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Datasheet for the decision of 19 February 2007

Case Number: T 1155/05 - 3.3.08

Application Number: 90914094.9

Publication Number: 0444181

IPC: C12N 15/12

Language of the proceedings: EN

Title of invention:
C-ERBB-2 External Domain: GP75

Patentee:
Bayer Schering Pharma Aktiengesellschaft

Opponents:
Pharmex A/S
F. Hoffmann-La Roche AG

Headword:
Soluble domain/BAYER SCHERING PHARMA

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

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

Decisions cited:
-

Catchword:
-

Case Number: T 1155/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 19 February 2007

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Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted 24 June 2005 concerning maintenance of European patent No. 0444181 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
T. Karamanli

Summary of Facts and Submissions

I. European patent no. 0 444 181 was granted on the basis of European patent application No. 90 914 094.9 (published as WO 91/02062, to be referred to in the present decision as the application as filed) and was opposed by two opponents on the grounds of Articles 100(a),(b) EPC. The patent was maintained in amended form by the opposition division on the basis of a second auxiliary request filed on 8 April 2005.

II. Opponents 01 and 02 (appellants I and II) lodged an appeal and filed the statements setting out their grounds of appeal.

III. The patentee (respondent) replied to the appellants' statements of grounds of appeal and filed as main request the claims as upheld by the opposition division and auxiliary requests 1 to 4.

IV. With the summons to the oral proceedings, the board sent a communication to the parties under Article 11(1) of the Rules of Procedure of the Boards of Appeal (RPBA). The parties were informed therein of the board's preliminary opinion on the relevant issues.

V. On 19 January 2007, the respondent and the appellant II replied to this communication and the former filed a new main request and auxiliary requests 1 to 4 in replacement of the corresponding previous requests.

VI. On the same date, the appellant I informed the board of its intention not to attend the oral proceedings.

VII. Oral proceedings took place on 19 February 2007 in the absence of the appellant I.

VIII. Claims 1, 3 and 10 of the **main request** read as follows:

"1. A method of testing mammalian body fluids for the presence of soluble gp75 at a level above normal, which comprises contacting a composition containing antibodies to gp75 proteins and/or polypeptides displaying epitopes of gp75, with a sample of a mammalian body fluid and determining whether said antibodies bind to a soluble protein in said sample, gp75 being the external domain of the c-erbB-2 protein extending from amino acid 22 (Ser22) to amino acid 653 (Ser653)."

"3. A diagnostic method for neoplastic disease associated with c-erbB-2 amplification employing an in vitro immunoassay to detect and quantitate soluble gp75 in human body fluids, wherein an elevated level above the level found in normal body fluids indicates the presence of tumor cells that overexpress the c-erbB-2 glycoprotein; said neoplastic disease being preferably a tumor of an epithelial origin or of an organ having a secretory function, gp75 being the external domain of the c-erbB-2 protein extending from amino acid 22 (Ser22) to amino acid 653 (Ser653)."

"10. An in vitro diagnostic method for detecting the presence of human tumor cells, which overexpress the c-erbB-2 glycoprotein, by detecting and quantitating soluble c-erbB-2 external domain extending from amino acid 22 (Ser) to amino acid 653 (Ser) of the c-erbB-2

glycoprotein, in a human body fluid which method comprises:

(a) contacting the body fluid with an antibody, preferably a monoclonal antibody, having specificity for the external domain of the c-erbB-2 glycoprotein; and

(b) detecting the amount of soluble c-erbB-2 external domain bound by the antibody, wherein an elevated level of binding above the binding level found in normal body fluids indicates the presence of tumor cells that overexpress the c-erbB-2 glycoprotein."

Claims 2 and 5 to 9 were directed to further embodiments of claim 1. Claims 4 and 11 related to embodiments of claims 3 and 10, respectively.

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

D3: T. Yamamoto et al., Nature, 16 January 1986, Vol. 319, pages 230 to 234;

D5: WO-89/06692 (publication date: 27 July 1989);

D26: G.K. Scott et al., Mol. Cell. Biol., April 1993, Vol. 13(4), pages 2247 to 2257;

D27: W. Weber et al., Science, 20 April 1984, Vol. 224, pages 294 to 297;

D28: A. Ullrich et al., Nature, 31 May 1984, Vol. 309, pages 418 to 425;

D30: G.T. Merlino et al., Mol. Cell. Biol., July 1985, Vol. 5(7), pages 1722 to 1734.

X. The appellants' arguments in relation to the main request may be summarized as follows:

Article 123(2) EPC

There was no formal support in the application as filed for combining the terms "*soluble gp75*" and "*at a level above normal*" in a method of testing (claim 1). The references to "*an elevated level ... above the normal background binding level*" found in the "Summary of the Invention" concerned gp75 in general and not soluble gp75. This was also shown by claim 55 as filed which was directed to a diagnostic method for detecting the presence of tumor cells based on the amplification of the c-erbB-2 receptor on the surface of these tumor cells and not on soluble gp75. Part (b) of claim 55 used the same wording as the "Summary of the Invention" and it referred exclusively to tumor cells, namely "*an elevated level of binding above the binding level of normal cells*". There was no reference in claim 55 to soluble gp75.

Neither claim 55 nor the description of the application as filed provided a formal basis for the subject-matter of present claim 10, since the application as filed always disclosed the binding level to gp75 in general in a human body fluid as the only level indicative of tumor cells and not the binding level to soluble gp75.

Article 56 EPC

Claim 1 was directed to a general method of testing the presence of soluble gp75 in any possible mammalian body fluid with any antibody. This broad subject-matter did not represent an inventive contribution over the prior art, it was only an obvious, non-surprising development over the prior art.

Monoclonal antibodies against the extracellular domain of the c-erbB-2 receptor were used for detecting this receptor and they were known to be useful in the diagnosis of human malignancies (cancer). Document D5 disclosed serological methods for the determination of the c-erbB-2 receptor in the form of immunoassay procedures like, *inter alia*, RIA and ELISA tests. These serological methods were characterized by the use of monoclonal antibodies directed against the extracellular domain of the c-erbB-2 receptor.

This prior art identified the c-erbB-2 receptor as a member of the epidermal growth factor (EGF) receptor family. This was acknowledged in document D3, which referred to documents D27 and D28 for the EGF receptor. The structural properties of both the c-erbB-2 and the EGF receptors were known to be very similar and, in tumor cells, long mRNA transcripts encoding the full-length sequence of these receptors were overexpressed. Shorter mRNA transcripts encoding truncated receptors (comprising only the extracellular domains) were also detected in tumor cells. Long and short mRNA transcripts were known to be translated in tumor cells, namely in A431 carcinoma cells for the EGF

receptor (documents D27 and D28) and in MKN-7 cells for the c-erbB-2 receptor (document D3).

By using antibodies specific to the extracellular domain of the EGF receptor, documents D27 and D28 disclosed the detection of truncated EGF receptor in the culture medium of tumor cells. The truncated EGF receptor was thus secreted and was present in soluble form in the medium. The importance of this soluble product unique to tumor cells (document D28) as a diagnostic marker for these tumor cells was obvious to the person skilled in the art and was explicitly acknowledged in document D27.

Since both the EGF and the c-erbB-2 receptors were members of the same family and had similar properties, it was plausible to expect that the translated product of the short mRNA transcript of the c-erbB-2 receptor (document D3) would be secreted by MKN-7 tumor cells and thus be present in the culture medium as a soluble truncated product in line with the findings for the soluble truncated EGF receptor. In fact, this suggestion was already made in document D3 and the skilled person had only to follow it for detecting and identifying – with no surprise, as expected – the secreted, soluble truncated product comprising the extracellular domain of the c-erbB-2 receptor, i.e. the soluble gp75 of the patent in suit. The use of this soluble product for diagnostic purposes was evident to the skilled person, since the advantages of (non-invasive) soluble diagnostic markers for detecting tumor cells were obvious to the skilled person (document D27).

Starting from document D3 as the closest prior art, the technical problem to be solved was the mere provision of an experimental confirmation that the extracellular domain of the c-erbB-2 receptor was indeed secreted by tumor cells. The actual contribution of the patent in suit was the experimental confirmation of what had already been plausibly suggested by document D3 in line with other prior art documents concerned with the related EGF receptor (documents D27 and D28).

Post-published evidence (document D26, cited as expert opinion) showed that, following the suggestion made in document D3, a soluble truncated c-erbB-2 product was indeed detected in the culture medium of MKN-7 tumor cells. Once this soluble c-erbB-2 product was detected in culture medium of tumor cells, its presence in (mammalian) body fluids could not be a surprise for the skilled person. In fact, these fluids were the only place were the skilled person could look for a soluble product. Thus, the claimed subject-matter was achieved in an inevitably manner. This was the only contribution of the patent in suit. Examples 5 and 6 detected a soluble truncated c-erbB-2 product in the supernatant of tumor cells and in human sera of cancer patients, respectively. Thus, no surprise was associated with these results and no inventive talent was needed to achieve them.

In accordance with the case law of the Boards of Appeal, no experimental proof was required if a proposal or suggestion was technically plausible. The same applied for a proposal made in the prior art, such as the one made in document D3 with regard to a soluble truncated c-erbB-2 receptor. This proposal was based on plausible

technical facts concerning the related EGF receptor. No surprise (or inventive contribution) was seen in the mere experimental confirmation of an already known plausible proposal.

References to post-published evidence (document D26) disclosing differences in the cellular mechanisms of secretion of truncated c-erbB-2 fusion products and truncated c-erbB-2 non-fused products as well as possible differences in their cellular and biological functions, were irrelevant for assessing inventive step. The patent in suit was silent on the mechanisms of secretion and it did not disclose any function for soluble gp75. The fact remained that the prior art (document D3) proposed the presence of a soluble c-erbB-2 product and that the patent in suit merely confirmed this suggestion. Post-published evidence of alleged difficulties was irrelevant and could not be used to demonstrate that the person skilled in the art person would not have followed a clear (obvious) suggestion made in the prior art.

In this respect, the presence of possible chromosomal aberrations in MKN-7 cells (document D3) was also irrelevant, since all tumor cells were expected to be different from normal healthy cells, i.e. to be in a sense aberrant and to have chromosomal aberrations. In fact, MKN-7 and A431 cells (documents D27 and D28) were used as suitable model systems for studying different types of cancer, in particular, MKN-7 cells were used as a model system for human stomach cancer. Although experimental results could not always be generalized, in the present case the results of document D3 (in the light of related prior art, documents D27 and D28) were

clearly of a general nature and not restricted to these MKN-7 cells. Likewise, the patent in suit was based only on the detection of soluble gp75 in human breast cancer and nevertheless, without further experimental evidence, this soluble gp75 was considered to be a suitable marker for many other cancer cells.

The present case was in line with the "try-and-see approach" as found in the case law of the Boards of Appeal, since the skilled person had only to follow what had previously been proposed by the prior art in an explicit and plausible manner. There was no need to ascertain the expectation of success, since by following the suggestion made in the prior art the skilled person would have inevitably achieved the expected result in a straightforward manner. Thus, the claimed subject-matter was not inventive.

XI. The respondent's arguments in relation to the main request may be summarized as follows:

Article 123(2) EPC

The application as filed stated that the concept underlying all aspects of the invention was the secretion of soluble gp75 into mammalian body fluids. The methods for detecting gp75 were all based on this concept and they were all used for diagnosis of neoplastic diseases (tumors). Figures 10 and 12 and Examples 5 and 6 of the application as filed showed the presence of soluble gp75 in human sera of normal healthy volunteers and elevated levels of soluble gp75 in sera of breast cancer patients. Claims 1 and 10 had

been amended so as to read in accordance with this concept disclosed in the application as filed.

Article 56 EPC

Although the c-erbB-2 and the EGF receptors were known to be members of the same structural family, it was also known that they had different properties. However, the claimed subject-matter was not directed to the c-erbB-2 receptor nor to the extracellular domain thereof but to diagnostic methods for tumor cells (neoplastic diseases) based on the soluble extracellular domain of this c-erbB-2 receptor (gp75). For this subject-matter, document D5 was the closest prior art. This document disclosed immunoassay methods for detecting cancer using the amplified expression of the c-erbB-2/HER2 receptor on the surface of tumor cells. The assays disclosed in document D5 were all based on cells and carried out on cell samples or tissues.

Starting from this closest prior art, the technical problem to be solved was the provision of an alternative method for the diagnosis of neoplastic diseases. The claimed methods provided a solution to this problem, which was not obvious from document D5 alone or in combination with document D3.

Document D3 did not relate to diagnostic methods and therefore, it could not motivate the skilled person to develop them. Reference was made in this document to the possible secretion of a hypothetical translation product of an aberrant 2.3-kb mRNA transcript found in MKN-7 cells. This hypothetical product comprised the

extracellular domain of the c-erbB-2 receptor fused to an unidentified protein that was unrelated to the c-erbB-2 receptor. However, MKN-7 was an established gastric cancer cell line maintained and passaged in culture for a long time and thus, prone to contain many artefacts (chromosome aberrations). No conclusions could be drawn on the presence and nature of proteins based only on detected mRNA transcripts from MKN-7 cells. The translation protein of the 2.3-kb mRNA transcript was only a hypothetical product of unknown length and amino acid composition, which was completely different from the soluble extracellular domain of the c-erbB-2 receptor (gp75) identified in the patent in suit. This hypothetical product, as stated in document D3 itself, could be the result of a strong chromosomal aberration specific to MKN-7 cells. There was no evidence for the actual secretion of this hypothetical product into the medium and there was no indication that the 2.3-kb mRNA transcript (or the hypothetical translated protein) could be present in other tumor cells let alone found in cancer patients.

In fact, post-published evidence (document D26, cited as expert opinion) showed that the translated product of the 2.3-kb mRNA transcript was different from the soluble extracellular domain of the c-erbB-2 receptor. The two products were produced by different cellular mechanisms and had different cellular distributions, structure and biological function. Thus, the skilled person could not rely on the suggestions made in document D3 for detecting the soluble extracellular domain of the c-erbB-2 receptor in the body fluids of mammals with neoplastic diseases associated with amplified expression of the c-erbB-2 receptor.

The deficiencies of document D3 were not remedied by the prior art concerned with the related EGF receptor (documents D27, D28 and D30). This prior art disclosed a soluble truncated EGF receptor in human A431 carcinoma cell line. However, this cell line had strong chromosomal aberrations (78 chromosomes) and the results obtained were considered to be unique to these A431 cells, thus unreliable for any conclusions regarding their neoplastic origin. The truncated transcript of the EGF receptor only encoded part of the extracellular domain of the EGF receptor and it was also fused to an unknown sequence. Thus, this truncated transcript diverged significantly in sequence from the normal transcript and it was associated with an abnormal chromosome of the A431 cell line (translocation chromosome M4).

Although c-erbB-2 associated cancers were known in the art (document D5) and document D3 as well as other prior art concerned with the related EGF receptor were long available and the advantages of non-invasive diagnostic methods based on soluble markers were also known to the person skilled in the art, non-invasive diagnostic assays based on the c-erbB-2 receptor had never been developed. It was only after the contribution of the patent in suit to the state of the art, i.e. the detection and identification of the soluble extracellular domain of the c-erbB-2/HER2 receptor (gp75) in the supernatant of culture tumour cells and in body fluids (human serum), that the use of this soluble gp75 for diagnostic purposes became obvious and possible.

XII. The appellants (opponents) requested that the decision under appeal be set aside and that the European patent No. 0 444 181 be revoked.

XIII. The respondent (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of either the main request, or auxiliary requests 1 to 4, all filed on 19 January 2007.

Reasons for the Decision

Main request
Article 123(2)EPC

1. The application as filed states in the "Summary of the Invention" that "*the invention ... is based on the detection of the external domain glycoprotein (gp75) or parts thereof encoded by the c-erbB-2 gene in the biological fluids of mammals carrying a tumor burden*" (cf. page 11, lines 13 to 17). The term "gp75" is defined as the glycoprotein that constitutes the external domain of the c-erbB-2 receptor and is different from "intact gp75" which is defined as "*the gp75 external domain expressed upon the surface of a cell*", i.e. still attached to the cell through the transmembrane region (cf. page 43, line 33 to page 44, line 21). In accordance with "*the concept underlying the many facets of this invention*", namely "*the discovery that c-erbB-2 overexpressing cells shed the c-erbB-2 external domain (gp75) into the body fluids of the host mammal*" (cf. page 20, lines 30 to 33), "gp75" is thus understood as corresponding to a "*soluble c-erbB-2 derivative (gp75)*" or "*shed antigen with*

affinity binding characteristics of the c-erbB-2 external domain" which, in the examples of the application as filed, is found in the supernatant of human tumor cells and in human sera from breast cancer patients (cf. page 20, line 34 to page 21, line 12).

2. The reference to "*an elevated level of gp75 in a host's body fluid, that is, above the normal background binding level*" in the "Summary of the Invention" (cf. page 11, lines 21 to 24) is thus understood as indicating the levels of "gp75", i.e. of soluble gp75, which is in line with the examples of the application as filed and the diagnostic methods and assays disclosed therein. In this respect, there is no need to refer to claim 55 as originally filed which was ambiguously formulated. The presence and detection in a human body fluid of the soluble gp75 corresponds to the "concept underlying" the invention as defined in the application as filed. This is now reflected by the claims of this request.

3. Therefore, the claimed subject-matter is considered to fulfil the requirements of Article 123(2) EPC.

Articles 123(3), 84, 83 and 54 EPC

4. The appellant did not raise any objections under Articles 123(3), 84, 83 and 54 EPC. The board sees no reason to take a different position. The claims are thus allowable pursuant to these articles.

Article 56 EPC

The patent in suit and the claimed subject-matter

5. The patent in suit relates to the human cell surface receptor c-erbB-2 which has an extracellular (ligand binding) domain, a transmembrane domain and an intracellular (tyrosine) kinase domain. This c-erbB-2 receptor has a molecular weight of 185 kd (gp185) and is closely related to but distinct from the epidermal growth factor (EGF) receptor (cf. pages 3 and 4, paragraphs [0008] and [0009]). The contribution of the patent in suit to the state of the art is said to be the "*discovery that c-erbB-2 overexpressing cells shed the c-erbB-2 external domain (gp75) into the body fluids of the host mammal*", in particular in "*human tumor culture supernates, and in human sera from breast cancer patients*". This contribution is said to have "*opened the way for the development of novel methods ... for the diagnosis ... of neoplastic disease in humans and other mammals.*" (cf. page 8, paragraph [0063]).

6. The claimed subject-matter is directed to diagnostic methods for neoplastic diseases associated with the amplification or overexpression of the c-erbB-2 receptor. These methods are based on the detection and quantitation of soluble gp75 in human body fluids (cf. claims 3 and 10, Section VIII *supra*). The subject-matter of claim 1 is directed to a "*method of testing*". However, by explicit reference to the presence of soluble gp75 in the mammalian body fluids "*at a level above normal*", i.e. above a normal (healthy) background level, it is considered to relate also to a diagnostic method.

The closest prior art

7. According to the established case law of the Boards of Appeal, the closest prior art is normally a document disclosing subject-matter conceived for the same purpose as the claimed invention (cf. "Case Law of the Boards of Appeal of the EPO", 4th edition 2001, I.D.3.1). The board considers that in the present case document D5 represents the closest prior art, since it is clearly directed to diagnostic methods of neoplastic diseases associated with the overexpression of the c-erbB-2 receptor.

8. Document D5 discloses "*immunoassay procedures to detect the presence of cancer in a patient or monitor the status of such cancer in a patient already diagnosed to have it*" based on exposing a tissue or a cell sample to antibodies specifically binding the extracellular domain of the c-erbB-2 receptor and determining the extent of binding of these antibodies to said cells (cf. page 7, lines 2 to 7 and page 24, lines 4 to 23 and claim 12). An overexpression of the c-erbB-2 receptor on the surface of the cell (a level above normal) indicates the presence of a neoplastic disease overexpressing the c-erbB-2 receptor (cf. *inter alia* page 13, lines 26 to 34). The patent in suit differs from document D5, since it does not measure the extracellular domain of the c-erbB-2 (HER2) receptor on the surface of the cell. Rather, the extracellular domain is measured only when shed (as such, alone) into the body fluids, i.e. as soluble gp75.

9. Contrary to the appellants' view (cf. Section X *supra*), document D3 is not suitable as the closest prior art because a diagnostic purpose is not derivable from this document (cf. *infra*).

The objective technical problem

10. Starting from document D5, the objective technical problem to be solved is the provision of an alternative diagnostic method of neoplastic diseases associated with the overexpression of the c-erbB-2 receptor.

11. Example 6 of the patent in suit shows the detection of shed soluble gp75 in sera of breast cancer patients (a neoplastic disease associated with an overexpression of the c-erbB-2 receptor) at levels above the ones found in sera from normal volunteers (cf. pages 32 to 33 of the patent in suit). Thus, the problem is solved by the claimed subject-matter.

Inventive step analysis

The prior art and the common general knowledge

12. Document D5 has a filing date (5 January 1989) close to the priority date of the patent in suit (4 August 1989) and thus, it illustrates the state of the art at the time of the invention. Document D5 refers to the involvement of growth factors and their receptors in the regulation of cell proliferation and oncogenesis. Of the known proto-oncogenes, one is related to a growth factor (PDGF) and two are related to growth factor receptors, namely the *c-fms* related to the macrophage colony-stimulating factor receptor (CSF-1R)

and the *c-erbB* encoding the EGF receptor. Both receptor-related proto-oncogenes are members of the tyrosine-specific protein kinase family. Document D5 also acknowledges that the c-erbB-2 gene is closely related but distinct from the EGF receptor gene (cf. page 2, line 10 to page 3, line 21).

13. Although document D5 refers to "*a variety of transformed cells* (that) *secrete factors which are believed to*" interact in both synergistic (stimulating growth) and antagonistic (antiproliferative) manner (cf. page 5, line 10 to page 6, line 9 and page 10, lines 21 to 25), there is no indication that the cited growth factor receptors (CSF-1R and EGF receptor) or fragments thereof are secreted by normal, tumor or transformed cells. In fact, there is no mention of fragments of these receptors at all, let alone of any soluble form thereof and of any possible relevance thereof for diagnostic purposes.

14. Document D5 also refers to the amplification of the EGF receptor gene in primary human tumors and established tumor-derived cell lines. Although reference is made to the detection of a few sequence rearrangements, in most tumors the amplified c-erbB-2 sequence does not differ from the normal c-erbB-2 sequence. There is, however, no indication on the nature of these rearrangements nor on the structure of the resulting encoded products and document D5 does not provide any bibliographic reference, either for the EGF receptor or for the c-erbB-2 receptor, for retrieving this information. The document is completely silent with regard to the relevance of these gene rearrangements and/or of the resulting encoded products for diagnostic purposes

(cf. page 3, line 23 to page 4, line 2). Thus, document D5 alone does not render obvious the claimed subject-matter.

The disclosure of document D3

15. The reference in document D5 to the rearrangements of the c-erbB-2 gene might draw the attention of the skilled person to the earlier document D3 (published on 16 January 1986). This document discloses the structural similarity of the cell surface c-erbB-2 and EGF receptors (cf. page 232, Figure 3). Two transcripts are identified in the established human gastric cell line MKN-7, a normal 4.6-kb mRNA transcript encoding the full-length c-erbB-2 receptor, and a truncated 2.3-kb mRNA transcript synthesized at elevated levels and predicted to encode only the extracellular domain of this c-erbB-2 receptor. It is indicated that this truncated 2.3-kb mRNA transcript may be caused by chromosomal aberration. The predicted encoded sequence has indeed, at its 3' extreme, a sequence of unknown origin caused by chromosomal translocation (cf. page 231, Figure 2 and page 234, left-hand column). Since the truncated transcript does not contain a signal for membrane anchoring, it is stated that the predicted polypeptide "*should be secreted by MKN-7 cells*" (cf. paragraph bridging pages 233 and 234). In this context, reference is also made to a truncated EGF receptor reported to be overexpressed concomitantly with the EGF receptor in human A431 cells, as a consequence of a strong chromosomal aberration (cf. page 234, left-hand column).

16. Document D3 does not disclose the isolation and characterization of the predicted aberrant, truncated c-erbB-2 receptor (fused with an unidentified sequence) nor does it verify whether or not it is actually secreted. Moreover, there is no indication of the possible relevance or significance of this aberrant, truncated c-erbB-2 fused receptor, in particular, whether it is specific to the established cell line MKN-7 or else to all tumor cells overexpressing the c-erbB-2 receptor. No implications can, therefore, be drawn from document D3 with regard to a possible use of this aberrant, truncated c-erbB-2 fused receptor for diagnostic purposes.

17. It was argued by the appellants that the relevance of the aberrant, truncated c-erbB-2 fused receptor can be directly derived from prior art cited in document D3 concerned with a similar aberrant, truncated EGF receptor, namely documents D27, D28 and D30 (cf. Section X *supra*).

The disclosure of documents D27, D28 and D30

18. Document D28 discloses "*the detection of an aberrant A431-specific chimaeric mRNA molecule which consists of a fragment of EGF receptor coding sequences recombined with sequences of unknown origin and function. This aberrant mRNA encodes only the external EGF binding domain of the receptor and lacks sequences which would encode the transmembrane and cytoplasmic domains*" (cf. page 418, right-hand column, first full paragraph and page 420, Figure 2). This aberrant, truncated receptor "*should be secreted by A431 cells*" and "*a monoclonal antibody ... which recognizes the extracellular domain*

of the EGF receptor, does in fact immunoprecipitate a secreted ... glycopeptide" which is thought to correspond to the aberrant, truncated EGF receptor (cf. page 423, left-hand column, last full paragraph). However, due to the "*unusual features*" of the A431 carcinoma cells (cf. page 418, paragraph bridging left- and right-hand columns), document D28 concludes that "*it seems likely that the A431 2.8-kb mRNA and the secreted EGF receptor external domain are* ***unique to A431 cells*** *and do not provide any clues to the neoplastic origin or character of this cell line*" (bold type introduced by the board). Moreover, it is stated that, since the A431 cell line "*has a multitude of defects exemplified by its 78 chromosomes, it is clearly best to regard this cell line with caution when exploring the mechanism of action of EGF or in characterizing changes which relate in a meaningful way to neoplasia*" (cf. paragraph bridging pages 424 and 425).

19. Document D30 does not go beyond the content of document D28. The aberrant 2.9-kb mRNA transcript found in A431 cells is characterized as "*a hybrid which does not contain the transmembrane-spanning region or the kinase domain, but instead possesses a divergent 600-bp sequence at its extreme 3' end and presumably encodes the EGF receptor-like protein secreted by A431 cells*" (cf. page 1732, left-hand column, first full paragraph). It further states that "*evidence ... suggests that the A431 EGF receptor gene is involved in a rearrangement with an unidentified piece of DNA. The nature of the DNA fused to the EGF receptor gene currently is unknown ... The relocation of this DNA to a region within the EGF receptor gene may affect the expression*

of the resulting aberrant receptor gene at the level of transcription or translation" (cf. page 1732, left-hand column, last paragraph). Thus, the 2.9-kb mRNA transcript is identified as an aberrant **A431-specific** secreted EGF receptor-like protein and no conclusions are drawn for other (established or primary) tumor cells. Therefore, no implications with regard to a possible diagnostic use of this aberrant A431-specific secreted EGF receptor-like can be derived from documents D28 or D30.

20. In fact, it is only in earlier document D27 that this suggestion is made. The document identifies a soluble extracellular EGF receptor-related protein (ERRP) in A431 carcinoma cells, which "*cannot be derived from membrane bound EGF receptor protein*" (cf. page 296, right-hand column, last sentence of first paragraph) and concludes that "*additional studies are required to determine whether production of ERRP occurs in normal cells or is restricted to tumor cells ... The secreted ERRP may provide a readily measurable marker of excessive production of EGF receptor and erb B proteins in human epidermoid carcinomas*" (cf. page 297, paragraph bridging left-hand and middle columns). There is, however, no evidence on file showing that, at the priority date of the patent in suit (4 August 1989) and thus, more than five years after the publication of document D27 (20 April 1984), these studies had actually been carried out and the results obtained thereby had demonstrated the suggested use of the ERRP as a diagnostic marker. In absence of this evidence, it cannot be assumed that the diagnostic use of the soluble ERRP was already well established in the state of the art.

21. It follows from the above considerations that the claimed subject-matter, namely a diagnostic method performed in mammalian body fluids based on the soluble extracellular domain of c-erbB-2 receptor (gp75), cannot be derived in an obvious manner from document D5 alone or in combination with document D3, and possibly also other prior art concerned with the related EGF receptor. To combine these documents with the expectation that the aberrant, truncated c-erbB-2 fused protein of document D3 (with an amino acid sequence different from gp75) would be a marker for diagnostic methods and provide an alternative to the methods disclosed in document D5, would require hindsight knowledge of the patent in suit.

Experimental verification of the disclosure of document D3

22. It has also been argued however that, even in the absence of any expectation of a diagnostic use for the aberrant, truncated c-erbB-2 protein disclosed in document D3, the skilled person had nevertheless a clear incentive to experimentally verify the suggestions made in that document (cf. Section X *supra*). When reading document D3, in line with the prior art concerned with the related EGF receptor (documents D27, D28 and D30), the skilled person expected the translated product of the aberrant, truncated 2.3-kb mRNA transcript of c-erbB-2 to be secreted by MKN-7 cells. The translated truncated product was expected to comprise the extracellular domain of the c-erbB-2 receptor fused with a sequence of unknown origin (cf. point 15 *supra*).

23. As a matter of fact, when using antibodies specific for the extracellular domain of the c-erbB-2 receptor for detecting the translated truncated (fused) product in the supernatant of MKN-7 cells (in line with the immunologic assays of documents D27, D28 and D30), an immunoprecipitate would have probably been detected and (wrongly) assumed to be the expected truncated (fused) product. However, only if the skilled person decided to go one step further and to characterize this product, he or she, contrary to all expectations, would have identified the soluble extracellular domain of the c-erbB-2 receptor alone (soluble gp75), not the expected truncated (fused) product. There is post-published evidence on file showing that the expected truncated (fused) product, which resulted from the translation of the aberrant, truncated 2.3-kb mRNA transcript, was an intracellular product (cf. document D26, cited as expert opinion). Under the said circumstances, the presence of soluble gp75 would have been an unexpected, surprising finding.

24. However, as stated above, the relevance of the detected immunoprecipitate or, if further characterized, of the soluble gp75, would have not become immediately apparent to the skilled person, since he or she did not know whether this product was specific only to the (chromosomal aberrations of the) established MKN-7 cell line or else to other tumor cells. This is all the more so, since the prior art concerned with the related cell surface EGF receptor was completely silent on the presence of this (non-fused) extracellular domain, either in tumor or in normal healthy cells. Thus, the skilled person would have found himself or herself in a situation similar to the one of document D27, wherein

"*additional studies are required*" so as to assess whether the secreted product "*may provide a readily measurable marker*" (cf. point 20 *supra*).

25. In fact, what is still missing in all cited prior art is the identification of these soluble extracellular domain forms in the transmembrane receptor systems, and in particular in the closely related cell surface EGF receptor and CSF-1R (cf. point 12 *supra*), as well as the recognition that these soluble proteins share a common physiological function (cf. page 2256, paragraph bridging left- and right-hand columns in document D26, cited as expert opinion). This important body of general knowledge was being established at the priority date of the patent in suit by scientific studies and contributions to the state of the art similar to the ones made by the patent in suit. However, this knowledge was still missing at the priority date of the patent in suit.

26. Although the interest of readily measurable soluble markers for non-invasive diagnostic purposes was obvious to the skilled person (document D27) and monoclonal antibodies specific for the extracellular binding domain of the c-erbB-2 receptor were available to the skilled person (document D5), in the absence of a body of knowledge recognizing the very existence of the soluble extracellular domains of these cell surface receptors as well as their biological relevance, there was no motivation to look for them in the body fluids, let alone to think of any possible diagnostic purpose.

Conclusion

27. In the board's judgement, the claimed subject-matter represents an inventive contribution over the prior art and therefore, fulfils the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent for all designated Contracting States with the following claims and a description to be adapted thereto:

   Claims No. 1 to 11 filed as main request with the letter of 19 January 2007.

The Registrar: The Chairman:

D. Magliano L. Galligani