DECISION
of 26 March 2002

Case Number: T 1038/00 - 3.3.4

Application Number: 95913946.0

Publication Number: 0755516

IPC: G01N 33/569

Language of the proceedings: EN

Title of invention:
A method for detecting and/or quantifying and/or separating apoptotic cells in or from a sample

Patentee:
NEXINS RESEARCH B.V.

Opponents:
(01) Boehringer Ingelheim GmbH
(02) Miltenyi Biotec GmbH
(03) Roche Diagnostics GmbH

Headword:
Apoptotic cells/NEXINS RESEARCH

Relevant legal provisions:
EPC Art. 52(4), 56, 105, 114

Keyword:
"Intervention admissible (no)"
"Inventive step (no)"
"Admissibility of late filed set of claims (no)"

Decisions cited:
T 0775/92, T 0385/86, T 0082/93, T 0329/94, G 0002/92,
T 0025/91, T 0234/92
Catchword:
-
Case Number: T 1038/00 - 3.3.4

DE C I S I O N
of the Technical Board of Appeal 3.3.4
of 26 March 2002

Appellant I: Boehringer Ingelheim GmbH
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Representative: -


Composition of the Board:
Chairwoman: U. M. Kinkeldey
Members: A. L. L. Marie
**Facts and Submissions**

I. European patent EP 0 755 516 was granted on the basis of a set of 32 claims, claims 1, 4 and 25 of which read:

"1. A method for detecting and/or quantifying and/or isolating apoptotic cells in a sample, comprising
a) contacting the sample with a detectable high-affinity reagent having a dissociation constant for phosphatidyl serine with Kd \(10^{-6}\)M and
b) qualitatively and/or quantitatively detecting cells that have reacted with the detectable reagent having high affinity for phosphatidyl serine, said detection occurring before or after the optional isolation step
c) isolating apoptotic cells from non-apoptotic cells on the basis of the apoptotic cells being bound to the said detectable reagent in step a)
said detectable reagent also being selectable."

"4. A method according to any of the preceding claims, wherein the cells can be distinguished into cells that have undergone lysis and intact cells through the use of a label for detecting cells that have undergone lysis."

"25. A method for determining the effect of a compound or a specific treatment on the degree of apoptosis in an individual and/or a sample comprising carrying out the method according to any of the preceding [sic] claims with a sample that has been subjected to the presence of the compound and/or the specific treatment to be tested and comparing the result to the result obtained carrying out the
method according to the preceding [sic] claims under the same conditions with a standard sample and/or with a sample taken prior to the presence of the compound and/or the specific treatment to be tested."

II. As a result of an opposition procedure, the patent was maintained in amended form on the basis of claims 1 to 32 of the second auxiliary request.

III. Appellant I (opponent 01) and appellant II (patentee) filed appeals against the decision of the opposition division, paid the respective fees and filed the respective statement of grounds of appeal.

IV. Opponent 02 did not file an appeal against said decision within the time limit laid down in Article 108 EPC and thus is a party as of right to these appeal proceedings according to Article 107(2) EPC (other party I). However after expiry of this time limit, proceedings for infringement of the German part of the European patent in suit were instituted against the other party I, who then, on 14 March 2001 filed a notice of intervention under Article 105 EPC, paid an opposition fee and an appeal fee, filed a statement of grounds of appeal, and requested reimbursement of the opposition fee and the appeal fee, on the grounds that one opposition fee had already been paid and the appeal fee was only paid as a precaution.

V. Opponent 03 had filed an appeal but withdrew this appeal by letter of 27 June 2001.

VI. The Board issued a communication under Article 11(2) of the rules of procedure of the boards of appeal giving
the Board's preliminary and non-binding opinion, in particular concerning the request of other party I under Article 105 EPC.

VII. Oral proceedings were held on 26 March 2002.

VIII. During the course of the oral proceedings the second and third auxiliary requests filed with letter of 19 March 2002 by appellant II were made the first and second auxiliary requests respectively, and a third auxiliary request was submitted.

In the first and the second auxiliary requests claim 1 was identical and corresponded to claim 1 as granted except for an additional step d) which read:

"d) distinguishing the detected cells into cells that have undergone lysis and apoptotic cells through use of a label for detecting cells that have undergone lysis."

Claim 25 of the first auxiliary request read:

"25. An in vitro method for determining the effect of a compound or a specific treatment on the degree of apoptosis in an individual and/or a sample, comprising carrying out the method comprising:

a) contacting a sample with a detectable high affinity reagent having a dissociation constant for phosphatidyl serine with \( \text{Kd} \leq 10^{-6} \text{M} \) and

b) qualitatively and/or quantitatively detecting cells that have reacted with the detectable reagent having high affinity for phosphatidyl serine, said detection step occurring before or after the optional isolation step c);
c) isolating apoptotic cells from non-apoptotic cells, on the basis of the apoptotic cells being bound to the said detectable reagent in step a), said detectable reagent also being selectable; with a sample that has been subjected to the presence of a compound and/or the specific treatment to be tested and comparing the result to the result obtained carrying out the same method under the same conditions with a standard sample and/or with a sample taken prior to the presence of the compound and/or the treatment to be tested."

Claim 25 of the second auxiliary request was identical to Claim 25 as granted, except for the addition of "in vitro" to characterize the claimed method.

As a third auxiliary request submitted during the oral proceedings it was requested to remit the case to the first instance on the basis of a set of claims 1 to 24. Claims 1 and 24 read:

"1. A method for evaluating the efficacy of an anticancer therapy by detecting and/or quantifying and/or isolating apoptotic cells in a sample, comprising
a) contacting the sample with a detectable high-affinity reagent which is a polypeptide or protein classified as an annexin having a dissociation constant for phosphatidyl serine with $K_d = 10^{-6}$ M and"

and steps b) and c) as in granted claim 1 (see section I above).
"24. Use of a kit suitable for carry [sic] out a method for detecting and/or quantifying and/or isolating apoptotic cells in a sample for separating suitable cells for transplantation from unsuitable cells, comprising
a) contacting the sample with a detectable high-affinity reagent which is a polypeptide or protein classified as an annexin having a dissociation constant for phosphatidyl serine with $K_d \approx 10^{-6}$ M and
b) qualitatively and/or quantitatively detecting cells that have reacted with the detectable reagent having high affinity for phosphatidyl serine, said detection occurring before or after the optional isolation step c)
c) isolating apoptotic cells from non-apoptotic cell on the basis of the apoptotic cells being bound to the said detectable reagent in step a) said detectable reagent also being selectable, comprising a reagent having a dissociation constant for phosphatidyl serine with $K_d \approx 10^{-6}$ M and is detectable or can be made detectable, said kit further comprising a label for distinguishing cells that have undergone lysis from intact cells."

Following a comment of the Board, appellant II offered to amend claim 1 of the third auxiliary request by replacing the word "...for..." by the word "...of...", so that claim 1 started: "A method of evaluating...".

IX. The following documents are cited in this decision:

(1) H.A.M. Andree et al., Journal of Biological Chemistry, 1990, Vol. 265, No. 9,
X. The arguments of appellant I and other party I can be summarized as follows:

Article 52(4) EPC: the vague wording of claim 25 of the main request extended to the determination in an individual of the effect of a compound or a treatment and thus covered diagnostic methods practised on human and/or animal body. Decision T 775/92 (7 April 1993) was cited in this context.

The different steps belonging to a diagnostic method as defined in Decision T 385/86 (OJ EPO, 1988, 308) were also to be found in claim 25 as granted. Furthermore, if the compound to be tested was an anti-cancer
substance acting on a solid tumor, then the method of claim 25 would have to be carried out on the patient by a medical doctor.

Article 123(2)(3) EPC: in claim 1 of the second and third auxiliary requests the step of distinguishing between apoptotic cells and cells that had undergone lysis was to be carried out after the step of detection of phosphatidyl serine (PS). Such an order of the steps was described neither in the application as filed nor in the claims as granted.

The expression "...evaluating the efficacy of an anticancer therapy by..." introduced into claim 1 of the auxiliary request submitted during the oral proceedings was not present in the claims as granted and contravened the requirements of Article 123(3) EPC.

Article 56 EPC: the closest prior art for the purpose of the main request was considered to be represented by either document (2) or document (3), even though these were not directly concerned with a method of determination of apoptotic cells, but rather with a study of the mechanisms leading to the phagocytosis of apoptotic cells by macrophages. However, since in both documents it was first necessary to determine the mechanisms leading to the recognition, and hence the determination, of the apoptotic cells by the macrophages, the disclosure of these documents related to the same technical field as the patent in suit.

Documents (2) and (3) further suggested that apoptosis was a general phenomenon, not restricted to the cells investigated. In this context, it was argued that claim 1 of all requests did not make any restriction on
the components contained in a sample, so that a sample only containing the specific cells described in documents (2) and/or (3) was embraced by said claim. Documents (2) and (3) made reference to the then standard method of determination of apoptotic cells based on the existence of the DNA fragmentation leading to so-called "DNA ladders", which implied the destruction of the apoptotic cells, thus hampering any further study of said cells. Both documents identified PS as a surface marker for apoptosis. The technical problem to be solved was to find a means reacting with PS, thus allowing to detect apoptotic cells without destroying them. The solution was to be found in documents (1) or (4), which described annexins, a group of molecules selectively binding to and having a high affinity for PS, which was able to carry a selectable fluorescent, radioactive label or a paramagnetic contrast reagent.

Another line of argumentation, when considering document (2) as the closest prior art, was that it described the derivatization of PS with fluorescamine. The technical problem was then to find an alternative molecule to said fluorescamine. The solution was again to be found in documents (1) or (4) in the form of annexin.

These arguments also applied to the first and the second auxiliary requests, since document (2) was also concerned with the distinction between apoptotic cells and cells that have undergone lysis, as shown by the use of trypan blue.

The third auxiliary request was not to be allowed into the proceedings under Article 114 (2) EPC as having
been filed too late and being a fresh case. It was not agreed to a remittal to the first instance because this would amount to an undue prolongation of the proceedings.

XI. Appellant II submitted essentially the following arguments:

Article 52(4) EPC: there was no ambiguity about the fact that none of the steps was carried out on the human body, since the wording of claim 25 related "in an individual" to the "degree of apoptosis" and not to "a method for determining". Furthermore, claim 25 clearly stated that the steps had to be carried out "on a sample". Therefore, the situation as underlying decision T 82/93 (OJ EPO 1996, 274) did not arise in relation to claim 25. Example 3.IV of the patent in suit was the only example, in which the method of claim 25 was carried out on the animal body. According to decision T 329/94 (OJ EPO 1998, 241), this example did not constitute a method excluded from patentability under this article. Furthermore, Example 3.IV was defined in column 9, lines 40 to 45 of the patent in suit as falling outside the scope of the claims.

Article 56 EPC: the search in the prior art and the argumentation of appellant I and other party I was basically based on hindsight, as seen by the fact that the relevant prior art as defined in the search report was represented by only four documents, one of them, document (17), was related to the then usual method of determination of apoptosis, ie the DNA fragmentation, which constituted the closest prior art.

The field of the invention described in the patent in
suit was related to the determination of apoptosis, ie
to medical diagnosis. On the contrary, documents (2)
and (3) related to the physiology of human beings
and/or animals. These fields were totally different
from each other and the skilled person involved in
medical diagnosis would not have looked for a document
in the field of physiology to solve the problem of the
determination of apoptosis. Thus, the finding of
documents (2) and/or (3) resulted from an ex post facto
analysis. Even if in possession of documents (2) and/or
(3), the skilled person involved in the field of
medical diagnosis would have only considered the part
under the heading "Materials and methods" concerned
with the then usual determination method, ie the "DNA
ladder" method (document (2), page 2208, right column,
heading "Evaluation of apoptosis"; document (3),
page 4030, right column, heading "Isolation and
apoptosis induction in thymocytes and lymphocytes").

Furthermore, the skilled person would also have had no
reasonable expectation of success, since documents (2)
and/or (3) were speculative. For instance,
document (2), on pages 2207 (right column, last
sentence) and 2208 (left column, lines 7 to 42) was of
hypothetical nature and expressed in the conditional
tense. Document (3) on page 4034, last paragraph
speculated on the mutual interaction of the three
described mechanisms for apoptosis. Document (3) on
page 4033 (Figure 6) showed that monocyte-derived
macrophages did not recognize PS on human neutrophils
and mouse thymocytes, contrary to phorbolester-treated
THP-1 cells.

Document (6) described the existence of three different
mechanisms used by the macrophages for the phagocytosis
of apoptotic cells. Furthermore, the authors of document (2) used three different methods to verify that macrophages recognized PS (pages 2211 and 2212). However, the fluorescent dye MC 540 did not interact with PS, but was used to show a possible loss of asymmetry of the membrane, the RVV test was an indirect test related to procoagulant activity and the specificity of fluorescamine for PS was questionable, since it in fact reacted with primary amines. Therefore, the combination of documents (2) or (3) with document (4) was neither obvious nor provided the basis for a reasonable expectation of success. As far as annexins were concerned, very little was to be retrieved from document (4) about sensitivity and selectivity of these molecules. The specificity of PS as a marker for apoptosis was also questionable, since document (4) demonstrated the presence of PS on platelets.

The introduction into claim 1 of the first and second auxiliary requests of the distinction step between apoptotic cells and cells that had undergone lysis necessitated the combination of the teaching of documents (3) and (4) with the teaching of a third document, such as that of document (14). This was according to the established Case Law of the boards of appeal a strong indication of non-obviousness.

Dr V. A. Fadok, one of the authors of documents (2) and (3), stated in her letter to the EPO (document 18) that neither document (2) nor document (3), the purpose of which was the study of the various recognition mechanisms utilized by macrophages in the removal of apoptotic cells and not a generalized characterisation of membrane changes and PS exposure in apoptotic cells,
provided a basis for generalized conclusions about apoptosis and there was no evidence to prove that PS was expressed on the outer leaflet of the membrane by all apoptotic cells. Furthermore, the presence of PS on red blood cells indicated that PS had functions unrelated to apoptosis and was not a specific marker for apoptosis. She also stated that the detection method of PS using annexin as published in 1994 was not obvious to her at the time of her publications and that, even after the publication of said method, she carried on working with alternative determination methods for apoptotic cells. Basing her opinion on the failure of other researchers in trying to utilize annexin staining of apoptotic cells, she finally concluded that this method was also not obvious for other researchers.

A survey in the scientific literature concerning apoptosis, PS and annexin showed that there was no connection between apoptosis, annexin and PS in a document until the publication of the patent in suit which had to be considered as a major break-through in the field of apoptosis.

XII. Appellant I (opponent 01) requested that the decision under appeal be set aside and that the European patent No. 0 755 516 be revoked.

XIII. The other party I (opponent 02) requested that it be recognized as intervener and appellant, that the opposition fee and appeal fee paid 14 March 2001 be repaid, that the decision under appeal be set aside and the European patent No. 0 755 516 be revoked.

XIV. Appellant II (patentee) requested that the decision
under appeal be set aside and that the patent be maintained as main request as granted or as first auxiliary request on the basis of the set of claims filed as 2nd auxiliary request on 19 March 2002 or as a second auxiliary request on the basis of the set of claims filed as 3rd auxiliary request on 19 March 2002, or as third auxiliary request that the matter be remitted to the first instance for further examination on the basis of the set of claims filed at the oral proceedings on 26 March 2002.

Reasons for the Decision

Parties to proceedings and admissibility of appeals

1. The appeals of appellant I (opponent 01) and appellant II (patentee) comply with the requirements of Articles 106 to 108 EPC and with Rule 64 EPC and are thus admissible.

2. Opponent 03 originally filed an appeal but withdrew this appeal. They remain a party as of right to the appeal proceedings pursuant to Article 107 EPC.

3. Other party I (Opponent 02), filed a notice of intervention under Article 105 EPC. However, this article only gives third parties who are sued for infringement a right to intervene. Other party I had filed an opposition requesting revocation of the patent in suit, and were a party adversely affected by the decision under appeal, and could themselves have appealed, but did not do so within the time limit laid down in Article 108 EPC. Pursuant to Article 107 EPC other party I are in any case a party to the appeals by
appellants I and II. As they are already a party they do not fulfill the requirement of Article 105 EPC of being a third party. The Board can see no good reason for simply ignoring this requirement of Article 105 EPC. An existing party can safeguard its rights within the ordinary framework for appeals. If a party neglects its right to file an appeal, Article 105 EPC cannot be used to give it a second chance: the purpose of this article was to allow those sued for infringement of the same patent to put forward for the first time their own arguments for invalidity in an already pending opposition, not to alter the status of an existing party who has already had the opportunity to put such arguments.

4. The Board would remark that though in this case the status of the other party I was not relevant to the outcome in view of the request for revocation by appellant I (opponent 01), in general an opponent whose request for complete revocation has not been granted by the Opposition Division, but who does not appeal this, must be certain that he is not at risk under the patent in the amended form that has been maintained. The extent of the maintenance of the patent by the Opposition Division will not be open to challenge by him even if the patentee does appeal (see Enlarged Board of Appeal Decision G 9/92 (OJ EPO 1994, 875, point 14). The existence of this jurisprudence is another reason for not ignoring the requirement of "third party" in Article 105 EPC.

5. The Board thus holds that the purported intervention by other party I has no basis under Article 105 EPC as an existing party cannot intervene. It follows that there was no basis for the payment by other party I of the
opposition fee and the appeal fee and these are to be reimbursed.

Main request

Article 52(4) EPC

6. Article 52(4) EPC excludes from the patent protection inter alia methods of diagnostics practised on the human or animal body. As stated in decision T 385/86 (cf supra), the expression "practised on the human or animal body" implies that both examination and establishing the symptoms on the basis of the examination results must be carried out on a living human or animal body.

In claim 25 of the main request, the wording "in an individual" relates to "the degree of apoptosis" and not to "determining". The claim specifies that the method for determining the effect of the compound and/or the treatment is to be carried out on a sample. The possibility seen by appellant I and the other party II of carrying out the determination in vivo in the case of a solid tumour is not covered by the claim.

Thus, claim 25 does not contravene the requirements of Article 52(4) EPC.

Article 56 EPC - Inventive Step

7. The invention relates to the field of medical diagnostic biochemistry, and more specifically to the detection of apoptotic cells. As stated in the patent, the presence of an abnormally large number of such
cells in a patient is an indication that one of a number of diseases may be present. At the priority date of the patent in suit there was used a method of detection of apoptotic cells based on the appearance of "DNA ladders". Of the documents cited in these proceedings, this method is described in document (2), page 2208, right column, heading "Evaluation of apoptosis"; and in document (3), page 4030, right column, heading "Isolation and apoptosis induction in thymocytes and lymphocytes". This method had the disadvantage that detection required destruction of the apoptotic cells. However as no other documents in the proceedings concern the detection of apoptotic cells, the Board chooses document (3) as the closest prior starting point for applying the problem and solution approach.

8. Compared to this prior art, claim 1 in its broadest aspect can be considered as solving the problem of providing a method of detecting apoptotic cells without destroying the cells. This alternative method of detection has the advantage that it also allows the further step of isolating apoptotic cells from non-apoptotic cells.

9. This problem is to be solved according to claim 1 of the main request by the use of a detectable high affinity reagent having a dissociation constant for phosphatidyl serine with $K_d 10^{-6}$M. The description of the invention, in particular the embodiment using labelled annexin as reagent makes it plausible that this problem has been solved, and this has not been disputed by the appellant I and the other party.

10. For the assessment of inventive step the question thus
needs to be posed whether given the problem of providing a method of detecting apoptotic cells by labelling them with a detectable label without destroying the cells, and starting from document (3), the skilled person would derive a solution from the prior art in an obvious manner, which solution falls within claim 1.

11. Given that the problem is one in the field of medical diagnostic biochemistry, the expertise and knowledge of the notional skilled person needs consideration. The skilled person must be deemed to have state of the art knowledge and skills both in the fields of physiology relating to apoptopic cells and the properties that distinguish them from other cells, and in the field of reagents and tests used for detecting cells and features thereof in biochemistry.

12. The starting point in the prior art, the methods section of document (3) is what the skilled person is trying to improve upon and does not yield any indication of a solution. But the other sections of document (3) will already tell the skilled person that PS is the marker that in nature macrophages home in on to identify apoptotic cells.

13. Document (3) concerns apoptosis and, although only studies on neutrophils, thymocytes and lymphocytes are reported, the abstract, and the introduction (page 4029) indicate that apoptosis occurs "in many tissues" or "a wide variety of tissues". The first sentence under the heading "Discussion" on page 4033 also states that "apoptosis has become widely recognized as a major mechanism in the regulation of
normal tissue growth. This programmed cell death is an ongoing process in many tissues." Further it is stated on page 4031, left hand column, last sentence: "These results suggested that expression of PS on the surface of apoptotic cells was not restricted to mouse cells and was not restricted to lymphocytes." The skilled person would therefore conclude from document (3) that apoptosis is a very general and probably even universal phenomenon.

14. Document (3) further states (page 4029, right column, second paragraph) that a characteristic feature of apoptotic cells is the loss of asymmetry of their cellular membrane, so that molecules which are present in the internal leaflet of the membrane of normal cells become exposed on the external leaflet of said membrane. One of these molecules is identified as PS (page 4029, right column; page 4031, left column; pages 4033 and 4034). PS is therefore a marker for apoptosis. This statement is made in positive and affirmative terms, leaving no room for doubts. Document (3) does refer to another scientific paper dealing with PS being also expressed on the surface of sickled red cells (page 4033, right hand column, first paragraph). That PS is also a marker for another type of abnormal cell (sickled red cell) would not put off the skilled person from treating PS as being a marker for apoptotic cells. That both abnormal cell conditions should occur in the same patient would seem improbable and no reason for not treating PS as a marker for apoptotic cells: certainly the solution as now claimed takes no precautions against errors arising due to the presence of sickled red blood cells.
15. Document (3) further indicates that three mechanisms can be used by the macrophages to recognize apoptotic cells, these mechanisms involving three different receptors (page 4033, right column) and hence three different markers. One of these mechanisms being based on the occurrence of PS on the surface of the apoptotic cells. Document (3) on page 4032 (left column) and page 4034 (right column) indicates that the nature of the mechanism used by the macrophages to recognize the apoptotic cells does not depend on the apoptotic cells, but is determined by the type of macrophage. From this teaching the skilled person would assume that the apoptotic cells simultaneously carry the markers for the three mechanisms of recognition. This implies that PS can be found on the surface of every apoptotic cell, whatever their nature or origin may be.

16. Document (3) thus establishes the direct relation between apoptosis and PS, hence suggesting that the quantitative determination of PS is also a quantitative measure of the degree of apoptosis in a sample.

17. Document (4) discloses annexins as reagents for detecting PS and distinguishing it from phosphatidyl choline. It is not in dispute that the use of annexins falls within the scope of claim 1, such use being exemplified in the embodiments.

18. The use of the annexins described in document (4), in the specific context of the structural modifications induced in platelets as a result of their activation for curing blood vessel injuries, is based on two characteristic cell membrane modifications also described in documents (2) and (3) for apoptotic cells. One of these modifications is the loss of the membrane
asymmetry resulting in the occurrence of PS on the external surface of the platelet cellular membrane. Since phosphatidyl choline and sphingomyelin are the major constituents of the external leaflet of the membrane (page 2, lines 9 to 13), document (4) provides a solution to the technical problem of finding a molecule which is specific for PS and does not react with phosphatidyl choline and sphingolmyelin. The subject-matter of document (4) aims at determining the presence of a medically meaningful sub-population of cells in a sample, namely the activated platelets, and thus relates to the field of medical diagnostics.

19. Figure 5 of document (4) shows that annexin (ie the molecule also used in the patent in suit) does not react with phosphatidyl choline and sphingomyelin. While annexin is not exclusively specific for PS, since it also reacts with cardiolipin, phosphatidylethanolamine, phosphatidyl inositol and phosphatidyl glycerol, this, in fact, is of no importance for the purpose of document (4), ie the demonstration of the platelet activation by reaction of annexin with PS, or for a reagent for use in detecting PS in apoptotic cells. While indeed, cardiolipin is a constituent of the mitochondrial membrane this does not interfere with the cellular membrane changes during platelet activation. Phosphatidylethanolamine and phosphatidyl inositol, as PS, are constituents of the internal leaflet of the membrane (page 2, lines 9 to 13) and if, as a result of the asymmetry loss, they appear on the external leaflet of the membrane, they will behave as markers for the activation of platelets, or for apoptotic cells just as PS does, and as a kind of "bonus effect", will amplify the signal given by PS.
20. The skilled person will realize from documents (3) and (4) that the mechanisms of platelet activation and apoptosis, at least as far as the loss of asymmetry of the cellular membrane is concerned, are the same, so that a reagent suitable for detecting one will be suitable for detecting the other.

21. Figure 5 of document (4) also shows that phosphatidyl glycerol reacts with annexin. No information about the localization of phosphatidyl glycerol in the membrane can be retrieved from document (4). However, since normal and non-activated platelets do not react with annexins which makes it possible to make a distinction between activated and non-activated platelets using annexins it cannot be a constituent of the external leaflet of the membrane of the living cells, in general, or of normal platelets, in particular. This would be in contradiction with the teaching of document (4). Again, as for sickled red cells discussed above, the skilled person would not expect to find activated platelets and apoptotic cells occurring in one and the same patient, so that PS being a marker for both conditions would not deter the skilled person from using a PS as a marker for apoptotic cells. Rather, in the Board's judgement it was obvious for the skilled person to use the same system to detect apoptotic cells.

22. In summary, annexins do not react with the normal constituents of the external leaflet of the cellular membrane when in non-apoptotic state and its use as a marker for loss of membrane asymmetry in apoptotic cells is not hampered by the presence of PS on sickled red cells and platelets. If there existed any confusion at all, then, as stated in point 14 supra, the patent
in suit does not provide any teaching how to resolve it either. Furthermore, claim 1 does not specify the nature and the origin of the sample to be tested which could, as in document (3), only contain lymphocytes, thymocytes or neutrophils. Therefore, the skilled person, on the basis of the teaching of document (4) would come to the conclusion that annexins are the molecules of choice to demonstrate the presence of PS on the external leaflet of the membrane.

23. The Board thus considers that the combination of the teaching of document (3), showing that apoptosis is characterised by the occurrence of PS on the external leaflet of the cellular membrane, and of document (4), identifying annexins as markers of choice for PS, led in an obvious manner to the subject matter of claim 1.

As a consequence, the Board considers that claim 1 does not fulfil the requirements of Article 56 EPC.

24. In view of some of the arguments put forward by appellant I the following further comments are made. A statement (document (18)) was made for the purpose of the opposition proceedings by Dr Fadok, one of the authors of documents (2) and (3). Her field of research appears to be physiology. She does not appear to have been interested or knowledgeable in the field of medical diagnostics, and was not acquainted with document (4). She thus does not meet all the characteristics of the skilled person who has notionally to be considered when assessing inventive step of the present invention. Here such skilled person would be a team including both a physiologist and someone skilled and knowledgeable in the field of medical diagnostics. Nor can her comments as an author
of documents (2) and (3) be considered as adding to or subtracting from the contents of these documents, as the reader of these documents would not be aware of views not expressed in them. That she considers that what is claimed would not have been obvious to her, thus cannot be decisive for the Board in assessing inventive step according to the established case law.

25. Less than one month before the date of oral proceedings Appellant II submitted a bibliographic survey of the frequency of the appearance of certain key words alone or in combination in some 5800 scientific journals, in the years before and after the priority date, and sought to make deductions therefrom as to whether or not it would have been obvious to a skilled person to connect apoptosis and annexins ie the disclosures of documents (3) and (4). Here again, the Board cannot treat the result of the survey as a substitute for assessing inventive step according to the approach used in the established case law. Such a survey does not relate to what is stated in Article 56 EPC or to the problem and solution approach developed in the established jurisprudence of the Boards of Appeal to assess inventive step under Article 56 EPC. Without a thorough and careful analysis of the some 5800 journal editions surveyed, first to eliminate duplications and quite irrelevant citations, and then an assessment of the significance, if any, of the remainder for inventive step, this material cannot be relied on. However, the chances of any useful information emerging seem infinitesimal, and certainly not such as would make the effort worthwhile even if the survey had been presented at a date early enough to allow such detailed investigation.
First and Second auxiliary requests

Article 123(2)(3) EPC

26. In view of the conclusions reached on inventive step (cf infra, points 28 to 30) no decision needs to be made on this aspect.

Article 56 EPC

27. Claim 1 is identical in both auxiliary requests and introduces into claim 1 of the main request a step of distinguishing between apoptotic cells and cells that have undergone lysis.

28. The Board considers that the introduction of this step requires the problem to be solved to be reformulated as being to provide a method of detecting apoptotic cells without destroying the cells, and of distinguishing them from cells that have undergone lysis. However this must be regarded as posing two independent problems to be solved. The solution to each of these is obvious. For the problem of detecting apoptotic cells without destroying cells the same reasoning applies as for the main request. For the problem of distinguishing apoptotic cells from those that have undergone lysis, the skilled person at the priority date of the patent in suit using his/her common general knowledge, which can be illustrated by document (14), which is a review article summarizing the common general knowledge of the skilled person on the distinction between apoptotic cells and cells that have undergone lysis and describes several methods therefor, would have straightforwardly been able to make the distinction.
29. As a consequence, to arrive at the subject matter of claim 1 of both these auxiliary requests was obvious for the skilled person in view of the combination of documents (3) and (4), together with his/her common general knowledge and thus does not meet the requirements of Article 56 EPC.

Auxiliary request submitted during oral proceedings

Article 114 EPC

30. According to the established case law of the Boards of appeal, auxiliary requests submitted at a very late stage of the procedure, for instance during oral proceedings, must clearly be admissible in the sense that they do not give rise to any formal objection under EPC and are a valid response to the objections which still remain to be answered. Following these principles, the concerned Board in decision T 25/91 (2 June 1992) refused to admit an amended set of claims, because, even from a preliminary examination of the facts, it was clear that the amended claims represented a radical departure from the claims previously on file. In decision T 234/92 (12 January 1995) the same conclusion was reached, because a feature present in the description had been introduced in new claim 1 and it was not clear whether this could have justified a request for a complementary search.
31. The present case is very close to the situations described in both decision T 25/91 and 234/92 (cf supra), since the new feature introduced in claim 1, ie "evaluating the efficacy of an anticancer therapy" can be found neither in the claims as granted nor in those maintained by the opposition division nor in any of the other auxiliary requests which had been put to the Board at an earlier stage. The introduction of this feature raises new factual issues, and has taken both the Board and the other parties to the proceedings by surprise. It cannot be concluded that all relevant prior art with respect to this aspect is on file, or that this aspect was searched. Certainly the other parties have not had an opportunity to deal with this aspect.

32. The Board is thus of the view that the conditions for allowing a request in at such a late stage are not here fulfilled, and in the exercise discretion under Article 114(2) EPC, does not allow it into the proceedings.

33. Appellant II offered to amend claim 1 of this auxiliary request by replacing "for" by "of" in order to meet an objection raised. However, this amendment would not change the conclusions reached under Article 114 EPC (cf supra, point 32).
Order

For these reasons it is decided:

1. The decision under appeal is set aside

2. The patent is revoked

3. The request by the other party I to be recognized as intervener and appellant is refused

4. The opposition fee and the appeal fee paid 14 March 2001 by the other party I are to be repaid.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey