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Datasheet for the decision of 15 September 2010

Case Number: T 0629/07 - 3.3.04

Application Number: 90301817.4

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Title of invention:
Detection of loss of the wild-type p53 gene

Patentee:
THE JOHNS HOPKINS UNIVERSITY

Opponent:
INTROGEN THERAPEUTICS, INC.
RHÔNE-POULENC RORER

Headword:
Detection of loss of p53/THE JOHNS HOPKINS UNIVERSITY

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request: inventive step (yes)"

Decisions cited:
T 0558/03

Catchword:
-

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Case Number: T 0629/07 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 15 September 2010

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 14 February 2007 revoking European patent No. 0390323 pursuant to Article 102(1) EPC 1973.**

Composition of the Board:

Chairman: M. Wieser
Members: R. Gramaglia
F. Blumer

Summary of Facts and Submissions

I. European patent No. 0 390 323 (application No. 90 301 817.4) claiming priority from US330566 filed on 29.03.1989 was filed on 20.02.1990. The patent having the title "Detection of loss of the wild-type p53 gene" was granted on the basis of 37 claims.

II. Notices of opposition against the present patent have been filed by opponents I and II on the grounds of Articles 100(a), 100(b) and 100(c) EPC.

III. The opposition division revoked the patent.

IV. A first appeal against the decision of the opposition division was lodged by the patent proprietor (appellant).

V. The present board in a different composition decided that claims 1 to 34 of Auxiliary Request IV filed on 27 May 2005 met the requirements of Articles 52(4), 83, 84, 123(2) and 123(3) EPC (see decision T 558/03, paragraph 33 of the reasons). However, the case was remitted to the opposition division for examination of the inventive step of the claims of this request.

Claims 1 to 34 of Auxiliary Request IV before the previous board read as follows:

"1. A method of diagnosing a neoplastic tissue of a human, comprising detecting loss of wild-type p53 genes or their expression products in isolated human tissue suspected of being neoplastic, wherein said loss leads to non-functional p53 gene products, loss of expression

of p53 mRNA or diminution of expression of p53 mRNA, said loss indicating neoplasia of the tissue, wherein the wild-type p53 gene sequence is shown in Zakut-Houri et al., EMBOJ., 4, 1251-1255, 1985.

2. The method of claim 1 wherein the expression products are mRNA molecules.

3. The method of claim 1 wherein the expression products are protein molecules.

4. The method of claim 1 wherein the loss of wild-type p53 genes is detected by sequencing all or part of the p53 gene using polymerase chain reaction.

5. The method of claim 1 wherein the loss of wild-type p53 genes is detected by identifying a mismatch between molecules (1) a p53 gene or p53 mRNA in said tissue and (2) a nucleic acid probe complementary to the human wild-type p53 gene, when molecules (1) and (2) are hybridized to each other to form a duplex.

6. The method of claim 5 wherein the nucleic acid probe is a RNA probe.

7. The method of claim 5 wherein the nucleic acid probe is a DNA probe.

8. The method of claim 5 wherein the mismatch is identified by enzymatic cleavage.

9. The method of claim 5 wherein the mismatch is identified by chemical cleavage.

10. The method of claim 8 wherein the enzymatic cleavage is performed using RNase A or S1 nuclease.

11. The method of claim 5 wherein the mismatch is identified by observing a shift in electrophoretic mobility of the duplex relative to the mobility of a duplex formed when molecule (2) is hybridized to a wild-type p53 gene or p53 mRNA.

12. The method of claim 1 wherein the loss of wild-type p53 genes is detected by amplification of p53 gene sequences and hybridization of the amplified p53 sequences to nucleic acid probes which are complementary to mutant p53 alleles.

13. The method of claim 1 wherein the loss of wild-type p53 genes is detected by molecular cloning and sequencing all or part of the p53 gene.

14. The method of claim 3 wherein loss of wild-type p53 protein molecules are detected by the loss of ability to complex with an antigen selected from the group consisting of SV-40 large T-antigen and adenovirus E1B antigen.

15. The method of claim 1 wherein the detection of loss of wild-type p53 genes comprises screening for a point mutation.

16. The method of claim 15 wherein the point mutation is a missense mutation.

17. The method of claim 1 wherein the detection of loss of wild-type p53 genes comprises screening for a frameshift mutation.

18. The method of claim 1 wherein the detection of loss of wild-type p53 genes comprises screening for a deletion mutation.

19. The method of claim 1 wherein the detection of loss of wild-type p53 genes comprises screening for a point mutation and screening for a deletion mutation.

20. The method of claim 1 wherein the neoplastic tissue is selected from the group consisting of: lung, breast, brain, colorectal, bladder, prostate, liver and stomach tumours.

21. The method of claim 20 wherein the neoplastic tissue is selected from the group consisting of: lung, breast, and colorectal tumours.

22. The method of claim 21 wherein the neoplastic tissue is a colorectal carcinoma.

23. A method of supplying human wild-type p53 gene function to a human cell which has lost said gene function by virtue of mutation in a p53 gene wherein said mutation leads to non-functional p53 gene products, loss of expression of p53 mRNA or diminution of expression of p53 mRNA, wherein the presence of said mutant p53 gene or expression product indicates the presence of a neoplastic tissue in the human, comprising:

introducing in vitro a wild-type p53 gene into the cell such that said gene is expressed in the cell, wherein the wild-type p53 gene sequence is shown in Zakut-Houri et al., EMBOJ., 4, 1251-1255, 1985.

24. The method of claim 23 wherein said wild-type p53 gene is expressed to a level higher than any mutant p53 gene present in the cell.

25. The method of claim 23 wherein the wild-type p53 gene introduced recombines with the endogenous mutant p53 gene present in the cell by a double recombination event to correct the p53 gene mutation.

26. A method of supplying human wild-type p53 gene function to a human cell which has lost said gene function by virtue of a mutation in a p53 gene wherein said mutation leads to non-functional p53 gene products, loss of expression of p53 mRNA, or diminution of expression of p53 mRNA, wherein the presence of said mutant p53 gene or expression product indicates the presence of a neoplastic tissue in the human, comprising:
introducing in vitro a portion of a wild-type p53 gene into the cell such that said portion is expressed in the cell, said portion encoding a part of the p53 protein which is required for non-neoplastic growth of said cell, wherein the wild-type p53 gene sequence is shown in Zakut-Houri et al., EMBOJ., 4, 1251-1255, 1985.

27. A kit for use in a method according to claim 1 for determination of the nucleotide sequence of a p53 gene by polymerase chain reaction, comprising:

a set of pairs of single stranded DNA primers, said set allowing synthesis of all nucleotides of the p53 gene coding sequences.

28. The kit of claim 27 wherein the primers have restriction enzyme sites at each 5’ end.

29. The kit of claim 27 wherein the set of pairs of primers comprise:

Primer pair 1: 5'-GGAATTCCACGACGGTGACACG-3' and
5'-GGAATTCGGTGTAGGAGCTGCTGG-3';
pair 2: 5'-GGAATTCCAGAATGCCAGAGGC-3' and
5'-GGAATTCATGTGCTGTGACTGCTTG-3';
pair 3: 5'-GGAATTCCACACCCCCGCCCG-3' and
5'-GGAATTCATGCCGCCCATGCAG-3';
pair 4: 5'-GGAATTCTGACTGTACCACCATCC-3' and
5'-GGAATTCTCCATCCAGTGGTTTC-3';
pair 5: 5'-GGAATTCCCAACAACACCAGCTCC-3' and
5'-GGAATTCAAAATGGCAGGGGAGGG-3'.

30. An allele-specific nucleic acid probe for use in a method according to claim 1 consisting of the nucleic acid sequence of a region a human mutant p53 gene or its ribonucleotide equivalent, said region containing a mutation.

31. The probe of claim 30 which is a RNA probe.

32. The probe of claim 30 which is a DNA probe.

33. A method of detecting the presence of a neoplastic tissue in a human, comprising:

isolating from a human a body sample selected from the group consisting of stool, urine and sputum; detecting in said sample a mutant p53 gene or expression product wherein said mutant p53 gene leads to non-functional p53 gene products, loss of expression of p53 mRNA, or diminution of expression of p53 mRNA, wherein the presence of said mutant p53 gene or expression product indicates the presence of a neoplastic tissue in the human, wherein the wild-type p53 gene sequence is shown in Zakut-Houri et al., EMBOJ., 4, 1251-1255, 1985.

34. A method of detecting genetic predisposition to cancer in a human comprising detecting loss of a wild-type p53 gene in DNA isolated from a human sample selected from the group consisting of blood and fetal tissue wherein said loss leads to non-functional p53 gene products, loss of expression of p53 mRNA, or diminution of expression of p53 mRNA, wherein the wild-type p53 gene sequence is shown in Zakut-Houri et al., EMBOJ., 4, 1251- 1255, 1985."

VI. The opposition division considered that the claims of the main request (i.e., Auxiliary Request IV filed on 27 May 2005) lacked an inventive step and that the claims of auxiliary request 1, filed at the oral proceedings of 27 September 2006, did not meet the requirements of Article 123(2) EPC and revoked the patent anew.

VII. In its decision to refuse the main request, the opposition division considered document D18 to represent the closest prior art and the technical problem to be formulated as the provision of an

alternative method for diagnosing of a neoplastic tissue of a human. However, the opposition division decided that the problem above had not been solved over the whole area covered by claim 1, which thus failed under Article 56 EPC.

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

IX. In a communication annexed to the summons to oral proceedings, the board indicated its preliminary non-binding opinion on some of the issues.

X. A reply dated 12 October 2009 was received from the appellant, which contained amended claims in the form of an amended Main Request and amended Auxiliary Requests 1 to 19 (labelled "1st Auxiliary Request" through "19th Auxiliary Request"). In this letter the appellant also inquired as to whether the board considered any of the claim requests before it to define allowable subject-matter. The appellant foreshadowed that it could be prepared to restrict the patent to subject-matter which the board considered allowable and thereby avoid the need for oral proceedings.

XI. In a further communication dated 3 November 2009, the board announced that the subject-matter of the Second Auxiliary Request (labelled "2nd Auxiliary Request") submitted with the letter dated 12 October 2009 defined allowable subject-matter.

XII. With letter dated 10 November 2009 the appellant withdrew the Main Request and the First Auxiliary

Request filed with the letter dated 12 October 2009 and stated that the Second Auxiliary Request submitted with the letter dated 12 October 2009 has become the new Main Request. The appellant also clarified that its request for oral proceedings only applied if the board was not inclined to set aside the decision of the opposition division and to maintain the patent on the basis of the new Main Request.

XIII. With letter dated 30 July 2009, respondent I (opponent I; hereafter: "the respondent") withdrew its request for oral proceedings, whereas respondent II (opponent II), which did not provide any submission during this second appeal phase, announced in a letter dated 29 October 2009 its intention not to attend the oral proceedings. Subsequently, the board cancelled the scheduled oral proceedings.

XIV. The claims of the new Main Request (i.e., the Second Auxiliary Request submitted with the letter dated 12 October 2009) were identical to those of Auxiliary Request IV filed on 27 May 2005 (see paragraph V supra), except for the deletion of former claims 27 to 32, directed to kits and probes, and renumbering of former claims 33 and 34 into claims 27 and 28, respectively.

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

D3 Benchimol S. et al., Cold Spring Harbor Meeting 7.9.-11.9.1988;

D5 Jenkins J.R. et al., Cancer Cells, pages 127-136 (1989);

D8 Green M.R., Cell, Vol. 56, pages 1-3 (13 January 1989);

D9 Hinds P. et al., J. Virology, Vol. 63, pp. 739-746 (February 1989);

D17 Eliyahu D. et al., Oncogene, Vol. 3, pages 313-321 (1988);

D18 Prokocimer M. et al., Blood, Vol. 68, pages 113-118 (1986);

D21 Zakut-Houri R. et al., EMBO J., Vol. 4, pages 1251-1256 (1985);

D38 Harris C.C., Carcinogenesis, Vol. 17, pages 1187-1198 (1996);

D54 Matozaki T., et al., Cancer Research, Vol. 52, pages 4335-4341, (1992);

D55 Bartek J., et al., Eur. J. Cancer, Vol. 29A, pages 101-107, (1993);

D56 Ruppert J.M. et al., Molecular and Cellular Biology, Vol. 13, pages 3811-3820 (1993);

D59 p53_lnfo_Soussi, 2006;

D61 IARC TP53 Database, 2006;

D62 Yamanishi Y. et al., Arthritis Research & Therapy, Vol. 7, pages R12-R18 (2005);

D66 Soussi T. et al., Human Mutation, Vol. 25, pages 6-17 (2005);

D72 Avigad S. et al., Oncogene, Vol. 14, pages 1541-1545 (1997);

D73 Lehman T.A. et al., Cancer Research, Vol. 60, pages 1062-1069 (2000);

D74 Soussi T. et al., Clin. Cancer Res., Vol. 12, No. 1, pages 62-69 (2006).

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

– Document D18 represented the closest prior art for the method according to claim 1. The technical problem to be formulated in view of document D18 was the provision of an alternative method for diagnosing a neoplastic tissue of a human.

– The method of diagnosis defined in the claims did enable one skilled in the art to reliably diagnose a neoplastic tissue in a human having the wild-type p53 gene sequence of Zakut-Houri et al (document D21).

– The method of claim 1 was only applicable to diagnosing neoplastic tissue in humans who had the wild-type p53 sequence shown in Zakut-Houri et al. and had lost the wild-type p53 sequence shown in

Zakut-Houri et al. in an isolated tissue suspected of being neoplastic.

– Silent mutations would not be considered to lead to non-functional p53 gene products as the same product would be encoded. Those skilled in the art were more than capable of distinguishing between silent mutations and inactivating mutations.

– With respect to polymorphisms, no evidence had been provided that such somatic polymorphisms in the p53 sequence occurred to such a level that the reliability of the claimed method was substantially affected.

– No evidence had been provided that individuals having the wild-type p53 sequence shown in Zakut-Houri et al. had mutations of the p53 wild-type sequence that were not associated with neoplastic tissue. Furthermore, even if such evidence was available, there was no indication that such "non-neoplastic" mutations occurred to such a level in the population as to significantly affect the reliability of the presently claimed method.

– Document D18 related to characterizing a role, if any, for p53 in cancers. According to this document, all malignant tissues studied expressed an elevated level of p53 compared with analogous normal tissues. Document D18 therefore taught away from a loss of p53 as being indicative of cancer.

XVII. The submissions in writing by the respondent, insofar as they are relevant to the present decision, can be summarized as follows:

– Document D18 represented the closest prior art for the method according to claim 1. The technical problem to be formulated in view of document D18 was the provision of a reliable method for the diagnosis of a neoplastic tissue of a human.

– Claim 1 of the main request lacked inventive step because it did not solve the problem above across the whole scope of the claim.

– The claimed methodology did not provide any technical advantage over the methodology of the prior art and was more complex and problematic than that used previously.

– Claim 1 related to the diagnosis of neoplastic tissue of all humans. However, approximately 60% of the population did not carry the wild-type p53 sequence shown in Zakut-Houri (D21).

– The methodology according to claim 1 would produce false positive results for all silent mutations.

– Applying the method of claim 1 would lead to an unacceptably high rate of false negative because only 50% of the tumours were associated with a p53 alteration.

– The method according to present claim 1 did not distinguish between mutants of the wild-type p53 sequence that caused tumours and mutants of the wild-type p53 sequence that did not cause tumours.

– The identification of a "non-functional p53 gene product" according to claim 1 by its ability to bind to either the SV4O T-antigen or the adenovirus E1B antigen could not provide a reliable method of diagnosing a cancerous tissue.

– The patent did not provide any evidence that the binding activities of wild-type p53 to either the SV4O T-antigen or the adenovirus E1B correlated with the ability of wild-type p53 to function as a tumour suppressor.

– The skilled person would have moved to the claimed methodology, insofar as it might be held to solve the present problem, from document D18, with a reasonable expectation of success, not least in the knowledge that p53 was an anti-oncogene, as disclosed, for example, in document D8.

XVIII. The appellant requested in writing that the decision under appeal be set aside and that the patent be maintained on the basis of the new Main Request (filed as Second Auxiliary Request with the letter dated 12 October 2009).

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

Reasons for the Decision

Articles 52(4) EPC 1973, 54, 83, 84, 123(2) and 123(3) EPC

1. The respondent (see paragraphs 18 to 57 of the submission dated 7 December 2007) raised objections under Articles 123(2), 84 and 83 EPC against claim requests comprising amendments over those found by the previous board not to add subject matter to application as filed, to be clear and to be sufficiently disclosed.

However, the claims of the new Main Request are now identical to those of Auxiliary Request IV filed on 27 May 2005, except for the deletion of former claims 27 to 32, directed to kits and probes, and renumbering (see paragraphs V and XIV supra), which claim request the previous board already found to meet the requirements of Articles 52(4) EPC 1973, 83, 84, 123(2) and 123(3) EPC (see decision T 558/03, paragraph 33 of the reasons). During the appeal procedure, the respondents did not raise any objection under Article 54 EPC, and the board also has no such objection. The only issue left is thus that of the inventive step (Article 56 EPC).

Inventive step (Article 56 EPC)
Closest prior art

2. Document D18 relates to investigations on the expression of p53 in human leukaemia and lymphoma and discloses that tissues from patients with B type lymphoproliferative diseases and the majority of patients with acute lymphoblastic leukemia express elevated levels of p53 (see page 116, r-h column,

lines 1-9). It is suggested on page 117, r-h column, last paragraph of this document to use p53 in these malignancies for monitoring cancer activity.

Document D17 relates to p53 mutations occurring in Meth A cells derived from a tumour induced in vivo by the exposure of mice to the carcinogen methylcholanthrene.

Document D3 relates to the rearrangement of the cellular p53 gene in Friend virus induced murine erythroleukaemia and mentions that rearrangement of the p53 gene has also been observed in certain human leukaemias.

Document D8 is a review article which presents no new experimental data of its own. This document refers to p53 as a "candidate anti-oncogene" and states that the transforming potential of p53 is activated by mutations in a wide variety of positions throughout the protein (see page 3, l-h column, lines 15-19 and 34-36).

3. In its decision to refuse the main request, the opposition division considered document D18 to represent the closest prior art for the method according to claim 1 because this document aimed at the same objective as the claimed invention, namely the diagnosis of cancer in human. Moreover, the diagnostic test suggested in document D18, like the claimed methodology, was based on the use of p53 as a marker. The parties and board agree as well to the choice of document D18 as representing the closest prior art.

Problem to be solved

4. In view of the technique described in document D18, the opposition division viewed the technical problem to be formulated as "the provision of an alternative method for the diagnosing of a neoplastic tissue of a human" (see page 7, 3rd paragraph of the decision under appeal). The board agrees to this formulation of the objective technical problem.

5. In this context, the respondent argued that the claimed methodology did not provide any technical advantage over the methodology of the prior art, besides being more complex and problematic than the one previously used because the description of the patent in suit lacked any substantive information as to how the method of diagnosis had to be carried out. It was also maintained by the respondent that the test of claim 1 was not 100% reliable (see paragraphs 98 to 103 of the submissions dated 7 December 2007).

The opposition division decided that the problem underlying the patent had not been solved after having apparently turned the formulation of the objective technical problem to be solved by the present invention from the original one ("the provision of an alternative method for the diagnosing of a neoplastic tissue of a human"; see page 7, 3rd paragraph of the decision under appeal) into "the provision of a method which can be used reliably for the diagnosis of a neoplastic tissue" (see paragraph 3.6 and the bottom of page 11 of the decision under appeal).

However, in the board's judgement, any deviation from the original formulation (see point 4 supra), such as to provide a very reliable (or an improved) test for determining whether a tissue from an individual was affected by cancer, is not justified and is without merit for the purpose of the present decision.

Problem solved/not solved?

6. The board is of the opinion that the claimed methodology solves the problem of providing an alternative diagnostic test to the one suggested in document D18. The molecular detection of cancer by detecting mutations occurring in this onco-suppressor according to the present invention may be more complex than morphological examination, however, it allows an earlier, non-morphological diagnosis and hence an earlier treatment, possibly leading to a better prognosis for the patient.

7. Since the vast majority of the arguments provided by the respondent (and partially upheld by the opposition division) aim at demonstrating that the subject-matter of claim 1 does not "credibly solve the problem of providing a reliable method for the diagnosis of a neoplastic tissue", the board will, nevertheless, deal with these issues in points 8 to 15 below.

8. According to post-published scientific literature summarized in document D59, approximately 40% of human individuals have the wild-type p53 sequence shown in Zakut-Houri (document D21), whereas 60% has the other normal wild type p53. Relying on these documents, the respondent concluded that present claim 1 did not

distinguish between mutants of the wild-type p53 sequence shown in Zakut-Houri (document D21) that cause tumours and mutants of the wild-type p53 sequence shown in Zakut-Houri (document D21) that do not cause tumours. In other words, the respondent interprets claim 1 as relating to the diagnosis of neoplastic tissue of **all** humans and concludes that since approximately 60% of the tissues taken from all humans would be misdiagnosed as neoplastic tissues, the presently claimed subject matter does not solve the problem of diagnosing a neoplastic tissue of "a human", as stated in claim 1.

However, the board cannot adhere to this respondent's interpretation of claim 1. This claim is directed to a method of diagnosing a neoplastic tissue of a human comprising detecting loss of wild-type p53 genes or their expression products in isolated human tissue, wherein the wild-type p53 gene sequence is shown in Zakut-Houri et al. (document D21). A critical feature of the method of claim 1 is that a loss (alteration) of the wild-type p53 gene sequence shown in Zakut-Houri et al. (document D21) or its expression products must be detected. Accordingly, before malignant transformation takes place, the tissue must have previously had the wild-type p53 sequence shown in Zakut-Houri et al. This implies that the method is only applicable to diagnosing neoplastic tissue in humans that have the wild-type p53 sequence shown in Zakut-Houri et al. and have possibly lost the wild-type p53 gene sequence shown in Zakut-Houri et al. or its expression products in an isolated tissue suspected of being neoplastic. Thus, given that the claimed methodology pertains to a well defined patient category, the fact that approximately 60% of the population does not have the

wild-type p53 sequence shown in Zakut-Houri et al. (document D21) is irrelevant to the claimed subject matter.

9. The opposition division (see page 8, last paragraph of the decision under appeal) and the respondent have also argued that applying the method of claim 1 would lead to an unacceptably high rate of false negative because, according to document D66, only 50% of the tumours were associated with a p53 alteration.

However, in the board's view, the method according to claim 1 is only applicable to a situation where the tissue under investigation had the wild-type p53 sequence shown in Zakut-Houri et al. (document D21), where a loss of said sequence or its expression product had possibly occurred. The claimed method does not apply to situations where cancer is caused by other agents and/or where no p53 alteration occurs. It can also not be derived from the wording of claim 1 that a possible lack of alteration of wild-type p53 sequence shown in Zakut-Houri et al. (document D21) or of its expression products indicates a healthy tissue, as cancer has many aetiologies.

Silent mutations

10. The opposition division concluded (see decision under appeal, page 8, second full paragraph) that the patent did not provide any teaching as to how to distinguish between polymorphisms, inactivating mutations and silent mutations. As regards the latters, i.e., those mutations that did not change the amino acid sequence of the encoded protein, the respondent pointed out that

the wording of claim 1 associated a loss or diminution of the mRNA encoded by the wild-type p53 sequence shown in Zakut-Houri et al. (document D21) to a finding that the tissue under investigation was neoplastic. Therefore, the respondent argued that since the mRNA encoded by the mutated DNA had a different RNA sequence from the mRNA encoded by the reference wild-type p53 sequence shown in Zakut-Houri et al. (document D21), said "loss or diminution of the mRNA encoded by the wild-type p53 sequence shown in Zakut-Houri et al. (document D21)" was necessarily detected in **all** cases in which the RNA contained a silent mutation. The respondent concluded that the methodology according to claim 1 produced false positive results for all silent mutations.

11. In the board's opinion, silent mutations may occur within an exon in a manner that does not alter the final amino acid sequence or in a non-coding region, this latter possibility being suggested in the patent in suit (see page 3, lines 24-25). All these silent mutations would lead to a functional p53 protein, as the same wild-type p53 would be encoded (hence the term "silent"). However, the p53 mRNA would exhibit an extra-intronic base change.

12. According to the respondent, any silent mutation would unavoidably result in a false positive result using the methodology according to claim 1. In the board's view, this conclusion follows from an interpretation of the terms "loss of expression of p53 mRNA" and "diminution of expression of p53 mRNA" in claim 1 that implies that the mRNA sequence of the actually expressed p53 mRNA have to be compared with that of the reference wild-

type p53 mRNA. However, the respondent's interpretation of claim 1 does not make technical sense and fails to take into account the whole disclosure of the patent. In fact, once claim 1 is interpreted in the light of the description (see page 3, lines 24-25 taken in combination with page 3, lines 40-41; page 6, line 30-31 ("normal amounts"), page 6, line 56 through page 7, line 1 ("little expression") and lanes 10-13 of Fig. 3), the terms "loss of expression of p53 mRNA" and "diminution of expression of p53 mRNA" clearly mean that it is the **levels** (not the sequences) of p53 mRNA expression that should be compared, namely the level of the actually expressed p53 mRNA (be it affected or not by point mutations) has to be compared with the original expression level of the reference wild-type p53 mRNA. This interpretation is in keeping with the view of the opposition division expressed on page 8, lines 1-4 of the decision under appeal. Moreover, this interpretation does not contradict point 5 of previous board's decision T 558/03, the latter being silent as to the specific embodiments covered by claim 1 where the "loss of expression of p53 mRNA" and "diminution of expression of p53 mRNA" have to be measured. Thus, contrary to the respondent's view, the methodology according to claim 1 would not lead to false positive results for all silent mutations. Rather, as illustrated in page 3, lines 24-25, in page 6, line 56 through page 7, line 1 and in Fig. 3 (lanes 10-13) of the patent in suit, a diminution the levels of p53 mRNA (or the absence of p53 mRNA), compared to the original expression level of the reference wild-type p53 mRNA, would indicate that an oncogenic mutation has occurred in a regulatory region.

13. Relying on documents D72 and D73, the respondent also maintained that silent mutations occurring in p53 introns and leading to tumorgenesis were known. It was the respondent's view that these intronic mutations could be detected neither in the expressed protein nor in a comparison using the Zakut-Houri sequence, as required by claim 1, because the Zakut-Houri sequence of document D21 was a cDNA sequence.

Document D72 deals with an intronic change in the p53 gene (G to A base substitution at 39 bp upstream to exon 7) which causes cancer. Document D73 is concerned with a p53 13964^{GC} intronic mutation causing familial breast cancer. As emphasized in the preceding point, the effects of silent mutations can be detected upon comparison of the expression levels rather than that of the sequences. An alteration of said expression levels was indeed noted by the authors of documents D72 (see page 1453, r-h column, lines 1-3) and document D73 (see page 1068, l-h column, lines 12-13 from the bottom). Moreover, it is stated in document D72 (see page 1543, r-h column, first full paragraph) that "the substitution is a rare polymorphism". As for the p53 13964^{GC} intronic mutation described in document D73, the fact that it was identified in 3 of 42 hereditary breast cancer patients, of which a third were of Ashkenazi ancestry (see Abstract) shows that this mutation is also a rare polymorphism.

Therefore, documents D72 and D73 do not show that tumorigenic intronic silent mutations cannot be detected or occur to such a level that the reliability of the claimed method is substantially affected.

14. The opposition division and the respondent also argued that a large number of mutations of p53 were not associated with neoplasms (see documents D61, D63 and D74). Document D61 listed 74 such mutations, document D63 disclosed non tumorigenic p53 mutations in rheumatoid arthritis synovium, whereas document D74 reviewed the mutations in a p53 database and determined that some mutations did not affect the normal activity of p53.

In the board's judgment, even accepting that documents D61, D63 and D74 show that some mutations of p53 are not associated with neoplasms, these non tumorigenic p53 mutations have to be balanced by the statement in the abstract of document D66 that "more than 15,000 tumours with TP53 mutations have been published, leading to the description of more than 1,500 different TP53 mutants" (see also document D38, Fig. 1 on page 1187). Moreover, the board observes that the mutations associated with normal onco-suppressor activity of p53 dealt with in document D74 are qualified as "infrequent" (see the abstract).

In conclusion, there is no evidence before the board that "non-neoplastic" mutations occur to such a level in the population to significantly affect the reliability of the presently claimed method.

Lack of function

15. Relying on documents D54 to D56, the opposition division and the respondent maintained that the identification of a "non-functional p53 gene product" (see claim 1) by its ability to bind to either the SV4O

T-antigen or the adenovirus E1B antigen (see page 4, lines 15-19 of the patent and claim 14) could not provide a reliable method of diagnosing a cancerous tissue. These documents indeed showed that mutant p53 polypeptides existed which were oncogenic but nevertheless bound to the SV4O T-antigen.

Document D54 indeed refers to two oncogenic p53 mutants (see page 4339, r-h column, first paragraph) which bind to the SV40 T-antigen. However, in the board's view, these two mutants have to be balanced with four other oncogenic p53 mutants, whose binding to the SV40 T-antigen was "greatly reduced" (see page 4339, r-h column, end of first paragraph). Moreover, according to document D55 (see page 105, r-h column, last paragraph) only 3 of 13 oncogenic mutations failed to abolish the binding activity to the SV40 T-antigen. Finally, Table 1 of document D56 (see page 3816) shows the effects of four mutations (V143A, R175H, R248W and R273H) on the SV40 binding activity of four p53 fragments ("fragments 25"). Mutants V143A and R175H abolished the binding activity, whereas mutant p53 fragments R248W and R273H did not. However, these two latter results pertaining to p53 fragments do not reflect the behaviour of the corresponding full-length p53 mutants: in fact, "Arg>Trp248" (see page 105, r-h column, last paragraph of document D55) and "273 Arg>His" (see document D55, Table 1) do not bind to the SV40 .

In summary, there is no evidence before the board that exceptions to the rule that oncogenic p53 mutants lose their binding activity to the SV40 T-antigen or the adenovirus E1B antigen occur to such a level to

significantly affect the reliability of the presently claimed method.

16. In the same context of the wild p53's ability to bind to either the SV4O T-antigen or the adenovirus E1B antigen, it was also argued by the opposition division and the respondent that the patent did not provide any evidence that these binding activities correlated with the ability of p53 to function as a tumour suppressor.

The board in fact notes that according to page 4, line 16, of the patent, the p53 functions still needed to be elucidated. However, in the board's view, the knowledge of the true mechanism underlying p53-dependent carcinogenesis was not necessary for reliably putting the claimed method into practice, as long as the skilled person could understand from the patent in suit that p53 mutations (or low levels/absence of wild-type p53) correlated with the presence of a tumour, and that this correlation rendered possible the molecular detection of cancer by detecting mutations occurring in this onco-suppressor, whatever the true cascade of events leading to carcinogenesis might have been. Thus, whereas the patent in suit (see page 4, lines 17-19) does not suggest that the loss of p53's ability to bind to either the SV4O T-antigen or the adenovirus E1B antigen correlates with its ability to function as a tumour suppressor, it recommends to use this loss of binding activities as a means to detect p53's mutations and hence tumours (see the wording "...indicates a mutational alteration"). The board notes in passing that the respondent agrees that the claimed method relates in essence to the teaching in the description, that a mutation in the wild-type p53 gene leads to loss

or diminution of expression of the wild-type p53 mRNA or a non-functional p53 gene product which, in turn, leads to a tumour (see paragraph 60 of the submissions dated 7 December 2007).

17. In view of the foregoing, the board concludes that these facts do not alter its view that the objective problem has indeed been solved.

Claimed solution obvious or not?

18. The relevant question in respect of inventive step is whether or not it was necessary for the skilled person departing from the teaching in document D18 to apply inventive skill in order to arrive at the claimed solution.

19. Document D18 itself did not provide any hint. This document related to investigating the role of p53 in human leukaemia and lymphoma. According to page 116, r-h column, lines 1-9, all leukaemia and lymphoma cell lines studied expressed an elevated level of p53 compared with analogous normal tissues. Document D18 therefore taught away from a loss of p53 as being indicative of cancer, the more so as this document held the involvement of p53 in the transformation in normal myeloid into leukemic cells as "unlikely" (see page 117, l-h column, lines 5-9).

20. The respondent maintained that the skilled person starting from document D18 would have arrived at the claimed methodology, with a reasonable expectation of success, in the knowledge that p53 was an anti-oncogene

(i.e., an onco-suppressor), as disclosed, for example, in document D8.

21. Document D8 taught that p53 was a candidate onco-suppressor. The board finds it doubtful whether the skilled person would have actually combined two contradictory documents (document D18: "excess p53 induces tumours"; document D8: "absence of the onco-suppressor p53 induces tumours").

22. If, nevertheless, the skilled person turned to document D8, he/she would find that it is a review article presenting no new experimental data of its own. This document refers to p53 as a "candidate anti-oncogene" and states that the transforming potential of p53 is activated by mutations in a wide variety of positions throughout the protein (see page 3, l-h column, lines 15-19).

Its disclosure in connection with p53 is based on data obtained from mouse erythroleukaemia cell lines generated by using the Friend virus. The Friend virus does not infect humans and is not found in naturally occurring human tumours. Accordingly, data generated using Friend virus-induced murine tumours is not indicative of the function of human p53 in naturally occurring human tumours. Moreover, the experiments referred to in document D8 deal with the cooperation of certain p53 mutants with the activated ras-oncogene to phenotypically transform primary cells. It cannot be derived from these experiments that it is specifically the loss of wild-type p53, without considering the role of the ras-oncogene, that would induce tumours in mice cells, let alone in human cells.

23. The respondent argued before the opposition division that many prior art documents, in addition to document D8, taught that p53 was an onco-suppressor.

However, the board observes that shortly before the priority date of the patent in suit, p53 was considered by certain authors as an oncoprotein (i.e. an oncogene inducing cancer; see document D5, page 134, lines 20-21). The scientific community was thus still awaiting the decisive experimental proof in favour of one of the above two hypothesis, namely the "onco-suppressor" theory versus the "oncogene" theory (see e.g., document D9, page 746, final sentence and document D5, page 134, line 11-13).

The break-through came from the present inventors, who provided the "decisive experimental proof" mentioned above and elucidated the role of human p53 in human carcinogenesis. First, the inventors analyzed 58 human carcinoma specimens and compared their DNA to DNA from adjacent normal colonic mucosa. Deletions on chromosome 17p were mapped to determine the area of overlap in the deletions. The mapping results showed that the smallest common region of deletion extended between 17p12 to 17p13.3 (see Figure 2). These authors demonstrated that in two different human tumours, the non-deleted p53 allele carried a point mutation at codons 143 and 175, respectively, which were detected by sequencing (see Examples 4 and 5 of the patent). Five additional subtle sequence changes on non-deleted p53 alleles in five human carcinomas were also detected (see Example 6 of the patent). Eight more human tumours were subsequently examined and found to contain point mutations in p53.

24. In summary, the subject matter of the claim 1 is not obvious in the light of the disclosure in the prior art documents on file. This conclusion extends to independent claims 23, 26, 27 and 28, all having in common with claim 1 the non-obvious link between loss of wild-type p53 and cancer, and to the dependent claims 2-22 and 24-25.

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 new Main Request, filed as Second Auxiliary Request with the letter dated 12 October 2009, and a description to be adapted.

The Registrar: The Chairman:

P. Cremona M. Wieser