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Datasheet for the decision of 13 January 2009

Case Number: T 0391/07 - 3.3.04

Application Number: 95939926.2

Publication Number: 0790835

IPC: A61K 39/02

Language of the proceedings: EN

Title of invention:
Specific immunotherapy of cancer using a live recombinant bacterial vaccine vector

Patentee:
The Trustees of The University of Pennsylvania

Opponent:
Anza Therapeutics, Inc.

Headword:
Immunotherapy of cancer/UNIVERSITY OF PENNSYLVANIA

Relevant legal provisions:
EPC Art. 56, 123(2)
EPC R. 139

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request; added subject-matter (no); inventive step (yes)"

Decisions cited:
G 0004/88, G 0002/04, T 1329/04

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0391/07 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 13 January 2009

Appellant: (Patent Proprietor)
The Trustees of The University of Pennsylvania
Center for Technology Transfer,
3700 Market Street
Suite 300
Philadelphia
Pennsylvania 19104-3147 (US)

Representative:
Jaenichen, Hans-Rainer
Vossius & Partner
P.O. Box 86 07 67
D-81634 München (DE)

Respondent: (Opponent)
Anza Therapeutics, Inc.
2550 Stanwell Drive
Concord, CA 94520 (US)

Representative:
Brasnett, Adrian Hugh
Mewburn Ellis LLP
33 Gutter Lane
London EC2V 8AS (GB)

Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 3 January 2007 revoking European patent No. 0790835 pursuant to Article 102(1) EPC 1973.

Composition of the Board:

Chair: U. Kinkeldey
Members: R. Gramaglia
R. Moufang

Summary of Facts and Submissions

I. European patent No. 0 790 835 (application No. 95939926.2, published as WO-A-96/14087) was granted with 8 claims. The patent relates to specific immunotherapy of cancer using a live recombinant bacterial vaccine vector.

II. Notice of opposition was filed by the opponent requesting the revocation of the European patent on the grounds of Article 100(a), (b) and (c) EPC. The opposition division revoked the patent on the grounds that the main and the auxiliary requests then on file did not fulfil the requirements of Article 56 EPC.

III. The opposition division considered the teaching of document D3 as the closest prior art. In its opinion, the problem underlying the patent in suit, namely the provision of an alternative tumour vaccine (main request) or the provision of an improved tumour vaccine (auxiliary request) had not been solved in an inventive manner.

IV. The patentee (appellant) filed an appeal against the decision of the opposition division.

V. In a letter dated 8 May 2008, the representative of the opponent filed a request for recording a transfer of opponent status from the original opponent Cerus Corporation to Anza Therapeutics, Inc., and submitted documentary evidence.

VI. Oral proceedings were held on 13 January 2009, during which the appellant submitted claims 1-8 of a new main request.

Independent claims 1 and 5 of this request read as follows:

" 1. A vaccine for the treatment of cancer or for inhibiting the formation of tumors by inducing an immune response to a tumor specific antigen in a human host, the vaccine comprising a recombinant ***Listeria monocytogenes*** produced by homologous recombination which is capable of expressing and secreting a tumor specific antigen or fragment thereof, wherein the homologous recombination is in the ***Listeria monocytogenes*** chromosome and does not disrupt bacterial genes necessary for the growth and spread of ***Listeria monocytogenes***."

"5. Use of a recombinant ***Listeria monocytogenes*** produced by homologous recombination which is capable of expressing and secreting a tumor specific antigen or fragment thereof, wherein the homologous recombination is in the ***Listeria monocytogenes*** chromosome and dose [sic] not disrupt bacterial genes necessary for the growth and spread of ***Listeria monocytogenes*** in the manufacture of a vaccine for the treatment of cancer or for inhibiting the formation of tumors by inducing an immune response to a tumor specific antigen in a human host."

Claims 2 to 4 and 6 to 8 related to specific embodiments of the vaccine according to claim 1 or the use according to claim 5, respectively.

VII. The following documents are cited in the present decision:

D3 Schafer R. et al., J. Immunol., Vol. 149, No. 1, pages 53-59 (1992);

D5 Kocks C. et al., Cell, Vol. 68, pages 521-531 (1992);

D6 Camilli A. et al., Molecular Microbiology, Vol. 8, No. 1, pages 143-157 (1993);

D7 WO-A-93/15212;

D8 Mollet B. et al., J. Bacteriology, Vol. 175, No. 14, pages 4315-4324 (1993);

D9 Lampson L.A. et al., Cancer Research, Vol. 53, pages 176-182 (1993);

D10 Ikonomidis G. et al., Abstracts of the 94th General Meeting of the American Society for Microbiology held in Las Vegas on May 23-27, 1994, page 159, Abstract No E-90;

D11 Huang A.Y.C. et al., Science, Vol. 264, pages 961-965 (May 1994);

D14 Gunn G.R. et al., Vaccine Delivery Strategies, Edited by Guido Dietrich and Werner Goebel, Horizon Scientific Press, UK, pages 315-348 (2002);

D16 Stahl M. et al., J. Bacteriology, Vol. 158, No. 2, pages 411-418 (1984);

D17 Brown D.P. et al., J. Bacteriology, Vol. 170, No. 5, pages 2287-2295 (1988);

D18 Shen H. et al., Proc. Natl. Acad. Sci. USA, Vol. 92, pages 3987-3991 (1995);

D19 Liau L.M. et al., Cancer Research, Vol. 62, pages 2287-2293 (2002);

D22 Wirth R. et al., J. Bacteriology, Vol. 165, No. 3, pages 831-836 (1986);

D28 Bodmer H.C. et al., Cell, Vol. 52, pages 253-258 (1988);

D29 WO-A-94/17192;

D44 Jensen E.R. et al., J. Virology, Vol. 71, No. 11, pages 8467-8474 (1997);

D45 Carbone F.R. et al., J. Exp. Med., Vol. 169, pages 603-612 (1989);

D46 Carbone F.R. et al., J. Exp. Med., Vol. 171, pages 377-387 (1990);

D51 Yangxin F. et al., Cancer Research, Vol. 50, pages 227-234 (1990);

D52 Repique C.J. et al., Cancer Investigation, Vol. 10, No. 3, pages 201-208 (1992);

D53 Young M.L.R. et al., Cancer Immunol. Immunother., Vol. 35, pages 14-18 (1992);

D54 Lejeune P. et al., J. Immunol., Vol. 152, No. 10, pages 5077-5083 (May 1994);

D55 Shimizu M. et al., Cancer Immunol. Immunother., Vol. 38, pages 272-276 (1994);

D64 Current Protocols in Immunology edited by Coligan J.E. et al., John Wiley & Sons, Inc., Vol. 3, pages 3.11.1-3.11.20 (1992);

D70 Bear H.D., Cancer Research, Vol. 46, pages 1805-1812 (1986);

D71 Weidt G. et al., J. Immunology, Vol. 153, pages 2554-2561 (1994);

D86 Bruhn K.W. et al., Vaccine, Vol. 23, pages 4263-4272 (2005);

D98 Lin C.-W. et al., Int. J. Cancer, Vol. 102, pages 629-637 (2002);

D99 Irvine K.R. et al., Semin. Cancer Biol., Vol 6, No. 6, pages 337-347 (1995).

VIII. The submissions by the appellant (patentee), insofar as they are relevant to the present decision, can be summarized as follows:

Article 123(2) EPC

– The skilled person would know that homologous recombination could be carried out with a number of different plasmids, insertion sites and under the control of promoters different from L. monocytogenes promoters. The skilled person would therefore not read the passages on page 10 of the original application as being limited to the preferred embodiments mentioned in this passage.

Inventive step (Article 56 EPC)

– The problem to be solved was the provision of an effective tumour vaccine for use in humans.

– The in vitro test used in document D10 (and also in document D3) were not predictive of a situation where a true aggressive tumour was implanted in vivo in mice.

– Document D70 merely demonstrated that in animals exhibiting an in vivo anti-tumour response, there was a corresponding CTL response. However, this finding did not mean that the reverse ("CTLs assays correlate with in vivo anti-tumour activity") was true.

– It was not obvious to use a Listeria or another intracellular bacteria expressing a tumor antigen for treating cancer because bacteria were believed to act primarily through MHC-II-restricted pathway suited to treat infections, whereas viruses were used as vectors for releasing tumour associated

antigens in vaccines suitable to treat cancer because viruses were known to elicit MHC-I immune response.

– The P815 cell line mentioned in document D3 was only used for determining CTL activity, a criterion which was not sufficient to establish protection and regression in vivo.

– There was no expectation of success that the claimed vector would elicit an anti-tumour immune response in view of the fact that many tumours were known to exhibit a variety of immunosuppressive functions.

Adaptation of the description

– The change of "MVC-1" in paragraph [0029] of the patent and in granted claim 3 and 7 to read "MUC-1" was evident (see document D99).

IX. The submissions by the respondent (opponent), insofar as they are relevant to the present decision, can be summarized as follows:

Article 123(2) EPC

– The wording in claims 1 and 5 "produced by homologous recombination... wherein the homologous recombination is in the ***Listeria monocytogenes*** chromosome and does not disrupt bacterial genes necessary for the growth and spread of ***Listeria monocytogenes***" was disclosed in the application as filed only in combination with additional

limitations regarding (i) a temperature-sensitive plasmid, (ii) a specific Listeria monocytogenes promoter, and (iii) the specific recombination site disclosed in document D6 (the Camilli reference). Therefore, the present wording represented a generalization in contravention of Article 123(2) EPC.

– The term "secreting" in claims 1 and 5 was in contravention of Article 123(2) EPC because secretion of the protein was disclosed in the original application only in the context of very specific situations.

Inventive step (Article 56 EPC)

– The recombinant Listeria vaccine defined by the claims differed substantially from the DP-L2028 strain actually used in the Examples. Moreover, the application as filed did not contain evidence that the claimed recombinant Listeria vaccine exhibited in vivo anti-tumour activity. In view of the differences/deficiencies highlighted above, it was not plausible that the claimed vaccine exhibited in vivo anti-tumour activity. This view was supported by post-published document D14, which showed that the integrated construct "Lm-E7" (obtained via homologous recombination) falling within claim 1 did not exert any anti-tumour effect on E7-expressing tumours.

– Since the present application as filed did not make it plausible that the claimed recombinant vaccine exhibited in vivo anti-tumour activity,

the conditions set out in decision T 1329/04 of 28 June 2005 for relying on post-published documents D19, D44, D98 and D86, as done by the appellant, were not satisfied.

– Hence, the problem to be solved was the provision of an alternative L. monocytogenes tumour vaccine.

– The claimed subject-matter was obvious in view of the teaching in document D10 alone or in combination with that of document D3 because the skilled person would have considered the in vitro CTL assays described in documents D10 and D3 to correlate with in vivo anti-tumour activity.

– The claimed subject matter could also be arrived at in an obvious way by starting from the teaching in document D9 or D29 that immunisation with the protein β-gal or tumour antigen conferred protection against tumor growth, by turning to the Listeria vectors known from document D7 or document D10.

Adaptation of the description

– The change of "MVC-1" in paragraph [0029] of the patent and in granted claims 3 and 7 to read "MUC-1" was not allowable.

X. The appellant (patentee) requested that the decision under appeal be set aside and the patent be maintained in amended form on the basis of the main request and the adapted description, both filed at the oral proceedings.

The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

Opponent status

1. The opponent status has been validly transferred from the original opponent Cerus Corporation to Anza Therapeutics, Inc. The documents on file show that the original opponent's part of business relating to Listerial vaccines was effectively transferred to Anza Therapeutics, Inc. This had the consequence that, following the opponent's corresponding request, Anza Therapeutics, Inc. acquired the procedural status as opponent and respondent in the present proceedings (see decisions G 4/88, OJ EPO 1988, 480 and G 2/04, OJ EPO 2005, 549).

Added subject matter (Article 123(2) EPC)

2. The respondent maintains that the original application disclosed the technique of homologous recombination only in combination with temperature-sensitive (ts) plasmids and that homologous recombination in general was not directly and unambiguously derivable from the application as filed. Therefore, the wording "homologous recombination" in claims 1 and 5 represented a generalization in contravention of Article 123(2) EPC.

3. The board agrees with the respondent that "homologous recombination" is explicitly disclosed in the WO application only in combination with the wording "with a temperature sensitive plasmid" (see page 10, lines 12-14 and 31-32 of the WO application). However, for the purpose of deciding under Article 123(2) EPC, the content of an application is to be considered as the whole disclosure that is directly and unambiguously derivable from the application, including information which is implicitly apparent to the skilled person as a clear and unavoidable consequence of what is explicitly mentioned. The question thus arises whether or not the technique "homologous recombination" in general is derivable in this sense from the application as filed.

4. According to page 4, lines 20-22 of the WO application, means necessary for carrying out the present invention are recombinant forms of Listeria monocytogenes capable of expressing a tumour specific antigen or fragment thereof. On page 9, lines 19-20 of the WO application, it is stated that several techniques for producing these recombinant L. monocytogenes, such as transposon insertion (line 22) or using a prfA-containing vector (line 28) are known. In the board's opinion, the skilled person would understand that the selected technique was not critical, as long as a recombinant form of Listeria monocytogenes capable of expressing a tumour specific antigen or fragment thereof could be obtained.

5. The WO application provides the further information on page 10, lines 12-14 that a more preferred method is homologous recombination with a temperature sensitive plasmid. In the board's opinion, the skilled person

reading this passage in the light of the whole application would perceive that, while homologous recombination with ts plasmids was a preferred and advantageous embodiment because the use of ts plasmids facilitated the screening, the remaining techniques mentioned above, **including** homologous recombination in general (i.e., relying on any screening method) also belonged to the palette of suitable techniques for obtaining the recombinant forms of Listeria monocytogenes. Therefore, it is the board's view that the disclosure of "homologous recombination" in general by the original application was a clear and unavoidable consequence of the disclosure of "homologous recombination with a ts plasmid" in the light of the original application, taken as a whole, and in the light of the common general knowledge (see documents D8, D16 and D17) that homologous recombination could also be carried out with plasmids not being ts.

6. In the respondent's view, the homologous recombination with a ts plasmid was disclosed in the original application only in combination with a Listeria monocytogenes promoter. The absence of the latter feature in claims 1 and 5 thus led to a generalization in contravention of Article 123(2) EPC.

Page 10, lines 17-20 of the WO application recites:

"This method [homologous recombination with a ts plasmid] allows for the routine insertion of any gene of interest into the chromosome of ***L. monocytogenes*** which is then expressed under the control of a ***L. monocytogenes*** promoter".

The respondent wants the board to read the sentence above as meaning that homologous recombination required the compulsory use of a Listeria monocytogenes promoter. The board firstly notes that the WO application (see claim 6, page 4, line 25 and page 10, lines 14-16) also provides a basis for claims referring to the expression/secretion of the protein of interest without any limitation to a Listeria monocytogenes promoter. Secondly, from the original application taken as a whole the skilled person perceived that neither the selected technique nor the selected promoter were critical, as long as the recombinant form of Listeria monocytogenes was capable of expressing/secreting a tumour specific antigen or fragment thereof. In fact, the application itself conveyed to the skilled person the information that heterologous promoters were also suited for performing the invention, since the plasmid pAM401 referred to on page 12, line 36 of the WO application (expressing antibiotic resistance genes under the control of E. coli or S. faecalis promoters: see document D22, page 834, under "Construction of pAM401") was introduced into L. monocytogenes (see page 13, line 5). Finally, the board observes that already a "natural" reading of the sentence quoted above excludes a compulsory connection of a particular gene with a particular promoter. In conclusion, the absence of a reference to a Listeria monocytogenes promoter in claims 1 and 5 is not in contravention of Article 123(2) EPC.

7. A further respondent's objection was that claim 1 infringed Article 123(2) EPC because there was a basis in the application as filed only for homologous recombination performed into the specific EcoRI

recombination site disclosed in document D6 (the "Camilli" reference), whereas claim 1 no longer comprised the restriction above to the specific "Camilli" recombination site.

The skilled person would read the passage on page 10, lines 25-29 as referring to any region which can act as a site for insertion, without disrupting bacterial genes necessary for the growth and spread of the organism and that the reference to document D6 ("Camilli") in the original application was made only to show an example of regions which have been shown to have these properties. In fact, document D6 discloses a further strain DP-L1552 disrupted at the plcA site (see Fig. 2 and page 147, r-h column, lines 9-19). Moreover, the skilled person was aware of other possible integration sites (see document D18, page 3991, first paragraph and document D5, page 526, l-h column, lines 5-9 and Fig. 1). Therefore, the skilled person would have understood the sentence on page 10, lines 27-29 ("...utilizes a region of its chromosome that can act as a site for insertion without disrupting bacterial genes necessary for the growth and spread of the organism...") to refer to any region that can be used without disrupting genes necessary for growth and spread, e.g. those disclosed in the application as filed and known from the prior art (documents D6, D18 and D5).

8. Finally, the respondent argues that the term "secreting" in claims 1 and 5 is in contravention of Article 123(2) EPC because secretion of the protein was disclosed in the original application only in the context of very specific situations (see page 7,

lines 15-21: fusion protein with LLO; page 9, lines 6-11: p210bcr-abl antigen from chronic myeloid leukemia and page 10, lines 23-25: LLO signal sequence).

Claims 1 and 5 require secretion of the tumor specific antigen and fragments thereof in the context of homologous recombination. There is a general statement on page 10, lines 12-16 of the WO application that homologous recombination with a ts plasmid can be used to produce transformants that secrete the protein of interest. In view of the conclusion arrived at by the board under point 5 supra that the disclosure by the original application of "homologous recombination with a ts plasmid" is an implicit disclosure of "homologous recombination" in general, there is a basis in the original application for the term "secreting" in the context of homologous recombination in claims 1 and 5.

Sufficiency of disclosure (Article 83 EPC)

9. During the oral proceedings, the respondent no longer maintained any objections under this Article and the board sees no need to deal with the question of sufficiency of disclosure.

Inventive step (Article 56 EPC)
Closest prior art
Document D3

10. This document, issued from the same inventor's team, discloses a recombinant Listeria monocytogenes wherein a DNA sequence encoding β-galactosidase (hereafter: β-gal) has been inserted via a transposon/plasmid-based technique. This inserted DNA construct expressed but

did not secrete β-gal into the cytoplasm. This recombinant L. monocytogenes (DP-L967) was used to determine if the anti-listerial immune response would also expand to include the β-gal antigen. The in vitro technique used for measuring the immune response of mice immunized with this live vaccine was a "chromium-51 release cytotoxicity assay" carried out with β-gal expressing P815 cells (see page 54, r-h column) used for the detection of β-gal specific CTLs (inter alia $CD8^+$).

Document D10

11. This document, also issued from the same inventor's team, describes a recombinant Listeria monocytogenes that expressed and secreted into the cytoplasm the influenza nucleoprotein antigen NP as a fusion protein. It is stated in this document that cells infected with this recombinant Listeria monocytogenes expressing NP became the targets for recognition by influenza-specific T cells. The method used for measuring the immune response of mice immunized with the live vaccine of document D10 relied on the in vitro detection of NP-specific CTLs in splenocyte cultures.

Document D7

12. This document relates to an attenuated mutant of L. monocytogenes incorporating in the act A gene or its promoter a mutation capable of blocking the expression of the protein encoded by the act A gene. It is stated on page 4, lines 21-34 of this document that an heterologous DNA can be inserted into this mutant for expression of bacterial or viral antigens such as HIV

gp120 (page 5, line 3). Reference is also made (see page 13, lines 11-16) to homologous recombination as a means for inserting the heterologous DNA, which could be preceded by the signal DNA sequence of the hly gene for enabling secretion of the expressed fusion protein (see claim 17).

Document D9

13. This document is concerned with a 9L/lacZ gliosarcoma cell line expressing the marker protein β-gal, permitting the visualisation of disseminated tumour cells within the brain. It is shown that immunisation with the protein β-gal itself could protect against tumour growth.

Document D29

14. It is stated on page 15, lines 16-19 of this document that studies indicated that vaccinia virus recombinant vaccines containing either the SV40 T antigen genes or the E6 and E7 genes from HPV or influenza nucleoprotein would protect animals against subsequent challenge with tumour cells that expressed these proteins as tumour antigens.

15. The analysis above shows that only document D9 (see page 179, l-h column, lines 4-5) and document D29 (see page 15, lines 16-19) are concerned with obtaining the biological effect stated in claim 1, namely reducing tumour growth or conferring tumour protection. However, the means for achieving this effect (document D9: immunisation with β-gal or a 9L/lacZ gliosarcoma cell line expressing the marker protein β-gal; document D29:

a viral vector) completely diverge from a recombinant Listeria vector.

Documents D3, D10 and D7 address a recombinant Listeria vaccine. The heterologous DNA to be inserted in the vector according to document D7, however, encodes a bacterial or viral antigen (see page 4, lines 21-34 and page 5, line 3), unlike that expressed by the vectors of document D3 and D10, which is a "pseudo cancer antigen" β-gal or NP (see point 21(i) infra).

Therefore, documents D3 and D10 come closer to the claimed subject-matter than documents D7 and D9. The biological effect described in the former documents is an immune response (β-gal specific or NP-specific CTLs) measured/detected in vitro (see points 10 and 11 supra). Given that both claim 1 and document D10 mention secretion of the expressed protein, while the construct referred to in document D3 is unable to achieve said secretion, the board considers that document D10 represents the closest prior art for the purpose of applying the problem-solution approach.

Problem to be solved

16. The claimed vaccine differs from the recombinant vector described in document D10 by the following features: (i) a tumor specific antigen (claims 1 and 5) vs. influenza nucleoprotein NP (document D10); (ii) human host (claims 1 and 5) vs. mouse (document D10); (iii) homologous recombination for inserting the heterologous gene (claims 1 and 5) vs. no information in this respect (document D10) and (iv) treatment of cancer or inhibition in vivo of cancer (claims 1 and 5) vs. in

vitro detection of NP-specific CTLs in splenocyte cultures of mice immunized with the live vaccine. Given that features (i) to (iii) above are not of a critical nature (see point 20(i) to (iii) infra), the board considers that feature (iv) represents the decisive difference over the closest prior art. Hence, the problem to be solved is the provision of an effective tumour vaccine for use in humans.

17. The respondent maintains that the problem to be solved was rather the provision of an alternative L. monocytogenes tumour vaccine because the feature in claims 1 and 5 that the recombinant vaccine exhibited in vivo anti-tumour activity could not be taken into account in view of the deficiencies explained in detail below.

18. It is argued by the respondent that the recombinant Listeria vaccine defined by the claims differed substantially from the DP-L2028 strain actually used in the Examples in the following features: (i) a tumor specific antigen (claims 1 and 5) vs. influenza nucleoprotein NP (Examples); (ii) human host (claims 1 and 5) vs. mouse (Examples) and (iii) homologous recombination (claims 1 and 5) vs. episomal expression via multicopy plasmids (Examples). Moreover, the application as filed did not contain evidence that the claimed recombinant Listeria vaccine exhibited in vivo anti-tumour activity. In view of the differences highlighted above, this in vivo anti-tumour activity was not plausible. This respondent's view was supported by post-published document D14, which showed that the integrated construct "Lm-E7" (obtained via homologous recombination) falling within claim 1 did not exert any

anti-tumour effect on E7-expressing tumours, in contrast to the plasmid-based construct "Lm-LLO-E7" (similar to the plasmid-based constructs exemplified in the patent). The respondent also cited decision T 1329/04 (supra) in support of its case. The rationale underlying this decision required that supplementary post-published evidence could only be taken into account if the disclosure in the application as filed made it plausible that its teaching indeed solved the problem it purported to solve. However, since the present application as filed did not make it plausible that the claimed recombinant vaccine exhibited in vivo anti-tumour activity, the conditions for relying on post-published documents D19, D44, D98 and D86, as done by the appellant, were not satisfied.

19. Since the respondent relied heavily on decision T 1329/04 (supra) to support its case, the board provides the following analysis. In this decision, the problem to be solved was isolating a further member of the TGF-β family. The solution provided was a DNA sequence encoding "protein GDF-9". The board in that case came to the conclusion that this problem had not plausibly been solved because the application underlying that decision lacked fundamental information which served to establish whether or not protein GDF-9 belonged to the TGF-β family, namely the "seven cystein residue pattern" (see point 7 of the "Reasons") and the "70-90% homology requirement" (see point 8 of the "Reasons"). Protein GDF-9 could thus not clearly and unambiguously be identified as a member of the TGF-β family on the basis of the application as filed and supplementary post-published evidence could not be

taken into consideration to establish the nexus between protein GDF-9 and TGF-β family.

20. In the board's judgement, the rationale underlying decision T 1329/04 (supra) has no bearing on the present case. This is because the present application as filed did provide all the necessary technical information for the skilled person to prepare (see the WO application on page 10, line 12 to page 11, line 13) and to test (see the in vivo challenge tests disclosed in the Examples) the claimed recombinant Listeria vaccine. The only missing data were the results of possible in vivo experiments performed with the claimed vaccine. However, the successful results of the exemplified in vivo tests could plausibly be extended to the claimed embodiment because the changes were not perceived as being such that the in vivo activity of the claimed vaccine would be abolished, in the light of the following facts:

(i) as for the difference in the expressed antigen (a tumour specific antigen (claims 1 and 5) vs. influenza nucleoprotein NP (Examples)), document D9 and document D11 showed that "pseudo cancer antigens" such as β-gal and NP (which were artificially introduced into the target tumour cell) could serve/behaved as tumour antigens for in vivo studies. According to page 176, l-h column, two last paragraphs of document D9, immunisation with the β-gal protein could indeed protect against tumour growth of tumour cell lines expressing the lacZ reporter gene (i.e. an E. coli-derived β-gal: see Abstract, lines 3-4). Likewise, the expression of NP did not change the in vivo growth characteristics of the tumor and the NP antigen was

capable of being recognized by T cells (see document D11, page 963, central column, lines 7-11) ;

(ii) as regards the selected expression/secretion technique (homologous recombination (claims 1 and 5) vs. episomal expression via multicopy plasmids (Examples)), this aspect was perceived as not being critical, as long as a recombinant form of Listeria monocytogenes capable of expressing and secreting a tumour specific antigen or fragment thereof could be obtained. In the decision under appeal (see point 7, fourth paragraph) the respondent is quoted that it agreed that the use of homologous recombination instead of transposon-based insertion did not result in improved properties of the tumour vaccine, but merely represented a well-known alternative way of introducing genes into the chromosome of a bacteria. On appeal, the respondent also argued that no advantageous technical effect resulted from this change in expression system (see bottom of page 10 of the submission dated 1 October 2007); and

(iii) as for the immunized host (human (claims 1 and 5) vs. mouse (Examples)), in vivo animal data could be used to show that treatment of humans was plausible.

21. In support of its view that the exemplified results could not be extrapolated to the claimed vaccine, the respondent further drew attention to post-published document D14, which showed that the integrated construct "Lm-E7" (obtained via homologous recombination) falling within claim 1 did not exert any anti-tumour effect on an E7-expressing tumour, in contrast to the plasmid-based construct "Lm-LLO-E7"

(similar to the plasmid-based constructs exemplified in the patent).

However, this possible failure described in document D14 has to be balanced with the numerous documents describing integrated constructs falling under claim 1 which are capable of conferring tumour protection or exerting tumour regression activity, such as documents D19 (see page 2288, l-h column, paragraph headed "rLm strains", wherein reference is made to document D18 (see the term "integration" on page 3988, l-h column, line 4)), document D44 (see page 8468, l-h column, line 14 from the bottom), document D98 (see page 630, r-h column, line 8, from the bottom: "integration") and document D86 (see page 4265, l-h column, line 15: "integrated plasmids").

22. In conclusion, the present situation differs from that dealt with in decision T 1329/04 (supra) in that the post-published evidence (documents D14, D18, D19, D44 and D98) cited by the appellant merely serves the purpose of confirming what was already disclosed and rendered plausible in the application as filed.

The problem to be solved is thus the one stated above (see point 16 supra) and not the one the respondent has proposed, i.e., the provision of an alternative L. monocytogenes tumour vaccine (see point 17 supra).

23. Example 2 of the patent shows that the anti-tumour responses elicited by a recombinant Listeria monocytogenes vector, LM-NP, impeded growth of a very large dose, 50 times the tumouricidal dose (column 10, line 47), of CT26-NP or RENCA-NP tumour cells.

Immunized mice were completely protected from tumour formation or growth, a result that indicated the efficacy and potency of this immunization technique. Further, Example 4 demonstrated regression of growth of a RENCA-NP tumour. Example 6 provided the technical information that $CD4^+$ T cells, in addition to $CD8^+$ T cells, were also important for protection from tumour challenge. Therefore, the board is satisfied that the claimed subject-matter solves indeed the problem of providing an effective tumour vaccine for use in humans.

24. The respondent argues that the claimed subject-matter would have been obvious in view of the teaching in document D10 alone or in combination with that of document D3 because the skilled person would have considered the in vitro CTL assays described in documents D10 and D3 to correlate with in vivo anti-tumour activity.

25. In support of its case, the respondent relies on document D70, which describes mice immunized with P815 mastocytoma cells. Response of the mouse immune system was determined using in vitro CTL assays performed on spleen cells as in document D10. A further document D71 was cited for confirming this line of argument.

26. It is stated in document D70 (see page 1810, l-h column, lines 10-14): "Our finding that resistance to challenge after a given regimen of immunization correlates with the in vitro ability to generate cytotoxic cells is in agreement with previous reports (16,17) that CTLs mediate immunity against this tumor". Page 1806, r-h column, lines 4-8 further recites: "The other 50-60% of syngeneic hosts demonstrated complete regression and

remained tumor free indefinitely (up to 4 mo). Spleen cells from these regressors demonstrated marked CTL activity after in vitro stimulation in MLTCs (Fig. 1)". Document D71 states (see page 2559, l-h column, lines 29-32: "... a well-established correlation exists between protection and suppression of virus replication in vivo, on the one hand, and activity of $CD8^+$ CTLs in vitro, on the other").

27. The board notes that the investigations described in document D70 merely demonstrate that in animals exhibiting an in vivo anti-tumour response (i.e., having the capacity of slowing down tumour growth or causing tumour regression), there was a corresponding CTL response. This also applies to the antiviral protection referred to in document D71. However, insofar as in vivo anti-tumour activity is concerned, this finding does not mean that the reverse ("CTLs assays correlate with in vivo anti-tumour activity") is true. This is because a CTL response is a necessary but not sufficient condition for in vivo anti-tumour response. In fact, document D45 demonstrates that elicitation of CTLs in vitro did not translate into in vivo efficacy (see bottom of page 603: "However, the ability of peptides to direct CTL recognition in vitro is not sufficient to predict their ability to prime CTL response in vivo"). This finding is confirmed by the last paragraph on page 605 of document D45, stating that "CN OVA, which can sensitize EL4 cells for lysis by OVA transfectant-primed or PT OVA-primed CTL, was not capable of priming the CTL response **when injected into mice** in doses up to 1 mg per animal" (emphasis by the board). Thus, the immune cells involved in these artificial in vitro assays (such as those described in

documents D3 and D10) could differ from those responsible for in vivo tumour protection or for any other type of in vivo efficaciousness.

Further, it should be noted that document D46 suggests that the use of β-gal expressing P815 cells (in the same assay as disclosed in document D3: see point 10 supra) might give misleading results (high CTL background response).

28. The respondent considers that the in vitro test described in document D3 uses a mastocytoma mouse cell line P815 as a tumour model indicative of tumour protection/regression.

In a few words, this test takes advantage of the release of radioactive ^{51}Cr by cells sensitized with the antigen upon attack by CTLs specific for the antigen. However, the board notes that the choice of the tumour cell P815 is only made by convenience (it takes up ^{51}Cr better than other cells) and not because it represents a tumour model. This cell was not used for inducing a tumour. Thus, the choice of this cell does not mean that the in vivo anti-cancer properties of a potential vaccine were at stake. The board's view above is supported by document D64 (see page 3.11.5: "Lymphoblasts, tissue culture cells, or **tumor** cells are recommended as source of target cells because they take up more ^{51}Cr with less subsequent spontaneous leakage than nonactivated, freshly explanted normal cells" (emphasis by the board). Hence, whilst it is true that document D3 (see point 10 supra) and document D28 (see page 257, under "Cytotoxic Assays") mention the P815 cell line, it is only for determining CTL activity, a

criterion which is not sufficient to establish protection and regression in vivo.

29. In conclusion, there is no evidence before the board that the in vitro tests used in document D10 (and also in document D3) to measure the immune response was ever used in the art in connection with tumour protection. The only acceptable standard indicative of tumour protection/regression was in vivo suppression of tumour growth and/or induction of tumour regression (see document D65, page 1207, r-h column, last paragraph discussing the orthotopic implantation of intact human tumour specimens in animals as a model for developing new cancer therapeutics).

30. Therefore, the respondent's line of argument that the claimed subject-matter would have been obvious in view of the teaching in document D10 alone or in combination with that of document D3 that the skilled person would have considered the in vitro CTL assays described in documents D10 and D3 to correlate with in vivo anti-tumour activity, is not convincing.

31. In a further line of argument, the respondent maintains that the claimed subject matter could be arrived at in an obvious way by starting from the teaching in document D9 or D29 (see points 13 and 14 supra) that immunisation with the protein β-gal conferred protection against tumour growth or that vaccinia virus recombinant vaccines expressing SV40 T or E6 and E7 HPV antigens or influenza NP protected animals against subsequent challenge with tumour cells that expressed these proteins as tumour antigens, and turning to the Listeria vectors known from document D7 or document D10.

32. However, the board first observes that before the present invention, only viruses were used as vectors for releasing tumour associated antigens in vaccines suitable to treat cancer. There is no evidence before the board of the use of an extracellular or intracellular bacteria (such as Listeria) for this purpose. But even if the skilled person, for the sake of argument, turned to Listeria monocytogenes as a vector for expressing a tumour antigen for treating cancer, there was no or too little expectation of success that such vector would elicit an anti-tumour immune response, let alone induce regression of an implanted tumour, in view of the fact that many tumours were known to exhibit a variety of immunosuppressive functions (see documents D51 to D55).

33. In summary, the subject-matter of claims 1 and 5 and dependent claims does not follow from the prior art in an obvious way.

Adaptation of the description

34. The respondent objected to the change of "MVC-1" in paragraph [0029] of the patent and in granted claim 3 and 7 to read "MUC-1". However, this correction satisfies the requirements of Rule 139 EPC since it was immediately evident that "MUC-1" was the correct acronym for mucin (a tumour specific antigen for breast and pancreatic carcinoma), not "MVC-1" (see reference "21" of document D99 on page 338, lines 7-10 and on page 344).

35. Furthermore the respondent took the view that the description had to make clear that the embodiments in the examples were not part of the claimed invention. However, the board notes that objections based on Article 84 EPC (lack of clarity or lack of support) can only be raised in opposition proceedings in so far as they are caused by amendments introduced by the proprietor, which is not the case here.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent in amended form on the basis of the following documents:

   - claims 1 to 8 of the main request filed at the oral proceedings;
   - pages 2 to 9 of the amended description, filed at the oral proceedings;
   - figures 1 to 8 of the patent specification.

The Registrar: Chair:

C. Eickhoff U. Kinkeldey