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Datasheet for the decision of 17 November 2017

Case Number: T 0151/14 - 3.3.08

Application Number: 02747916.1

Publication Number: 1434874

IPC: C12Q1/68, G01N33/53, G01N33/543, G01N33/551, C07K16/44

Language of the proceedings: EN

Title of invention:
IMMUNOAFFINITY ISOLATION OF MODIFIED PEPTIDES FROM COMPLEX MIXTURES

Applicant:
Cell Signaling Technology, Inc.

Headword:
Antibodies specific post-translationally modified (PTM) peptides/CELL SIGNALING TECHNOLOGY

Relevant legal provisions:
EPC Art. 83, 84, 113(1), 114(2)
RPBA Art. 12(4)

Keyword:
Main request and auxiliary request 1 - admission into the appeal proceedings (yes)
Main request and auxiliary request 1 - clarity and sufficiency of disclosure (no)
Auxiliary requests 2 to 4 - admission into the appeal proceedings (no)

Decisions cited:
G 0010/93

Catchword:

Europäisches Patentamt
European Patent Office
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Beschwerdekammern

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Case Number: T 0151/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 17 November 2017

Appellant: (Applicant)	Cell Signaling Technology, Inc. 166B Cummings Center Beverly, Massachusetts 01915 (US)
Representative:	CMS Cameron McKenna Nabarro Olswang LLP Cannon Place 78 Cannon Street London EC4N 6AF (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 24 July 2013 refusing European patent application No. 02747916.1 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	P. Julià
	D. Rogers

Summary of Facts and Submissions

I. European patent application No. 02 747 916.1 (published as International patent application WO 03/000931; hereinafter "*the patent application*") was refused by an examining division of the European Patent Office. The examining division decided that the main request and the auxiliary request did not fulfil the requirements of Articles 54 and 84 EPC.

II. Claims 1 and 29 of the main request underlying the impugned decision read as follows:

"1. A method for isolating a post-translationally-modified protein fragment from a crude cell lysate, said method comprising the steps of:

(a) providing an unpurified crude cell lysate or a crude cell lysate that has been subjected to a purification to remove non-protein elements;
(b) digesting the crude cell lysate from step a) to produce a complex mixture of a plurality of different post-translationally-modified peptide fragments and containing many different types of modified as well as unmodified peptides;
(c) contacting said complex mixture with at least one immobilised post-translation modification-specific antibody; and
(d) isolating said modified protein fragment specifically bound by said immobilized modification-specific antibody in step (c).

29. A method for isolating a protein fragment comprising at least one phosphorylated amino acid from

a crude cell lysate, said method comprising the steps of:

(a) providing an unpurified crude cell lysate or a crude cell lysate that has been subjected to a purification to remove non-protein elements;
(b) digesting the crude cell lysate of step a) to produce a complex mixture of phosphorylated peptide fragments;
(c) contacting said complex mixture with at least one immobilised motif-specific, context-independent antibody that binds a motif comprising at least one phosphorylated amino acid;
(d) isolating a phosphorylated protein fragment specifically bound by said immobilized antibody in step (c); and
(e) characterizing said phosphorylated protein fragment isolated in step (d) by mass spectrometry (MS), tandem mass spectrometry (MS-MS), and/or MS3 analysis."

III. Claims 1 and 29 of the auxiliary request read as claims 1 and 29 of the main request, except for steps (a) in claims 1 and 29 and step (b) in claim 29 which read as follows:

"(a) providing an unpurified crude cell lysate",

"(b) digesting the crude cell lysate from step a) to produce a complex mixture of phosphorylated peptide fragments from said different proteins;"

IV. The examining division considered that the methods of claim 1 and 29, defined as "*comprising*" steps (a) to (d), did not exclude the possibility of treating or purifying the crude cell lysate provided in step (a) before performing the digestion according to step (b).

Therefore, the protein mixture digested in step (b) could be different from the initial crude cell lysate of step (a). Indeed, such a method was disclosed in document (11), wherein the step of proteolytic digestion was carried out on purified proteins and not on an unpurified/crude cell lysate subjected to a simple purification to remove non-protein elements. Thus, the examining division considered the disclosure of document (11) to anticipate the methods of claims 1 and 29 (Article 54 EPC). The examining division raised also an objection under Article 84 EPC against the subject-matter of claim 9, a claim dependent on claim 1 which referred to a "said proteinaceous preparation" of claim 1, whilst there was no reference to any "proteinaceous preparation" in claim 1.

V. The appellant (appellant) lodged an appeal against the decision of the examining division. With the statement setting out the grounds of appeal, the appellant filed a new main request and new auxiliary requests 1 to 4. As a subsidiary request, the appellant requested oral proceedings.

VI. The appellant was summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), the appellant was informed of the board's provisional, non-binding opinion on substantive matters of the case. In the board's opinion, the main request and auxiliary request 1 could be admitted into the appeal proceedings, but not auxiliary requests 2 to 4. The board was also of the provisional opinion that the main request and auxiliary request 1 contravened Articles 83 and 84 EPC.

VII. Without submitting substantive arguments, the appellant informed the board that it did not intend to attend the scheduled oral proceedings.

VIII. Oral proceedings were held on 17 November 2017 in the absence of the appellant.

IX. Claims 1 and 28 of the main request and the auxiliary request 1 read as claims 1 and 29 of the main request and the auxiliary request before the examining division, respectively, except for the deletion of the words "from said different proteins" in step (b) of claim 28 of auxiliary request 1.

X. Claims 1 and 28 of the auxiliary requests 2 and 3 read as claims 1 and 28 of the main request, except for the the presence of the following half-sentences at the end of step (e) of these claims:

"wherein the complex mixture comprises a digested tissue or fluid" (auxiliary request 2).

"wherein the complex mixture comprises a digested crude cell lysate of more than one cell type" (auxiliary request 3).

XI. Claim 1 of the auxiliary request 4 reads as claim 1 of the main request, except for the presence of an additional step (e) which read as follows:

"(e) characterizing said modified protein fragment isolated in step (d) by), [sic] tandem mass spectrometry (MS-MS), and/or MS3 analysis."

Claim 28 of auxiliary request 4 reads as claim 28 of auxiliary request 1.

XII. The following documents are cited in this decision:

(6): WO 01/27624 (publication date: 19 April 2001);

(7): WO 00/14536 (publication date: 16 March 2000);

(10): Y. Zhao and B.T. Chait, Anal. Chem. 1994, Vol. 66, pages 3723 to 3726;

(11): M.S. Kalo and E.B. Pasquale, Biochemistry 1999, Vol. 38, pages 14396 to 14408.

XIII. Since claim 9 of the requests before the examining division, the sole claim objected under Article 84 EPC (*supra*), had been deleted in the claim requests filed in appeal proceedings, appellant's submissions in the statement of grounds of appeal concerned only Article 54 EPC. No submissions have been filed by the appellant regarding the objections raised under Articles 83 and 84 EPC by the board in its communication pursuant to Article 15(1) RPBA.

XIV. In the statement of grounds of appeal, the appellant requests to set aside the decision under appeal and to grant a patent on the basis of the main request or, in the alterative, any of auxiliary requests 1 to 4, all of them filed with the statement of grounds of appeal.

Reasons for the Decision

Article 113(1) EPC

1. In the decision under appeal, the examining division decided in substance only on Articles 54 and 84 EPC. The objection under Article 84 EPC concerned only previous claim 9. No further objections under Article 84 EPC or under Article 83 EPC are mentioned in the decision under appeal (cf. point IV *supra*).

2. According to decision G 10/93 (OJ EPO 1995, page 172), "*In an appeal from a decision of an examining division in which a European patent application was refused the board of appeal has the power to examine whether the application or the invention to which it relates meets the requirements of the EPC. The same is true for requirements which the examining division did not take into consideration in the examination proceedings or which it regarded as having been met. If there is reason to believe that such a requirement has not been met, the board shall include this ground in the proceedings*".

3. In the communication pursuant to Article 15(1) RPBA attached to the summons to oral proceedings, the board informed the appellant that, for the reasons given, it had serious doubts whether the main request and the auxiliary request 1 fulfilled the requirements of Articles 83 and 84 EPC (cf. point VI *supra*). The board also gave reasons why it was of the provisional opinion that auxiliary requests 2 to 4 could not be admitted

into the appeal proceedings and that, therefore, the appeal was likely to be dismissed.

4. By its decision not to attend the scheduled oral proceedings and not to submit substantive arguments in reply to the board's communication, the appellant has chosen not to avail itself of the opportunity to comment or present its observations on the board's provisional opinion (Article 113(1) EPC).

5. The present decision is based on the same grounds and evidence on which the board's provisional, non-binding opinion - as expressed in points 11 to 31, and 36 of its communication pursuant to Article 15(1) RPBA - was based.

Admission of the main request and the auxiliary request 1

6. The function of an appeal is to give a judicial decision upon the correctness of a separate earlier decision taken by a department of first instance. Appeal proceedings are not an opportunity to re-run the proceedings before the first instance. Article 12(4) RPBA empowers the board not to admit facts, evidence or requests which could have been presented in the first instance proceedings (cf. "Case Law of the Boards of Appeal of the EPO", 8th edition 2016, IV.E.4, page 1127).

7. Except for the deletion of previous claim 9 and the replacement of the terms "*proteinaceous preparation*" and "*protein preparation*" by "*crude cell lysate*" in claim 20 and dependent claims 21 to 23, the new main request and auxiliary request 1 are identical, respectively, to the main request and auxiliary request underlying the decision under appeal. There is also a

deletion of the term "*from said different proteins*" in step (b) of claim 28 of auxiliary request 1 (cf. point IX *supra*).

8. In view of these amendments, the board decides to admit the main request and the auxiliary request 1 into the present appeal proceedings.

Main request and auxiliary request 1

9. The method of claim 1 is directed to the isolation of a post-translationally-modified protein fragment from a crude cell lysate characterized by a step in which peptide fragments, produced by digesting the crude cell lysate and having a specific post-translation modification (PTM), are contacted with at least one immobilised PTM-specific antibody, before isolating these peptide fragments and, optionally, characterizing them.

The method of claim 28 is directed to the isolation of protein fragments comprising at least one (PTM) phosphorylated amino acid from a crude cell lysate characterized by a step in which the digested crude cell lysate is contacted with at least one immobilized motif-specific, context-independent antibody that binds a motif comprising at least one phosphorylated amino acid, before isolating and characterizing said phosphorylated protein fragments.

According to the patent application, motif-specific, context-independent antibodies bind short (typically comprising 1 to 6 invariant amino acids), modified motifs comprising one or more amino acids including at least one modified residue in a manner that is highly independent of the surrounding (flanking) amino acid

sequence, i.e. the differing protein context in which the motif occurs in multiple signaling proteins within a genome. These motif-specific, context-independent antibodies do not substantially recognize peptides containing the unmodified form of the motif. For the production and applications of the motif-specific, context-independent antibodies, the patent application explicitly refers to the disclosure of document (7) (cf. page 19, last paragraph, and page 22, third paragraph of the patent application).

The methods of claims 1 and 28 are exemplified in the patent application by using a predefined mixture of phosphorylated and non-phosphorylated peptides (in the absence or presence of a digested crude cell extract; Examples I and II, respectively) and by isolating peptide fragments with a phosphorylated amino acid from several crude cell extracts (A431 cells, 3T3 mouse fibroblasts, Jurkat cells and COS-1 cells; Examples III to VII). Examples VIII to X are theoretical examples describing how to isolate phosphorylated or acetylated peptides from a crude cell extract (Example VIII and IX), and how to isolate phosphorylated peptides from a crude extract from tumor tissue (Example X). Whilst the digestion of crude cell extracts is carried out with trypsin in Examples III and IV, endoproteinase Glu-C is used in Examples V, VI and VII.

Articles 84 and 83 EPC

10. According to the established case law, a claim must be not only comprehensible from a technical point of view, but it must also define the object of the invention clearly, i.e. indicate all the essential features thereof. All features which are necessary for solving the technical problem with which the application is

concerned have to be regarded as essential (cf. "Case Law", *supra*, II.A.3.2, pages 272).

11. The methods disclosed in the patent application are bottom-up or shotgun proteomic PTM analyses of crude cell extracts, wherein an immobilized, PTM-specific (motif-specific, context-independent) antibody is used for isolating specific PTM peptide fragments from the crude cell lysate (steps (c) and (d) of claims 1 and 28). In the statement of grounds of appeal, the appellant argues that, as a consequence of the use of an antibody with this specificity in steps (c) and (d), the digestion of the crude cell lysate in the preceding step (b) can be carried out on unpurified or semi-purified (removal of non-protein elements) crude cell lysates, thereby generating a complex mixture of modified and unmodified peptides (cf. page 24, lines 15 to 27 of the application; page 3, last two paragraphs of the statement of grounds of appeal).

12. The board observes however that, according to the Examples, the digestion of step (b) is not directly carried out on the unpurified or semi-purified crude cell lysate as such but that, before carrying out said digestion, the cell lysate is further processed by denaturation and clearing of the lysate by centrifugation. This intermediate step between steps (a) and (b) is an essential step of the claimed methods (cf. page 75, lines 8 and 9; page 77, lines 3 and 4; page 81, lines 20 and 21; page 93, lines 5 and 6; page 100, lines 16 and 17; page 106, lines 8 and 9; page 107, line 22; page 109, lines 21 and 22 of the patent application) and its absence from the claims is thus not in line with the case law relating to Article 84 EPC as referred to in point 10 *supra*.

13. The board agrees with the examining division that the wording of claims 1 and 28 does not exclude the presence of further purification steps between steps (a) and (b). According to the preamble of these claims, the claimed methods **comprise** steps (a), (b), (c) and (d), with step (a) merely specifying the removal of non-protein elements. In fact, the possibility of carrying out further "simple purifications" between steps (a) and (b) to remove non-signaling or structural proteins by standard methods is explicitly mentioned in the patent application (cf. page 24, line 27 to page 25, line 2, and the flow-diagram shown in Figure 1).

14. This interpretation is not in contradiction with the appellant's argument that the skilled person reading pages 32 to 34 of the patent application would understand that intermediate purification steps can be carried out after digestion but prior to the immuno-chromatography, i.e. after step (b) and prior to step (c) (cf. page 4, first paragraph of the statement of grounds of appeal). However, if the appellant considers that the wording of claim 1 allows for the presence of intermediate steps between steps (b) and (c), the same must be true for the presence of intermediate steps between steps (a) and (b). The more so when taking into account that step (c) refers to "contacting said complex mixture" and "said complex mixture" is the complex mixture obtained in step (b), whilst the back-reference to the "crude cell lysate" in step (b) does not contain the qualifier "said".

15. In any case, the considerations above show that claims 1 and 28 are open to interpretation and thus, ambiguous and unclear. Hence, the requirements of Article 84 EPC are not fulfilled.

"Digesting the crude cell lysate"

16. The term "*digesting*" in step (b) of claims 1 and 28 is not limited to a proteolytic/enzymatic digestion of a crude cell lysate but comprises also chemical cleavage (cf. page 25, lines 19 and 20 of the patent application, and dependent claim 6). Whilst most of the tryptic peptides have a length of 6 residues or lower (average MW 1.8 - 2.0 kDa), the length of peptides resulting from a chemical cleavage usually is greater, making them more suitable for a middle-down proteomics approach (3.0 - 15 kDa *vs*. 0.5 - 3.0 kDa in a bottom-up approach). Although peptide mixtures of a middle-down approach are less complex and the generated peptides nearly always have a unique sequence, there are important technical difficulties for handling these polypeptides (such as chromatographic separation, increased acquisition times required for high resolution measurements, etc.) and deficiencies arising from the limited availability of bioinformatic methods for these mixtures. It is also well known that chemical cleavage, such as with cyanogen bromide (CNBr), can be very toxic. There is no information in the patent application as regards a chemical cleavage nor a teaching on how to overcome the shortcomings associated with such a cleavage.

17. It is also worth noting that, contrary to the short tryptic peptides, longer peptides may easily adopt secondary and tertiary structures preventing or hindering the recognition of antigenic epitopes by the immobilized PTM-specific (motif-specific, context-independent) antibody used in step (c) of claims 1 and 28. The patent application refers to the method disclosed in document (7) for the production of these antibodies (cf. *inter alia*, page 19, last paragraph;

page 22, third paragraph; page 34, lines 13 to 15; pages 35 and 36, second paragraph). This method relies on highly diverse peptide libraries of about 6 to 14 residues in length (cf. page 36, lines 10 to 12, 17 and 18 of the patent application at issue; page 13, last paragraph, Examples, claim 8 and Sequence Listing of document (7)). The motif-specific, context-independent antibodies resulting from the method disclosed in document (7) may bind/recognize specific short-motifs in a context-independent manner but only as linear epitopes, not necessarily as conformational, non-linear antigenic epitopes (cf. document (10)). In this context, it is also noted that the average length of the peptide libraries referred to in document (7) is comparable to the average length of tryptic fragments but a far cry from the average polypeptide length resulting from standard chemical cleavages.

18. In view of these considerations, the board concludes that the patent application may support and sufficiently disclose the enzymatic (trypsin) digestion of crude cell lysates but it is not sufficient to support a generic digestion (which includes multi-enzymatic digestions, chemical cleavage, etc.) of the crude cell lysates (Articles 83 and 84 EPC).

"Spectra of known peptide sequences"

19. Although, for carrying out the digestion of step (b), the application refers to the use of several proteolytic enzymes and mixtures thereof, such as trypsin, endoproteinases Lys-C, Glu-C, Asp-N, chymotrypsin, and thermolysin (cf. page 25, lines 20 to 24), only trypsin and endoproteinase Glu-C are exemplified. Indeed, tryptic peptides are over-sampled in the available peptide databases, the number of

tryptic peptide data sets representing more than 95% of all the available deposited data sets. The information available for other proteases is very scarce (Glu-C only 0.6% of deposited data).

20. The identification of a parent protein(s) of a PTM peptide fragment obtained by digestion with other proteases, mixtures thereof or by chemical cleavage, is therefore not readily possible (cf. *inter alia*, dependent claims 4, 24 and 29). The less so in view of the fact that there is no limitation in claim 1 of the type and number of PTMs ("*at least one immobilised PTM-specific Ab*"), nor of any intermediate purification between steps (a) and (b) and/or steps (b) and (c) of claims 1 and 28. Protein inference caused by the presence of multiple proteins and/or isoforms of similar sequences in the crude cell lysates (the so-called protein parsimony problem) is also not to be underestimated, particularly in lysates with highly abundant protein species (high proteome diversity/ width). In Examples V, VI and VII of the patent application, endoproteinase Glu-C is used only for a specific type of PTM (phosphorylation) and substrate proteins (phospho-(Ser) PKC substrates, Example V; phospho-(Ser/Thr) Akt substrates, Example VI; phospho-(Ser) 14-3-3 binding substrate motif, Example VII).

"PTM specific antibodies"

21. Step (c) of the method of claim 1 is based on the use of an immobilized PTM-specific antibody which, according to the application, may be produced by the method disclosed in document (7). Although reference is made in document (7) to a large number of PTMs, such as methylation, ubiquitination and glycosylation (cf. page 14, second paragraph; page 23, last paragraph of

document (7)), only two types of PTMs are exemplified (phosphorylation, Examples I-V; acetylation, Example V). These two PTMs are also the ones exemplified in the application (phosphorylation, Examples I-VIII; acetylation, theoretical Example IX).

22. Likewise, most of the prior art on file is concerned with phosphorylation as PTM (cf. *inter alia*, document (11)). Document (6) refers to a large number of PTMs (cf. page 5, last paragraph; page 22, last paragraph; pages 23 to 28; claims 12 to 18 and 32) and PTM-specific antibodies (cf. *inter alia*, page 30, lines 31 and 32; page 31, lines 21 and 22), including phospho(Tyr)-, phospho(Ser)- and phospho(Thr/Pro)-specific antibodies (cf. page 23, lines 8 to 10), acetylated-lysine and methylation specific antibodies (cf. page 24, lines 19 and 29-30, respectively), ADP-ribosylation specific antibodies (cf. page 25, lines 18 and 19), (poly)ubiquitin-specific antibodies (cf. page 26, lines 8 to 10) and antibodies against a specific carbohydrate group (cf. page 28, lines 3 to 5). However, most of the examples concern only phosphorylation (cf. pages 45 to 56, Table 1).

23. Thus, the board concludes that the skilled person is not in a position to readily and without undue burden obtain antibodies specific for many of the possible post-translational modifications (cf. points 26 and 27 *infra*).

"Motif-specific context-independent antibodies"

24. None of the antibodies described in the prior art on file, and in particular in document (6), is defined as motif-specific and context-independent. While not a

feature of claim 1, it is however a limiting feature in step (c) of claim 28.

25. According to the patent application, the definition of a "modification-specific antibody" includes "motif-specific, context-independent antibodies" (cf. page 21, line 27 to page 22, line 2; in particular, page 22, lines 1 and 2), the definition of the latter type of antibodies being narrower than the former type of antibodies (cf. page 22, lines 8 to 20). It is thus questionable whether the advantages allegedly associated with the motif-specific, context-independent antibodies may also be present and/or achieved using modification-specific antibodies in general (cf. *inter alia*, page 34, lines 16 to 22 of the application).

26. The board further observes that the method of document (7) for producing motif-specific, context-independent antibodies is suitable only for a very specific type of PTMs, namely those modifications which provide a relatively small change in a specific amino acid residue, such as the exemplified phosphorylation and acetylation. However, it is questionable whether other PTMs in which more bulky groups are involved, such as ubiquitination (cf. page 26, lines 10 to 13 of document (6); ubiquitin has 76 amino acid residues, MW 7 kDa), glycosylation with carbohydrates of medium/large size, etc. are suitable for incorporation in a "highly diverse peptide library" as defined in document (7). Let alone that motif-specific, context-independent antibodies may be thereby achieved in a straightforward manner.

27. Since the advantages of motif-specific, context-independent antibodies are not associated with PTM-specific antibodies in general and, due to the fact

that motif-specific, context-independent antibodies may not be produced for all PTMs referred to in the patent application, the patent application does not disclose the methods of claims 1 to 11 and 17 to 27 in a manner sufficiently clear and complete for them to be carried out by a skilled person across the whole breadth of the claims (Article 83 EPC).

28. Moreover, step (c) of claims 1 and 28 encompasses the use of more than one PTM-specific (motif-specific, context-independent) antibody ("at least one"), including the use of antibodies having different specificity (phospho(Ser)-, phospho(Thr)- or phospho(Thr/Pro)-specific) for the same PTM type (phosphorylation) as well as antibodies specific for different PTM types, such as phosphorylation, acetylation, etc. (wherein at least one of them must be phosphorylation in claim 28). None of these embodiments is exemplified in the patent application and there is no information how to carry out any of them. As a consequence, the disclosure of the patent application is insufficient to support all embodiments falling within the methods of claims 1 and 28, such as (simultaneous/serial/parallel) immuno-chromatography with several PTM-specific antibodies, with or without intermediate purification steps, with (multi-)protease digestion or chemical cleavage, etc. and further characterization of the modified protein fragments (dependent claim 2) and use of a search program for identifying their parent protein(s) (dependent claim 4) (Article 83 EPC).

29. Example X of the patent application refers to the use of several types of antibody-resins in series, such as a resin with a general phospho(Tyr)-specific antibody followed by an Akt substrate motif-specific antibody on

a second support (cf. page 109, line 25 to page 110, line 7). According to this Example, the claimed method may be used to characterize the phosphorylation states of specific proteins involved in cellular signaling pathways of target (tumor) cells. Example X is the sole example in the patent application which, as a starting material, refers to crude cell lysates derived from tumor tissues or biopsy samples. However, this example is only theoretical and does not provide specific technical details nor does it disclose any result. Indeed, the subject-matter of dependent claims 5 (which apart from a digested crude cell extract in general refers to a digested tissue sample, a digested serum sample, a digested urine sample, a digested synovial fluid sample, and a digested spinal fluid sample), 18 (which requires that at least one of the said modified protein fragments isolated in step (d) of claim 1 corresponds to a known marker of disease) and 21 to 27 (which refer to comparisons of normal/untreated and disease/treated samples), is not exemplified in the patent application.

30. In view of the nature and character of some PTMs, such as their sub-stoichiometric abundance, transience and reversibility (variability depending on type/stage of target cells and difficulty to find suitable controls), the high proteomic complexity (width) and dynamic range of protein concentrations of some cell lysates (human plasma, serum, blood cells), the extensive amount of data acquired from peptide analysis and the need to define appropriate criteria for selecting from this data a subset of possible peptides associated with or resulting from a specific disease (cancer) and/or treatment thereof (biomarker candidates), the disclosure of the patent application is insufficient, i.e. does not put the average skilled person in a

position to perform the methods of claims 21 to 27 in a straightforward manner and without undue burden (Article 83 EPC).

31. In view of all the above considerations, the board concludes that neither the main nor the auxiliary request 1 fulfil the requirements of Articles 83 and 84 EPC.

Admission of auxiliary requests 2 to 4

32. Auxiliary requests 2 to 4 are new in the proceedings. In the statement of grounds of appeal, the appellant did not give any reasons why these auxiliary requests could not have been filed in the course of the examination proceedings (Article 12(4) RPBA). Nor did it provide reasons in reply to the explicit indication in the board's communication that it was not minded to admit them.

33. The amendments introduced into these requests intend to delimit the claimed methods from the method disclosed in document (11). However, none of these requests overcomes all the objections raised under Articles 83 and 84 EPC above. Moreover, the amendments introduced into these auxiliary requests raise new issues under Articles 123(2) and 84 EPC.

The use of different terms in claim 1 of auxiliary request 2 leads to a lack of clarity. While step (a) refers to the provision of an unpurified crude cell lysate or a crude cell lysate, the feature newly added to step (d) of claim 1 of auxiliary request 2 requires that the complex mixture obtained in step (d) comprise a digested tissue or fluid. Likewise, there is inconsistency between the use of the term "crude cell

lysate" in step (a) and the term "crude cell lysate of more than one cell type" referred to in step (d) of claim 1 of auxiliary request 3 (cf. point X *supra*).

34. In view of these considerations, the board, in the exercise of its discretion, decides not to admit the auxiliary requests 2 to 4 into the appeal proceedings (Article 114(2) EPC and Article 12(4) RPBA).

Order

For these reasons it is decided that:

The appeal is dismissed

The Registrar: The Chairman:

A. Wolinski B. Stolz

Decision electronically authenticated