Case Number: T 1091/02 - 3.3.04
Application Number: 92305862.2
Publication Number: 0520794
IPC: C12Q 1/68
Language of the proceedings: EN
Title of invention:
Methods for detection of carcinoma metastases by nucleic acid amplification
Patentees:
F. HOFFMANN-LA ROCHE AG, et al
Opponents:
Akzo Nobel N.V.
Vysis Inc.
Headword:
Methods for detection II/HOFFMANN-LA ROCHE
Relevant legal provisions:
EPC Art. 54, 56, 107
EPC R. 65(1)
Keyword:
"Admissibility of appeal (yes)"
"Main request: novelty (no)"
"Auxiliary request: inventive step (no)"
Decisions cited:
G 0002/04, T 0711/99
Catchword:
-
Case Number: T 1091/02 - 3.3.04

DECISION
of the Technical Board of Appeal 3.3.04
of 28 June 2006

Appellant: Akzo Nobel N.V.
(Opponent 01)
Velperweg 76
NL-6824 BM Arnhem (NL)

Alternative Appellant: bioMérieux B.V.
Boseind 15
NL-5281 Boxtel (NL)

Common Representative: Van Someren, Petronella F. H. M.
Arnold & Siedsma
Advocaten en Octrooigemachtigden
Sweelinckplein 1
NL-2517 GK Den Haag (NL)

Other Party: Vysis Inc.
(Opponent 02)
Downers Grove
IL 60615-5400 (US)

Representative: Naylor, Kathryn May
Mathys & Squire
120 Holborn
London EC1N 2SQ (GB)

Respondents: F. HOFFMANN-LA ROCHE AG
(Proprietors of the patent)
Grenzacherstrasse 124
CH-4002 Basel (CH)

The Regents of the University of California
300 Lakeside Drive
22nd Floor
Oakland, CA 94612-3550 (US)

Common Representative: Bizley, Richard Edward
HLBBshaw
Merlin House
Falconry Court
Baker's Lane
Epping, Essex CM16 5DQ (GB)
Decision under appeal: Decision of the Opposition Division of the European Patent Office posted 16 August 2002 rejecting the oppositions filed against European patent No. 0520794 pursuant to Article 102(2) EPC.

Composition of the Board:

Chair: U. Kinkeldey
Members: R. Gramaglia
         R. Moufang
Summary of Facts and Submissions

I. European patent No. 0 520 794 with the title "Methods for detection of carcinoma metastases by nucleic acid amplification" and claiming priority from US 720061 of 26 June 1991 was maintained as granted by the opposition division. Claim 1 as granted read as follows:

"1. The use in a method of detecting metastatic disease in body tissue or fluid by nucleic acid amplification of oligonucleotide primers suitable for amplifying a target carcinoma associated nucleic acid sequence, which sequence is expressed by carcinoma cells as well as in healthy cells from which the tumors arise but not by the resident non-carcinoma cells normally present in a sample of said body tissue or fluid."

II. Two oppositions were filed in the name of Akzo Nobel N.V (opponent 01) and Vysis Inc. (opponent 02) challenging the patent in suit under Article 100(a) EPC for lack of novelty and inventive step (Articles 54 and 56 EPC).

III. After rejection of the oppositions, an appeal was filed on 25 October 2002 in the name of bioMérieux B.V. It was submitted that bioMérieux B.V. then owned the diagnostic activities of Akzo Nobel N.V. to which the opposition pertained. As a precautionary measure in case the appeal in the name of bioMérieux B.V was considered inadmissible, it was requested that the appeal be treated as being in the name of Akzo Nobel N.V.
IV. A declaration signed by representatives of Akzo Nobel N.V., bioMérieux B.V. and bioMérieux S.A. was submitted with the notice of appeal. Its content may be summarised as follows: Akzo Nobel N.V. had diagnostics as part of its business, which had been concentrated in its business unit Organon Teknika B.V.. The opposition was filed by Akzo Nobel N.V. in the interest of its European diagnostics business, as conducted on its behalf by Organon Teknika B.V.. An agreement was reached effective from 30 June 2001 between Akzo Nobel N.V. and bioMérieux S.A. to transfer the diagnostic activities of Organon Teknika B.V. from Akzo Nobel N.V. to bioMérieux S.A.. Since then Organon Teknika B.V. has continued its diagnostic business as a 100%-affiliate of bioMérieux S.A., first under its old name and since February 2002 under the name of bioMérieux B.V..

V. Oral proceedings were held on 29 July 2003 to hear the matter relating to the transfer of opponent status and to the admissibility of the appeal.

VI. The board issued interlocutory decision T 1091/02 dated 23 July 2004, referring to the Enlarged Board of Appeal (EBA) questions relating to the transfer of opponent status and to the admissibility of an appeal. The EBA answered these questions in decision G 2/04 (OJ EPO 2005, 549).

VII. Further oral proceedings before the present board to hear the parties on all remaining issues were held on 28 June 2006, during which the respondents filed a new Main request (claims 1 to 11) and Auxiliary request I (claims 1 to 9).
Claim 1 of the Main request read as follows:

"1. The use of oligonucleotide primers suitable for amplifying a target carcinoma associated nucleic acid sequence, which sequence is expressed by carcinoma cells as well as in healthy cells from which the tumors arise but not by the resident non-carcinoma cells normally present in a sample of said body tissue or fluid in a generally applicable method of detecting metastatic disease in body tissue or fluid by nucleic acid amplification."

Claim 1 of Auxiliary request I read as follows:

"1. The use in a method of detecting metastatic disease in body tissue or fluid by nucleic acid amplification of oligonucleotide primers suitable for amplifying a target carcinoma associated nucleic acid sequence, which sequence is expressed by carcinoma cells as well as in healthy cells from which the tumors arise but not by the resident non-carcinoma cells normally present in a sample of said body tissue or fluid, wherein said carcinoma cells are cells from small cell lung carcinoma, non-small cell lung carcinoma, prostatic carcinoma, gastrointestinal system carcinoma, genitourinary system carcinoma, testicular carcinoma, endocrine carcinoma or melanoma, or wherein the target carcinoma associated sequence is selected from nucleic acids encoding chromogranin A, neuron specific enolase, synaptophysin, L-dopa decarboxylase, neurophysin I, neurophysin II, bombesin, calcitonin, CGRP, parathyroid-related hormone, KS1/4, prostate specific antigen, prostatic acid phosphatase, melanoma associated antigen p97 or melanoma antigen gp75."
VIII. The following documents are cited in the present decision:


A2 Neville A.M., Cytopathology, Vol. 1, pages 223-231 (1990);


A25 English translation of A25a;


IX. The submissions by the appellant, insofar as they are relevant to the present decision, can be summarized as follows:
Admissibility of the appeal

- When filing the appeal, it was not clear whether or not an opposition could be transferred to a subsidiary company. Therefore there was the justifiable legal uncertainty required by the EBA in its decision G 2/04 for a party to file an appeal in one name, and at the same time, as an auxiliary request, in the name of a different person. Thus the appeal was admissible.

Main request and Auxiliary request I

Novelty (Article 54 EPC)

Document A1

- The use according to claim 1 of these requests was anticipated by document A1, which disclosed the use of oligonucleotide primers suitable for amplifying keratin-19 mRNA in order to detect micrometastases of breast carcinoma in a body tissue or fluid by nucleic acid amplification.

Auxiliary request I

Inventive step (Article 56 EPC)

- The use according to claim 1 of this request was rendered obvious by combining the teachings of document A26 with that of document A2 or by combining the teachings of document A25 with that of document A1, document A14 or document A4.

- An arbitrary selection of carcinoma cells or cell markers according to claim 1 could not confer inventive step.
X. The submissions by the respondents (patentees), insofar as they are relevant to the present decision, can be summarized as follows:

Admissibility of the appeal by Akzo Nobel N.V.

- When the notice of appeal was filed, there was no justifiable legal uncertainty as to how the law was to be interpreted in respect of the question of who the correct party to the proceedings was. It followed from the established case law that no transfer of opponent status could have taken place in the present circumstances. If there was any uncertainty at all, it arose from the actions of the appellant Akzo Nobel N.V. who has to be held responsible for it.

Main request and Auxiliary request I

Novelty (Article 54 EPC)

Document A1

- Document A1 was not prior art.

- Document A1 did not provide a clear and unambiguous technical teaching of the use of claim 1, as the skilled person was not taught without any doubt that keratin-19 mRNA could be used for the detection of breast carcinoma micrometastasis in bone marrow samples.

- The "faint product", which, according to document A1 was detected upon amplification could not unambiguously be identified as resulting from keratin-19 mRNA because (i) it could be an artefact due to subsequent laboratory contamination of the bone marrow samples; (ii) the identity of the "faint product" could not be
verified owing to the lack of information in document A1 about any probe specific for keratin-19 mRNA being used and (iii) it could have been the result of illegitimate transcription (see page 8, lines 11-24 of the patent).

- The technique according to claim 1 was generally applicable, unlike the previous ones, relying on the identification of abnormal nucleotide sequences (gene mutations, aberrant oncogene/tumor suppressor DNA, chromosome translocation (hematopoietic malignancies), tumour cell-specific high expression). This generally applicable technique was novel over the specific and uncertain teaching of document A1.

**Inventive step (Article 56 EPC)**

- Starting from the specific and uncertain teaching of document A1, the skilled person could not derive in an obvious way the generally applicable technique according to claim 1, which no longer relied on too diverse, poorly characterized or infrequent abnormal nucleotide sequences.

X. Opponent 02 (Vysis Inc.) did not make any substantive submission during the appeal proceedings and, although duly summoned, did not take part in the oral proceedings before the board.

XII. The appellant (opponent 01) requested that the appeal of Akzo Nobel N.V. be declared admissible, the decision under appeal be set aside and the European patent No. 0 520 794 be revoked.
The respondents (patentees) requested that the appeal be rejected as inadmissible or, in the alternative, the decision under appeal be set aside and the patent be maintained in amended form on the basis of claims 1 to 11 of the "Main request" or claims 1 to 9 of the "Auxiliary request I", both filed at the oral proceedings.

Reasons for the Decision

Opponent status of Akzo Nobel N.V. and admissibility of the appeal

1. In its decision G 2/04 (OJ EPO 2005, 549) the Enlarged Board of Appeal held that the status as an opponent cannot be freely transferred. It furthermore held that a legal person who was a subsidiary of the opponent when the opposition was filed and who carries on the business to which the opposed patent relates cannot acquire the status as opponent if all its shares are assigned to another company. According to Article 112(3) this decision is binding on the board of appeal in respect of the appeal in question. Therefore the present board has to conclude that, notwithstanding the assignment of all the shares of its former subsidiary Organon Teknika B.V. (the name of which being later changed into bioMérieux B.V.) to bioMérieux S.A., Akzo Nobel N.V. could not transfer its opponent status and therefore remains party in the present opposition appeal proceedings.
2. It follows from the above conclusion that bioMérieux B.V. never acquired the status as an opponent in these proceedings and was therefore not adversely affected by the appealed decision. An appeal filed alone on behalf of bioMérieux B.V. would therefore have to be rejected as inadmissible pursuant to Article 107, first sentence, and Rule 65(1) EPC. However, in the present case, the notice of appeal contained the explicit proviso that the appeal should be treated as being in the name of Akzo Nobel N.V. if the appeal in the name of bioMérieux B.V was considered inadmissible.

3. In its decision G 2/04 the Enlarged Board of Appeal answered Question 3 referred to it by this board as follows:

"If, when filing an appeal, there is a justifiable legal uncertainty as to how the law is to be interpreted in respect of the question of who the correct party to the proceedings is, it is legitimate that the appeal is filed in the name of the person whom the person acting considers, according to his interpretation, to be the correct party, and at the same time, as an auxiliary request, in the name of a different person who might, according to another possible interpretation, also be considered the correct party to the proceedings."

4. The content of the notice of appeal in the present case puts it beyond reasonable doubt that, from a subjective point of view, the representative of the appellant was uncertain as to which one of bioMérieux B.V. and Akzo Nobel N.V was the right party. This conclusion is in line with the view taken in decision G 2/04
(point 3.2.2 (d) of the reasons) according to which "The author of the declaration wanted to file it in the name of the correct person, but was, however, not sure who the correct party to the proceedings was."

5. Nevertheless, as correctly emphasised by the respondents, subjective uncertainty as such is not sufficient. There must have been "justifiable legal uncertainty as to how the law is to be interpreted". The respondents argued that this requirement should be very narrowly construed and that, in order to justify the filing of an appeal in the manner done by bioMérieux B.V. and Akzo Nobel N.V., a high standard for proving legal uncertainty had to be applied. Furthermore, the respondents took the position that, in view of the commercial decision to assign the shares of its subsidiary to BioMérieux S.A., opponent 01 was directly responsible for any possible uncertainty arising in the present case and that, notwithstanding the later referral decision of the board, the law itself had been clear when the appeal was filed.

6. The board does not agree with these arguments put forward by the respondents. When the notice of appeal was filed by the representative of the appellant, the legal issues relating to the substantive requirements for the transfer of opponent status were far from clear, in particular with respect to the factual situation as presented by the appellant, i.e. the sale of a subsidiary company carrying on the business to which the opposition relates. In this respect the board refers to the detailed reasons set out in its referral decision.
7. The board is not aware of any appeal decision which, prior to the filing of the notice of appeal in the present case (25 October 2002), addressed this particular question. It is noted that the decision T 711/99 (OJ EPO 2004, 550) where a similar issue was at stake is dated 21 October 2003. The board is therefore convinced that it was seriously arguable at the relevant point of time that the opponent status of Akzo Nobel N.V. could be transferred to bioMérieux B.V.. This amounted to a justifiable legal uncertainty as to the person entitled to appeal.

8. The respondents have referred to a passage in the decision G 2/04 (point 3.2.5 (b) of the reasons) according to which "a request indicating an appellant in the alternative to the main request is restricted to a situation in which the party in question cannot be held responsible for the legal uncertainty". However, the board does not interpret this passage as suggesting that a commercial decision such as the selling of a subsidiary makes a party "responsible" for possible legal uncertainties arising out of it in the context of opposition proceedings before patent offices and courts. It is thus irrelevant that the above uncertainty would not have occurred at all if opponent 01 had not assigned all the shares of its subsidiary to another company.

9. The board concludes that the appeal (in the name of Akzo Nobel N.V.) is admissible.
Main request

Novelty (Article 54 EPC)

Document A1

10. The respondents submitted for the first time during the second oral proceedings, i.e., many years after the submission of document A1, that this document was not prior art because it had been published after 26 June 1991, i.e., the filing date of the priority document US 720061 underlying the patent in suit. However, taking into account that the respondents had never contested the public availability of document A1 before the above date, that this new factual submission came as a surprise both to the board and the appellant and that considering it would require further investigations and delay of the proceedings, the board uses its discretion under Article 114(2) EPC to disregard it at such a late stage of the proceedings. Document A1 is thus considered to represent prior art pursuant to Article 54(2) EPC.

11. Document A1 is an abstract having the title "DETECTION OF MICROMETASTASES IN BREAST CANCER BY THE POLYMERASE CHAIN REACTION: A FEASIBILITY STUDY" reading:

"We investigated whether polymerase chain reaction (PCR) analysis could be used to detect micrometastases in breast cancer using primers and probes for keratin. In an initial study of 5 epithelial tumors and 5 normal lymph nodes for the presence or absence of keratin-18 mRNA, all ten samples yielded hybridizable product. The keratin-18 signal in lymph nodes was probably due to keratin-18-positive stromal cells, which were demonstrated by immunohistochemistry. The RNA samples
were next amplified using primers for keratin-19 mRNA; the epithelial tumors gave a strong signal, while the lymph nodes were negative. Two samples of normal peripheral blood and two samples of normal bone marrow were also negative for keratin-19. To test the sensitivity of the PCR method, MCF-7 cells (keratin-positive) were mixed at various dilutions with HL-60 cells (keratin-negative) and keratin-19 mRNA was amplified. The keratin signal from the carcinoma cells could be detected in dilutions as high as 1:100,000. Bone marrow samples from two of three patients with breast cancer, microscopically free of carcinoma, revealed a faint product upon amplification of keratin-19 mRNA. Parallel amplification of actin mRNA was used as internal control. We conclude that PCR amplification of keratin-19 mRNA, but not keratin-18, may be useful in the detection of micrometastases of breast carcinoma."

12. The board observes that keratin-19 mRNA referred to in document A1 encodes a protein belonging to the family of the epithelium-specific cytokeratins expressed in both normal epithelia and epithelium-derived tumors (carcinomas) (see document A26, page 69, r-h column, first full paragraph and line 14 from the bottom; see also page 81, r-h column, line 13). Moreover, according to document A1, "5 normal lymph nodes" and "two samples of normal peripheral blood and two samples of normal bone marrow" were negative for keratin-19 mRNA upon PCR amplification. Therefore, the board considers that keratin-19 mRNA is "a target carcinoma-associated nucleic acid sequence expressed by carcinoma cells as well as healthy cells from which the tumor arises", which target sequence is also "not expressed by the
resident non-carcinoma cells normally present in a sample of said body tissue or fluid" as claimed. In summary, keratin-19 mRNA is a target carcinoma-associated nucleic acid sequence according to the wording of present claim 1.

13. Document A1 discloses the use according to present claim 1 of oligonucleotide primers suitable for amplifying the above target carcinoma associated nucleic acid sequence (keratin-19 mRNA) in order to detect breast carcinoma micrometastases in a body tissue or fluid by nucleic acid amplification. Therefore, the board concludes that the subject matter of claim 1 of this request is anticipated by document A1.

14. The opposition division dismissed document A1 as being a non-enabling disclosure. The respondents also argue that document A1 did not provide a clear and unequivocal technical teaching of the use of claim 1, on the grounds that the skilled person was not taught without any doubt that keratin-19 mRNA could be used as a target carcinoma associated nucleic acid sequence for the detection of breast carcinoma micrometastasis in bone marrow samples because the "faint product" detected upon amplification could not unambiguously be identified as being keratin-19 mRNA. This was because (i) it could be an artefact due to subsequent laboratory contamination of the bone marrow samples; (ii) the identity of the "faint product" could not be verified because document A1 provided no information about any probe specific for keratin-19 mRNA being used and (iii) it could have been the result of illegitimate transcription (see page 8, lines 11-24 of the patent).
15. As regards possibility (i) (artefact due to contamination of the bone marrow samples), the board notes that document A1 reports that five normal lymph node samples, two samples of normal peripheral blood, two samples of normal bone marrow and one bone marrow sample from one of the three patients with breast cancer turned out negative for keratin-19 mRNA, i.e., they did not reveal any "faint product" upon amplification of keratin-19 mRNA. Therefore, the fact that ten (5+2+2+1) samples do not exhibit such faint band pleads against the "faint product" being an artefact due to a (somehow selective) contamination of only two (of the 12 in total) samples subjected to amplification.

16. As for possibility (ii) (the identity of the "faint product" could not be verified), it is true that abstract A1 does not disclose any size of the amplified product nor discloses the DNA sequence of the specific probes, however, it is stated by the authors of document A1 that they used primers and probes specific for keratin-19 mRNA. They were thus able to distinguish between the amplification products from keratin-18 mRNA and keratin-19 mRNA by comparing the sizes of the amplified products and/or by hybridization with the specific probes. Therefore, they were also in a position, inter alia, to compare the size of the "faint product" with that of the above amplification products, a conventional approach in the field of PCR also taken in the patent in suit (see e.g., page 16, line 31 and page 14, lines 41-43).
17. As for possibility (iii) (illegitimate transcription), page 8, lines 11-24 of the patent prescribes that "the amplification and/or detection methods have to be modified to distinguish between low basal level of expression in a non-carcinoma cell and positive expression in a carcinoma cell". However, the board firstly notes that present claim 1 does not relate to an amplification and/or detection method incorporating the above feature. Secondly, in the board's view, this attack has the character of a mere allegation not supported by any plausible facts as to why this kind of transcription might have occurred. But in any case, the fact that no such illegitimate transcription or anything else (see point 15 supra) could be detected in five normal lymph node samples, in two samples of normal peripheral blood, in two samples of normal bone marrow and in one bone marrow sample from the three patients with breast cancer (ten (5+2+2+1) samples) does not assist the respondents arguing that the "faint product" could have been the result of illegitimate transcription: the latter would have to (somehow selectively) turn up only in two samples and not in the remaining ten.

18. In view of the foregoing, the board concludes that document A1 provided all the technical information for the skilled person to reliably reproduce the use according to present claim 1. The board further observes that the above technical information also included the expedient of using the "parallel" amplification of actin mRNA as an internal control, an expedient also used in the Examples of the patent in suit as a positive control for monitoring that nucleic acid amplification is working (see e.g., page 13,
line 39: "β-actin was included as a positive control") and a check for the sensitivity of the PCR method by testing mixtures of MCF-7 cells (keratin-19-positive) with HL-60 cells (keratin-19-negative) at various dilutions and amplification of keratin-19 mRNA. The keratin-19 signal from the carcinoma cells could be detected in dilutions as high as 1:100,000. This is exactly the test used (and more importantly, the sensitivity level obtained) in the patent in suit (see Example 4).

The "generally applicable" feature in claim 1

19. Relying on the passage bridging pages 2 and 3 of the patent, the respondents argue that the selection of the specific target carcinoma associated sequences and the corresponding oligonucleotide primers according to present claim 1 render possible a method for detecting micrometastases of carcinomas which is generally applicable, unlike the known target carcinoma associated sequences. The latter techniques relied on too diverse or infrequent abnormal nucleotide sequences (gene mutations, aberrant oncogene/tumor suppressor DNA, chromosome translocation (hematopoietic malignancies), tumor cell-specific high expression). It is the respondents' view that this generally applicable technique was novel over the specific and uncertain teaching of document A1.

20. However, keratin-19 mRNA referred to in document A1 is in fact a target carcinoma-associated nucleic acid sequence fulfilling the requirements of present claim 1 (see point 12 supra). Moreover, the skilled person was in a position to reproduce the teaching of this
document (see point 18 supra). Therefore, the "generally applicable" language in present claim 1 cannot confer novelty on the claim.

Auxiliary request I

21. In claim 1 of this request, the "said carcinoma cells" are further defined as being cells from small cell lung carcinoma, non-small cell lung carcinoma, prostatic carcinoma, gastrointestinal system carcinoma, genitourinary system carcinoma, testicular carcinoma, endocrine carcinoma or melanoma, or the target carcinoma associated sequence is better defined as having been selected from the specific nucleic acids encoding chromogranin A, neuron specific enolase, synaptophysin, L-dopa decarboxylase, neurophysin I, neurophysin II, bombesin, calcitonin, CGRP, parathyroid-related hormone, KS1/4, prostate specific antigen, prostatic acid phosphatase, melanoma associated antigen p97 or melanoma antigen gp75.

Novelty (Article 54 EPC)

22. Claim 1 includes neither breast carcinoma in the list of the carcinoma cells, nor keratin-19 in the list of the proteins encoded by the target carcinoma associated sequence. Therefore, the subject matter of claim is novel over document A1.

Inventive step (Article 56 EPC)

23. The closest prior art is represented by document A1, which discloses the use of oligonucleotide primers suitable for amplifying the target carcinoma associated
nucleic acid sequence keratin-19 mRNA in order to
detect breast carcinoma micrometastases in a body
tissue or fluid by nucleic acid amplification.

24. Since keratin-19 mRNA referred to in document A1 is
indeed a target carcinoma-associated nucleic acid
sequence fulfilling the requirements of present claim 1
(see point 12 supra), the respondents' argument that
the claimed subject matter purports to solve the
problem of finding a "generally applicable" technique
for detecting micrometastases based on the
identification for the first time of such family of
target carcinoma-associated nucleic acid sequence, must
fail. Hence, the objective problem to be solved vis-à-
vis the disclosure of document A1 can be seen as the
provision of further target carcinoma associated
sequences within the meaning of present claim 1 and the
corresponding oligonucleotide primers, to be used to
detect micrometastases of carcinomas other than breast
carcinoma in body tissues or fluids by nucleic acid
amplification.

25. In the board's judgement, it would be obvious to the
skilled person departing from the teaching of document
A1 and coming across e.g., document A5, disclosing the
prostate specific antigen (PSA), to use PSA and the
corresponding oligonucleotide primers as a further
target carcinoma associated sequence within the meaning
of present claim 1, and hence to detect micrometastases
from e.g. prostatic carcinoma according to claim 1 of
this request.

26. In view of the foregoing, the subject matter of claim 1
does not satisfy the requirements of Article 56 EPC.
Order

For these reasons it is decided that:

1. The appeal of Akzo Nobel N.V. is admissible.

2. The decision under appeal is set aside.

3. The patent is revoked.

The Registrar: P. Cremona

Chair: U. M. Kinkeldey