to overcome the objections raised by the opponents under Articles 123(2), 83 and 56 EPC.

III. The appeals lie from this decision by the opposition division.

IV. In response to a communication pursuant to Article 11 of the rules of procedure of the boards of appeal giving the board's preliminary, non-binding opinion and to the arguments presented by appellants II and III (opponents 1 and 2), appellant I (patentee) filed with his letter of 25 May 2001, besides 3 auxiliary requests, a new main request with the following claims 1, 2, 4, 14, 15 and 33:

"1. A temperature-resistant pseudorabies virus obtainable by recombinant DNA techniques which fails to produce any functional TK as a result of a deletion in the tk-gene of about 10 to 1,500 bp in size, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5."

"2. A temperature-resistant pseudorabies virus which fails to produce any functional TK as a result of a deletion in the tk-gene of about 10 to 1,500 bp in size, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5, obtainable by the following process steps:

(1) constructing a hybrid plasmid comprising a cloning vector and a DNA fragment of PRV containing substantially all of the PRV tk-gene;

(2) co-transfecting, in tk^+ host cells, the hybrid plasmid of step (1) with DNA from a temperature-resistant PRV tk^- mutagen-induced mutant;

(3) selecting, in tk^- host cells, for PRV tk^+ from the virus produced in step (2);

(4) deleting DNA sequences from the hybrid plasmid of step (1) such that less than substantially all of the PRV tk-gene is present, while retaining PRV DNA sequences adjacent to each side of the deletion;

(5) co-transfecting, in tk^+ host cells, PRV tk^+ DNA derived from the PRV tk^+ obtained in step (3) with the resulting hybrid plasmid of step (4);

and

(6) selecting, in tk^- host cells, for PRV tk^- from the virus produced in step (5) to produce temperature-resistant PRV tk^- deletion mutants."

"4. Pseudorabies virus according to claim 1, wherein said virus had the identifying characteristics of PRV-(BUK-dl 3) (ATCC No. VR-2074)."

"14. A modified live virus vaccine for pseudorabies disease comprising:

(1) a pharmaceutically acceptable amount of a temperature-resistant pseudorabies virus obtainable by recombinant DNA techniques which fails to produce a functional TK as a result of a deletion of about 10 to 1,500 bp in size of the tk-gene, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5; and

(2) a pharmaceutically acceptable carrier or diluent."

"15. A modified live virus vaccine for pseudorabies disease comprising:

(1) a pharmaceutically acceptable amount of a temperature-resistant virus which fails to produce any functional TK as a result of a deletion of about 10 to 1,500 bp in size in the tk-gene, said tk-gene corresponding essentially to the tk-gene sequence shown in Fig. 5, obtainable by the following process steps:

(a) constructing a hybrid plasmid comprising a

cloning vector and a DNA fragment of PRV containing substantially all of the PRV tk-gene;

(b) co-transfecting, in tk^+ host cells, the hybrid plasmid of step (a) with DNA from a temperature-resistant PRV tk^- mutagen-induced mutant;

(c) selecting, in tk^- host cells, for PRV tk^+ from the virus produced in step (b);

(d) deleting DNA sequences from the hybrid plasmid of step (a) such that less than substantially all, of the PRV tk-gene is present, while retaining PRV DNA sequences adjacent to each side of the deletion;

(e) co-transfecting, in tk^+ host cells, PRV tk^+ DNA derived from the PRV tk^+ obtained from step (c) with the resulting hybrid plasmid of step (d); and

(f) selecting, in tk^- host cells, for PRV tk^- from the virus produced in step (e) to produce temperature-resistant PRV tk^- deletion mutants; and

(2) a pharmaceutically acceptable carrier or diluent."

"33. The use of the pseudorabies virus according to any of claims 1 to 13 for the preparation of a vaccine to combat diseases caused by pseudorabies virus."

In dependent claims 3, 5 to 13 some of the features of

the claimed pseudorabies virus were more precisely defined, whereas in dependent claims 16 to 32 the same was done for the vaccines.

These sets of claims contained some errors, the correction of which were requested under Rule 88 EPC.

V. The documents relied on during the appeal procedure are the following ones:

(P1) Ben-Porat, T. et al., Virology, 1983, Vol. 127, pages 194-204,

(P35) Wigler, M. et al., Cell, 1977, Vol. 11, pages 223 to 232,

(D65) Kit, S. et al., Journal of Medical Virology, 1983, Vol. 12, pages 25 to 36,

(D67) Sanders, P.G. et al., J. Gen. Virol., 1982, Vol. 63, pages 277 to 295,

(D72) Tenser, R.B. et al., Journal of General Virology, 1983, Vol. 64, pages 1369 to 1373,

(D73) Tenser, R.B. et al., Journal of Clinical Microbiology, 1983, Vol. 17, pages 122 to 127,

(D99) Baskerville, A. et al., The Veterinary Bulletin, 1973, Vol. 43, pages 464 to 480,

(D100) Bass, E.P., Proceedings of the US Animal Health Association, 1978, pages 426 to 431,

(D101) Tatarov, G., Tijdschrift voor Diergeneeskunde,

1983, Vol. 108, pages 204 to 209,

(D104) Lomniczi, B. et al., Abstracts 8th International Herpes Workshop, 31 July to 5 August 1983, Oxford,

(K2) Skoda, R. et al., Acta Virol., 1964, Vol. 8, pages 123 to 134,

(K3) Thompson, R.L. et al., Virology, 1983, Vol. 131, pages 180 to 192,

(K8) Medical Virology, 2nd edition; Eds. F. Fenner et al., Academic Press, 1976, page 230,

(K9) Virus and Immunity, Eds. C. Koprowski et al., Academic Press, 1975, page 122,

(K30) Skoda, R. et al., Acta Virol., 1962, Vol. 6, page 189,

(K52) Howarth, J.A., Proceedings of the 74th A.l Meetings: US Animal Health Association, 1971, pages 371 to 384,

(K57) EP-A-0 141 458,

(K62) Mock, R.E. et al., Can. J. Comp. Med., 1981, Vol. 45, pages 56 to 59,

(K80) Mocarski, E.S. et al., Cell, 1980, Vol. 22 (part I), pages 239 to 251,

(K123) Tatarov, G. et al., Veterinary Science, 1981, Vol. XVIII, No. 1, pages 3 to 12,

(K126) Declaration of Dr. C.E. Jacobs

VI. Oral proceedings were held on 26 and 27 June 2001.

VII. The admissibility of some late-filed submissions was questioned under Article 114(2) EPC by the three appellants.

VIII. The submissions by appellants II and III (opponents 1 and 2) are summarized as follows.

Article 123(2) EPC: it was argued that claim 4 of the new main request corresponded to claim 6 as granted which was directly dependent on claim 1, whereas claim 4 of the new main request depended on claim 1, which in turn was a combination of claims 1 and 4 as granted. The subject-matter of claim 1 as granted was therefore different from that one resulting from the combination of claims 1 and 4 as granted, so that claim 4 of the new main request defined a new subject-matter as compared to that of claim 6 as granted, which was not disclosed in the application as filed.

Claim 14 of the new main request embraced deletions in the flanking sequences of the tk-gene (thymidine kinase), which were not encompassed by the application as filed (page 23, lines 19 to 30).

Article 123(3) EPC: it was argued that the claims of the new main request encompassed the possibility to create deletions in the tk-gene flanking sequences which was not within the scope of protection of the claims as granted.

Claim 16 as granted mentioned that the tk^- phenotype had

been obtained "...as a result of a deletion ***of*** the tk-gene..." and thus encompassed a single deletion mutant. On the contrary, corresponding claim 14 of the new main request stated that the tk^- phenotype is the "...result of a deletion ***of about 10 to 1,500 bp in size of*** the tk-gene..." and hence encompassed a plurality of deletion mutants. Attention was drawn in this context to the different formulations of claims 1 and 16 as granted, which could be indicative of differences in the scope of said claims: whereas claim 1 stated that the deleted mutant virus failed to produce "***any***" functional TK, claim 16 only mentioned that said mutant failed to produce "***a***" functional TK, not excluding that other functional TK could still be present. Furthermore, the necessity, in this context, for a claim to make sense or for the dependence of the claims to each other to be correct was questioned.

Article 84 EPC: it was considered that the skilled person would be at guess about the nature of the "...identifying characteristics..." of the deposited strain mentioned in claim 4 of the main request, since, due to the dependence of claim 4 upon claims 1 or 2, the deposited strain was already known to be temperature-resistant and to show a tk^- phenotype as a result of a deletion in the tk-gene of about 10 to 1500 bp.

It was also argued that the expression "...about 10 bp...", because of the inherent imprecision of the term "about", was not suited to make a distinction between the vaccines of the patent in suit and the MK-25 and/or MK-35 vaccines (documents (D101) and (K123)), which appeared, according to document (K126), to have a 1 bp

deletion in the tk-gene.

The skilled person was also considered to be at guess in view of the upper limit of the deletion ("1,500 bp"), since this value exceeded the length of the tk-gene coding region.

Further, a 10 bp deletion in the flanking sequences of the tk-gene, although encompassed by the claims, had also not been described in the patent in suit.

Article 83 EPC: it was objected that Figure 5, which had been introduced into the claims in the examination phase (patentee's letter of 24 January 1991) as an essential feature of the claimed subject-matter enabling the skilled person to prepare tk^- deletion mutant and was thus considered as crucial for the enablement of the claimed subject-matter of the patent in suit, was incorrect not only in the nature/presence/absence of some nucleotides, but also in the position of the reading frame, so that the sequence disclosed therein did not correspond to the PRV tk-gene. As a consequence, it was impossible to perform the subject-matter of the claims mentioning Figure 5, since they required a deletion mutation to be made in a "tk-gene" which was not the tk-gene.

Further, claims 1, 2, 14 and 15 of the main request, for instance, were considered as misleading, because they required that the PRV "*fails to produce any functional TK*", although the deletion mutants of Table 3 of the patent in suit still exhibited a TK activity. Furthermore, said Table 3 gave no indication on the units used for the measurement of the TK activity and showed that other mutations than deletions

may also lead to a tk^- phenotype.

It was also considered that a single deletion mutant lacking 148 bp in the coding sequence of the TK gene had been described in the patent in suit and that this teaching was not a suitable basis for a generalization concerning the position and/or the nature of the deletions leading to a non-functional TK.

Since the position of ATG was incorrect, the teaching of the patent in suit could not have been carried out for the part of the nucleotide sequence lying between the incorrect and the correct ATG.

Article 54 EPC: an objection was raised in view of document (K57) under Article 54(3) EPC, whereas the fact that MK-25/MK-35 were shown by document (K126) to have 1 bp deletion in the tk-gene as compared with the wild-type NIA3 PRV gave rise to an objection under Article 54(2) EPC.

Article 56 EPC: appellant III was of the opinion that the starting materials and the methods used in the patent in suit, the importance of the tk-gene for the virulence of PRV, examples of deletions of/in the tk-gene of PRV-related viruses and their implication in the resistance to subsequent viral infection had already been at the disposition of the skilled person at the priority date as demonstrated by the analysis of the prior art made in the patent in suit (page 3, line 10 to page 7, line 59). The problem to be solved in view of this prior art was then defined on page 3, lines 40-50 of the patent in suit as overcoming in the case of PRV the risk of reversion, the latency and the virulence. Since document (P1) demonstrated the

presence of the tk-gene on the BamHI-fragment No. 11, it was obvious for the skilled person, in view of the prior art teachings on the involvement in viruses related to PRV of the tk-gene in the virulence and on deletions leading to avirulent tk^- mutants, to delete parts of said PRV tk-gene in order to obtain with a reasonable expectation of success avirulent PRV tk^- deletion mutants, susceptible to be used as vaccines.

Appellant II considered document (D65) as being the closest prior art, since it defined, using MarHV as an example, a strategy (preparation of deleted tk^- MarHV used as vaccines) also applicable to PRV and saw the technical problem to be solved as the adaptation of said strategy to PRV. Since the localisation of the PRV tk-gene on the BamHI fragment No. 11 had been demonstrated by document (P1), the combination of the teachings of documents (D65) and (P1) obviously led to the solution of the claims of the main request.

IX. The submissions of appellant I (patentee) can be summarized as follows.

Article 123(2) EPC: it was argued that claim 4 of the new main request did not extend the subject-matter of the application as originally filed, since the deposit with its deletion of 148 bp in the tk-gene fell within the limit of "about 10 to about 1,500 bp" also found in the application as filed.

In view of claim 14 embracing deletions in the flanking sequences, the application as filed, read as a whole, already embraced such deletions by stating the upper limit of the deletions to 1,500 bp, while the tk-gene coding sequence was said to be about 1,000 bp.

Furthermore, the expression "...about 10 to 1,500 bp..." should be read together with the other features of claim 14, in particular the one requiring that the mutated PRV "...fails to produce a functional TK...".

Article 123(3) EPC: in view of the objection against claim 14 of the new main request, it was indicated that corresponding claim 16 as granted and its depending claims (concerned with partial deletions in the tk-gene) read as a whole would not make sense, if the expression "...deletion ***of*** the tk-gene..." was assumed to solely imply a deletion of the ***whole*** tk-gene. In particular, the dependence of claim 21 as granted (claiming the specific 148 bp deleted deposited PRV mutant) on claim 16 as granted would make no sense in this context. It was emphasized that this was a case, where, according to Article 69 EPC, the claims had to be read in the light of the description. The argument put forward about the different formulations of claims 1 and 16 as granted was considered as purely speculative and not based on any indication in the prior art about the possible existence of concomitant functional TKs.

Article 84 EPC: the expressions "...identifying characteristics..." and "...about 10 to 1,500 bp..." had already been in the claims of the application as filed and of the patent as granted and thus could not be again considered under Article 84 EPC, since they did not result from amendments.

Article 83 EPC: the patent in suit was considered as defining a concept fit for generalisation, since it described a specific, deposited 148 bp deleted PRV

mutant, which could be used as an "anchor" for further deletions extending on both sides of the 148 bp deletion up to the claimed 1,500 bp.

The errors in the sequence of Figure 5 were not considered as of important meaning, because the purpose of the patent in suit did not require the knowledge of the precise sequence or of the precise boundaries of the tk gene. Only the knowledge of restriction sites was required.

In view of the misleading character of the claims as far as the expression "fails to produce any functional TK" was concerned, it was argued that Table 3 of the patent in suit gave a negative control (the value of the mock-infected Rab(BU) cells) showing the "zero level" of TK activity, ie the "background noise" of the activity determination test in the absence of TK activity. Furthermore, this test was defined as a "relative test" allowing the skilled person to make a distinction between tk^+ and tk^- mutants, rendering a precise definition of the TK activity units unnecessary.

Article 54 EPC: novelty had never been a ground for opposition and applying decisions G9/91 (OJ EPO 1993, 408), G 10/91 (OJ EPO 1993, 420) and G7/95 (OJ EPO 1996, 626) of the Enlarged Board of Appeal, Appellant I did not give his consent for an analysis of the impact of documents (K126) and (K57) on the novelty of the subject-matter of the patent in suit.

Article 56 EPC: it was submitted that the patent in suit described a pioneer invention, for which no close prior art existed. If, for the sake of the problem-

solution approach, a closest prior art had to be identified, then document (P1) should be chosen and the technical problem to be solved of view of document (P1) seen in that preparation of a safe, effective and not over-attenuated PRV vaccine which cannot revert.

The patent in suit tremendously differed from the prior art in view of the safety, because of the absence of reversion due to the deletion contrary, for instance, to document (D65), the closest prior art cited by appellant II, which was not safe when injected intracerebrally in mice as shown by Table IV. The fact that Herpes Virus Simplex (HSV), Marmoset Herpes Virus (MarHV) and PRV, although classified as herpesviruses, were very different from each other was stressed, as demonstrated by the hosts (primate/human vs pigs and cattle), the symptoms induced and the fact that HSV and PRV only showed a 8% homology spread over the whole genome and no cross hybridisation between their tk-genes (document (P1)). Documents (P1) and (D65) did not give any information about the localisation, the restriction map and/or the DNA sequence of the PRV tk-gene. Furthermore, PRV TK differed in its function and specificity from HSV/MarHV TK. No information could have been derived from the prior art about a possible over-attenuation of PRV, although this phenomenon was quite common in the preparation of live vaccines, as shown by documents (K8) and (K9). Document (K3) also suggested the involvement of genes other than the tk-gene in the PRV virulence.

Thus, there could not have been at the priority date of the patent in suit a reasonable expectation of success in extrapolating the results of documents (P1) and/or (D65) to PRV.

Furthermore, the field of vaccines offered several possibilities to the skilled person, who could have chosen, besides the deletion mutant, the insertion mutants, the subunit vaccines, the killed vaccines, the temperature-sensitive live vaccines, the antibody-idiotype based vaccines. Therefore, the skilled person ***could*** have chosen the deletion mutation route, but nothing proved that he ***would*** have done so.

X. Appellants II and III requested that the decision under appeal be set aside and that the European Patent No. 0 149 040 be revoked.

XI. Appellant I requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request or 1st, 2nd or 3rd auxiliary requests, all filed on 25 May 2001, and amended pages 8, 9, 10 and 28 of the description submitted at the oral proceedings on 27 June 2001.

Reasons for the Decision

Rule 88 EPC.

1. Appellant I requested that the following corrections in the new main request were admitted under Rule 88 EPC:

- in claim 8: "*bp*" instead of "*pb*" for "base pair",

- in claims 9 and 28: "*SacI-C*" instead of "*SacD-C*",

- in claim 31: "*Rab-9*" instead of "*Rab9*".

No objection was raised by appellants II and III against this request. The board also considers that these corrections are obvious in the sense that nothing else would have been intended than what is offered as the correction. In particular, no "*SacD*" restriction endonuclease is known by the skilled person, who is only aware of the existence of a "*SacI*" one. The replacement of "**pb**" by "**bp**" is self-explaining and that of "**Rab9**" by "**Rab-9**" is the evident correction of a pure typographical error. Therefore, these corrections fulfil the requirements of Rule 88 EPC.

Article 114 EPC.

2. The board after consideration of the late-filed submissions of appellant I during the opposition procedure and of appellants II and/or III in answer to the communication of the Board decides in view of their *prima facie* relevance not to disregard them under Article 114(2) EPC.

Article 123(2) EPC.

3. As far as the objection raised under Article 123(2) EPC against claim 14 of the new main request is concerned, the board considers that the question to be answered is whether the expression "*...a deletion **in** the tk-gene...*" as found in the application as filed (page 16, line 10 to page 17, line 2) in relation to temperature-resistant PRV mutants may embrace the expression "*...a deletion **of about 10 to 1,500 bp in size** of the tk-gene...*" of said claim 14. The answer to this question lies in the meaning given to the preposition "***in***" and, in the given technical context, whether it only refers

to the coding sequence or may also be understood as encompassing the flanking sequences of the tk-gene. The application as filed (page 23, lines 20-30) states that such a deletion may follow two purposes: either a frame shift caused by a deletion of 10 to 100 bp or the prevention of the correct folding and/or substrate binding of/to TK as a result of a 75 to 1,500 bp deletion, which is further specified as being in "***...an appropriate coding region of the tk gene...***". On said page 23 of the application a filed (line 19), the tk gene is further said to be "***...approximately 1,500 bp in size...***". The application as filed hence encompasses deletions of up to 1,500 bp, which correspond to deletions of the whole tk-gene. Next, it has to be determined what falls within a 1,500 bp long tk-gene. The application as filed states on page 51 (lines 1 to 9) that the putative PRV ATG is at position 122 of the sequence given in Figure 5, whereas a putative stop codon is at position 1229. This results in a coding sequence of 1107 bp. Therefore, the tk gene which is said on page 23, line 19 to be about 1,500 bp long must contain the flanking sequences, so that the up to 1,500 bp deletion mentioned on page 23, lines 20-23 of the application as filed also encompasses the deletion of the flanking sequences. It can hence be concluded that the preposition "***in***" in the expression "...***in the tk gene***..." of the application as filed (page 16, line 10 to page 17, line 2) embraces the flanking sequences of the tk-gene. Thus, claim 14 of the new main request does not extend beyond the content of the application as filed and does not contravene the requirements of Article 123(2) EPC.

4. Claim 4 of the new main request had been considered as

defining a subject-matter extending beyond the content of the application as filed, because of its dependence on claim 1 of the new main request, which resulted from the introduction of the feature of claim 4 as granted into claim 1 as granted, which amounted to a restriction of the scope of the latter to a deletion in the tk-gene of about 10 to 1,500 bp. The scope of claim 1 of the new main request thus has to be compared with that of claim 1 as granted, which only stated that the deletion was "***in*** *the tk-gene*". The question in view of Article 123(2) EPC is whether this defines a subject-matter, which extends beyond the content of the application as filed. The answer to this question is negative, since, as seen above (point 3, above), the application as filed encompasses deletions "***in***" the tk-gene and the preposition "***in***" must be understood as meaning "from about 10 to 1,500 bp", since these values are the limits of the range for deletion length defined on page 23, lines 19-30. Therefore, claim 1 of the new main request does not define any subject-matter different from that of claim 1 as granted and thus does not extend beyond the content of the application as filed. As a consequence, claim 4 of the new main request, which makes the deposited strain PRV-(BUK-dl 3) dependent upon claim 1, defines neither a subject-matter different from that already defined by claim 6 as granted (dependent upon claim 1 as granted), nor a subject-matter, which extends beyond the content of the application as filed.

Article 123(3) EPC.

5. Claim 16 as granted, because of the expression "*...as a result of a deletion* ***of*** *the tk gene...*", was considered

to refer to a single deletion mutant having the ***whole*** tk-gene deleted, whereas claim 14 of the new main request encompassed a plurality of deletion mutants and hence contravened the requirements of Article 123(3) EPC.

6. Independent claims are usually directed to the essential features of the invention, whereas dependent claims concern particular embodiments of said invention as defined in the independent claims, so that a tight link exists between independent and dependent claims. Articles 69 (interpretation of the claims in the light of the description) and 84 EPC (support of the claims in the description) in turn define a tight link between the claims and the description. Description, independent and dependent claims are therefore related to each other in such a way that no contradiction should arise between them.

7. In this context, when seen together with claim 21 as granted for instance, which is supposed as a dependent claim to relate to a particular embodiment of claim 16 on which its depends, the deletion of claim 16 must also encompass the 148 bp partial deletion of the specific mutant. Therefore, the tight link between the description, the independent and the dependent claims leads the skilled person to interpret the expression "*...as a result of a deletion **of** the tk gene...*" as encompassing not only a complete deletion of the tk-gene, but also partial deletions of said gene.

8. Therefore, claim 14 of the new main request does not contravene the requirements of Article 123(3)EPC.

9. The presence in claim 14 of the new main request of the

characterizing feature "*...of a deletion of about 10 to 1,500 bp in size of the tk-gene...*" had led to an objection under Article 123(3) EPC, since the patent as granted makes in claims 16 and/or 18 reference to deletions "in" (claim 18) or "of" (claim 16) the tk-gene without specifying whether the flanking sequences are embraced. The skilled person could interpret said claims as not encompassing deletions in the flanking sequences, so that claim 14 of the new main request might be considered as extending the protection conferred.

10. It has already been shown (cf. points 5 to 8, above) that claim 16 as granted does encompass both partial and complete deletion mutants. When seen together with its dependent claim 19 (with the feature "*...deletion of about 10 to 1,500 bp*"), claim 16 implies a deletion of the flanking sequences of the tk-gene, since these flanking sequences are contained in the 1,500 bp long sequence (cf. point 3, above).

11. Furthermore, if the claims as granted are considered as presenting some ambiguity as far as the involvement of the flanking sequences in the deletion is concerned, they have to be interpreted in the light of the description, as suggested by Article 69 EPC. Since the patent as granted contains on page 10, lines 32 to 50 and page 21, lines 17 to 25 the same information as the application as filed (page 16, line 10 to page 17, line 2; page 23, lines 19 to 30 and page 51, lines 1 to 9), it can be concluded that it encompasses the same deletions as the application as filed, ie. deletions also involving the flanking sequences (cf. point 3, above). As a consequence, claims 16 and/or 18 as granted, as claim 14 of the new main request, also

encompass deletions in the flanking sequences of the tk-gene.

Therefore, claim 14 of the new main request does not contravene the requirements of Article 123(3) EPC.

Article 84 EPC.

12. The objected features "temperature-resistant", "about 10 to 1,500 bp in size" and of "identifying characteristics of PRV-(BUK-dl 3)" were already in the claims as granted and therefore not susceptible to objection under Article 84 EPC.

Article 83 EPC.

13. The expression "*...which fails to produce any functional TK...*", which appears for instance in the independent claims of the new main request, was considered as misleading and thus non-reproducible in view of Table 3 of the patent in suit, which shows that the tk^- deletion mutants still exhibit a residual TK activity, suggesting the presence of a functional TK. Table 3 is the result of an assay described in Example 4 of the application as filed for the determination of the TK activity based on the phosphorylation of ^{3}H-deoxythymidine as measured by scintillation spectrophotometry. The "zero level" is given by the value obtained with a strain known to display the tk^- phenotype, in case of the patent in suit the mock-infected Rab(BU) cells, and is not exactly "zero", because there is always some amount of unspecific contamination by radioactivity which cannot be washed out and leads to a certain "background noise". The difference between the "zero level" and the

value given by a tk^+ sample should be significant, since it is an indication of the sensitivity of the assay. This condition is met by the present assay: the value given by PRV(BUK), a tk^+ strain, is about 50 times higher than the "zero level". This assay does not require that specific units are given, because it is a "relative assay", in which a given sample is compared to a strain known to have the tk^- phenotype and defining the "zero level". Since it is a "relative assay", it does not even require that the "zero level" has always the same value, as seen in the fluctuations exhibited by the various tk^- strains in Table 3: indeed, the value given by the mock-infected Rab(BU) cells is about three times higher than that of the three deletion mutants PRV(BUK-dl 2/3/4) or of the mutagen-induced tk^- mutant, PRV(BUK-5A). This qualitative difference is actually meaningless, since such a "relative assay" only requires that a distinction can be made between a tk^+ strain and a tk^- strain. For this purpose, it is enough to have a negative control, ie a strain which is definitely known as exhibiting the tk^- phenotype. Therefore, the expression "*...which fails to produce any functional TK...*" is not misleading and hence reproducible in view of Table 3.

14. There is also no contradiction between the feature of the claims, according to which the production of a non-functional TK is the result of a deletion in the tk-gene and Table 3 of the application as filed and/or the patent as granted, which shows that the tk^- phenotype can also be obtained by other methods, such as a mutagen-induced mutation as in the case of PRV(BUK-5A). The claims do not imply that, basically, the tk^- phenotype can only be obtained by deletion. They only require that, in the specific case, said phenotype be

directly recognized as having been obtained by a deletion, as is the case of the mutants PRV(BUK-dl 2/3/4) which have been obtained by deletion of certain fragments of the tk-gene of PRV(BUK-5A-R1).

15. Figure 5 of the patent in suit has led to an Article 83 EPC objection, because it is incorrect not only as far as insertion, deletion or substitution of nucleotides are concerned, but also in view of a mis-identification of the boundaries and position of the tk-gene, so that the skilled person would allegedly not be able to reproduce the teaching of the claims, in which Figure 5 is mentioned. In this context, it was pointed to the fact that the reference to said Figure 5 was introduced as an essential feature of the claims during the examination phase to favour the acknowledgement of inventive step over the prior art. Figure 5 was said (patentee's letter of 24 January 1991, page 5) to be "*...a pre-requisite to enable the construction of PRV tk deletion mutants which fail to produce any functional thymidine kinase.*". Since Figure 5 is not the nucleotide sequence encoding TK, the skilled person was said to be unable to reproduce the claimed subject-matter.

16. This objection has to be seen in its context: the nucleotide sequence of Figure 5 is not claimed *per se* in any of the claims of the new main request or of the claims as granted or as filed, it is only mentioned in the claims as a means to identify the tk-gene. Furthermore, the formulation of the claims does not require a complete identity of the sequence of the tk-gene with the sequence of Figure 5. It is sufficient when both sequences ***essentially*** correspond to each other.

17. The crucial question to be answered is whether the correct sequence of the tk-gene is required for the purpose of the patent, ie the provision of tk^- deletion mutants of PRV.

18. The expression of a given nucleotide sequence in order to produce a biologically active protein imperatively demands that said nucleotide sequence be correct, since a modification of a single amino acid may lead to an inactive protein. Preparing a deletion mutant, on the contrary, is a much coarser and less demanding target and amounts to nothing else than destroying the gene, so as to avoid the expression of an active protein. For this purpose, the knowledge of the correct sequence is not so important as that of some restriction sites, which could be used to cut off all or parts of the nucleotide sequence. This is exactly the teaching of the application as filed and of the incriminated Fig 5.

19. The Board's view is fully corroborated by the declaration of Dr. Aguirre submitted as Annex B of appellant II's letter of 25 May 2001, who also considers that "*...the information of Figure 5 was not important to the ordinarily skilled person who wanted to make deletion mutants of PRV,...*" (page 4).

The Board is, therefore, of the opinion that the application as filed, despite the errors of Figure 5, does enable the skilled person to reproduce the teaching described therein.

20. As emphasized by appellants II and III, the patent in suit only gives a single example: a tk^- strain obtained by deletion of a 148 bp fragment in the tk gene. The board is nevertheless of the opinion that the teaching

of the patent in suit seen as a whole gives a concept fit for generalization, which enables the ordinary skilled person to achieve the envisaged result without undue difficulty within the whole ambit of the claims as required eg. by decision T 435/91 (OJ EPO 1995, 188). Indeed, besides said specific deletion mutant, Figure 5 of the patent in suit provides the skilled person with numerous correct restriction sites all along the nucleotide sequence, so that the skilled person could "walk" along the sequence extending the 148 bp deletion on both sides. On a scientific basis it is reasonable to assume that deletions larger than the known 148 bp one would result in the same tk^- phenotype. The skilled person would also have been confident as far as deletions smaller than the known 148 bp one, down to the claimed 10 bp deletion, are concerned, due to the theoretical tremendous effect of even short deletions on an expressed protein, because of a possible frame shift and/or the impact of the disappearance of some amino acids on the spatial structure and the biological properties of such a protein. The Board is also of the opinion that, due to the disclosure of numerous correct restriction sites all along the nucleotide sequence of Figure 5, the skilled person aware of the theory behind the concept of deletion would have been able to reproduce the teaching of the patent in suit, ie the production of tk^- deletion mutants, without slavishly starting from the described and deposited 148 bp deletion mutant.

Article 54 EPC.

21. Novelty objections had been raised in view of document (K57) and of the vaccines MK-25/Mk-35 as described in documents (D101) and (K123). Appellant I, referring to

the Decisions of the Enlarged Board of Appeal G 9/91 (OJ EPO 1993, 408), G 10/91 (OJ EPO 1993, 420)and G 7/95 (OJ EPO 1996, 626), did not give his consent arguing that novelty had never been an implicit or explicit ground of opposition against the patent in suit. Thus, novelty is not an issue of this decision.

Article 56 EPC

22. The appellants and the opposition division have considered documents (P1) and/or (D65) as the closest prior art. The Board disagrees therewith, because, since the subject-matter of the patent in suit is concerned with a vaccine to prevent PRV disease based on the use of a PRV deleted in the tk gene, the closest prior art should be, if available, an already existing vaccine against PRV disease. Neither document (D65), which is concerned with MarHV, nor document (P1), which is concerned with PRV, but not with vaccines, fulfill this condition. However, such a prior art is indeed available and even rather abundant, since numerous prior art publications are concerned with more or less successful attempts to prepare PRV vaccines: for instance, documents (D72), (D73), (K2), (K30), (K52), (K62), (D99), (D101), (D104), (D100) and (K123). These vaccines have been attenuated either by serial passages on host cells (mainly chick embryo cells) or culture in presence of bromo-, iododeoxyuridine or ara-T. Although each of them could be used as the closest prior art, the board considers document (K123) for this purpose. The reason therefor does not lie in the nature and particular properties of the PRV vaccines described in this document, but much more in the quality of its disclosure. Document (K123) describes MK-35, a tk^- PRV mutant obtained by selection on a medium containing

5-bromo-deoxyuridine and which is avirulent in rabbits, mice, sheep, suckling pigs while retaining its immunogenic properties, so that it can be used for vaccination purpose. The disadvantage of the tk^- PRV mutant of document (K123) is that the reason, on the molecular level, for the attenuation is unknown, as is its reversion rate. However, since the selection has been made by passage in presence of 5-bromo-deoxyuridine, the skilled person would assume that it is most probably a point mutation susceptible of reversion at a relatively high frequency. This, of course, renders such a PRV mutant unsuitable for use as a safe vaccine. This assumption is corroborated by document (K126), which indicates the presence of a single base deletion in the sequence of MK25/MK35 as compared to that of the tk-gene of NIA3 wild type strain. However, said NIA3 strain was not the "starting material" used in document (K123) to prepare MK25/MK35.

23. The technical problem to be solved starting from document (K123) as the closest prior art is to prepare a vaccine to prevent diseases caused by PRV, which is efficient (ie not over-attenuated) and safe, ie which presents an extremely low frequency of reversion.

24. This problem is solved by the vaccines based on the tk^- PRV mutants of the patent in suit, which have been obtained by deletion within the tk-gene. The nature of the deletion can easily be determined, since the patent in suit gives enough information on the molecular structure of said tk gene (sequence, restriction sites) and the reversion frequency of a deletion is extremely low, a feature which renders the vaccine safe. The vaccines also appear to be efficient and not over-attenuated as demonstrated by the results obtained with

mice and calves in Examples 5 and 6 of the patent in suit.

25. A first consideration when examining the inventive step of the subject-matter of the patent in suit is that the skilled person at the priority date had other attractive possibilities to solve the technical problem mentioned above than a deletion in the tk-gene. For instance, document (K80), efficiently inactivated the HSV tk-gene by insertion therein of fragments of various regions of the viral genome. The history of vaccination would also have suggested at least the possibility of a killed vaccine. Therefore, the skilled person at the priority date of the patent in suit was not bound to prepare a tk^- deletion mutant. This route was a **deliberate choice**, which was not necessarily related to a reasonable expectation of success, since document (D67) indicated that tk^- HSV deletion mutants poorly grow (page 278, first paragraph).

26. In the context of this deliberate choice in favour of the "tk^- deletion mutant route", the fundamental question in view of the assessment of inventive step of the solution of the patent in suit is whether the ordinary skilled person starting from document (K123) as the closest prior art (first step of this route) would have found indications in the prior art and/or the common general knowledge, when considered in combination with document (K123), leading in an obvious manner to the solution described in the patent in suit with a reasonable expectation of success.

27. Part of the answer to this question would have been found in document (D65), the second step of this route, which describes the deletion of part of the coding

region of the MarHV tk-gene. The so obtained tk^- MarHV deletion mutant confers a protection on mice after subcutaneous inoculation (Tables III and IV) and is safe, because it cannot revert to the tk^+ phenotype (page 35). A possible use as an attenuated, live vaccine is envisaged (page 35). Document (D65) thus describes in the context of MarHV exactly what the skilled person is looking for in the case of PRV. This explains why the skilled person would take document (D65) into consideration and try to adapt the results described therein to PRV.

28. The skilled person at this stage, however, would still lack an important information to be able to adapt the results described in document (D65) with the MarHV tk-gene to the PRV. This information is the localisation of the tk-gene in the genome of PRV. Document (D65) is silent about this point.

29. The necessity of getting this information would have prompted the skilled person, in a third step, to take document (P1) into consideration. This document discloses the localisation of the tk-gene in the genomes of HSV-1 and PRV by marker rescue of tk^- mutants using BamHI fragments of the HSV and PRV wild type strains and tentatively identifies the BamHI fragment No. 11 as carrying the PRV tk-gene, which is localized between map coordinates 0.43 and 0.45 of the PRV genome. Document (P1) also demonstrates that HSV and PRV tk-genes are collinear, although PRV and HSV only share 8% homology spread over the whole genome.

30. However, apart from the fact that the skilled person would have had to make three steps and to take three prior art documents into consideration, it is doubtful

whether he would have had a reasonable expectation of success in combining the teachings of documents (K123), (D65) and (P1) to reach the solution of the patent in suit. This is due to the fact that the molecular modifications responsible for the tk^- phenotype in document (P1) are not described. Therefore, the skilled person could not have been sure that the BamHI fragment No. 11 contains the tk gene. The tk^- phenotype could have been the result of a modification of a gene regulating the expression of the tk-gene. The only conclusion which could have been drawn from document (P1) is that BamHI fragment No. 11 is able to rescue the tk^- phenotype.

31. In order to be reasonably sure that said BamHI fragment No. 11 does contain the tk gene, the skilled person would have to make a fourth step and take a fourth document into consideration, namely document (P35), which identified by biochemical and serological techniques the TK produced by mouse cell after transfection with a 3.4 kb BamHI fragment from HSV as being of viral origin. Due to the collinearity between HSV and PRV demonstrated by document (P1), the skilled person could, despite the low homology between HSV and PRV and the absence of cross-hybridization between PRV and HSV tk-genes (document (P1)), have come to the conclusion that the BamHI fragment No. 11 of PRV also contains the tk gene as high. However, the skilled person would have needed four steps and would have had to consider the teaching of four documents to come to the solution proposed in the patent in suit. This is more than the skilled person can be expected to derive for himself in an obvious manner from the prior art, so that inventive step must be acknowledged for this subject-matter.

32. For these reasons the Board considers that the claims of the new main request submitted on 25 May 2001 meet the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the claims filed as main request on 25 May 2001, pages 3 to 7, and 11 to 27 of the description as granted, pages 8, 9, 10 and 28 of the amended description filed on 27 June 2001 and the Figures as granted.

The Registrar: The Chairwoman:

U. Bultmann U. Kinkeldey