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Datasheet for the decision of 20 December 2019

Case Number: T 0401/14 - 3.3.01
Application Number: 01986941.1
Publication Number: 1346217
IPPC: G01N33/50, C12N5/06
Language of the proceedings: EN

Title of invention:
REVERSIBLE MHC MULTIMER STAINING FOR FUNCTIONAL PURIFICATION OF ANTIGEN-SPECIFIC T CELLS

Applicant:
IBA GmbH

Headword:
Reversible cell staining/IBA GmbH

Relevant legal provisions:
RPBA Art. 13(1), 13(3)
EPC Art. 123(2), 84, 83, 111(1)
Keyword:
Late-filed request - admitted (yes)
Amendments - allowable (yes)
Claims - clarity (yes)
Sufficiency of disclosure - (yes)
Appeal decision - remittal to the department of first instance (yes)

Decisions cited:

Catchword:
Case Number: T 0401/14 - 3.3.01

DECISION
of Technical Board of Appeal 3.3.01
of 20 December 2019

Appellant: IBA GmbH
(Applicant)
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Decision under appeal: Decision of the Examining Division of the European Patent Office posted on 16 September 2013 refusing European patent application No. 01986941.1 pursuant to Article 97(2) EPC

Composition of the Board:
Chairman A. Lindner
Members: T. Sommerfeld
P. de Heij
Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division, in which European patent application 01986941.1, based on an international application published as WO 02/054065, was refused under Article 97(2) EPC.

II. The decision of the examining division is based on the sets of claims of the then main request and auxiliary requests 1 and 2, all filed by letter of 5 June 2013.

The examining division decided that claim 1 of all requests on file contravened Article 84 EPC and that claim 1 of auxiliary request 2 also contravened Article 83 EPC. Further, claim 14 of the main request and auxiliary request 1, and claim 13 of auxiliary request 2, were found to contravene Article 123(2) EPC. In addition, the examining division decided not to admit six late-filed documents into the proceedings.

III. The applicant (hereinafter "the appellant") lodged an appeal against the decision of the examining division, requesting that the decision be set aside and that a patent be granted according to a main claim request or, alternatively, one of auxiliary requests 1 to 3, all filed with the statement of grounds of appeal. It further requested that the six documents not admitted by the examining division be admitted into the appeal proceedings, and that the appeal not be remitted to the department of first instance for further prosecution.

IV. In a communication pursuant to Rule 100(2) EPC and Article 17(1) RPBA 2007, dated 14 December 2018, the board gave its preliminary opinion as to Articles 123(2), 84 and 83 EPC, as well as the admission of
documents. With its reply dated 24 April 2019, the
appellant submitted a new claim request to replace the
previous requests on file.

V. The board issued a summons to oral proceedings
accompanied by a further communication pursuant to
Article 15(1) RPBA 2007. By letter dated
31 October 2019, the appellant submitted a new main
request and requested that the appealed decision be set
aside and the case be remitted to the examining
division for further prosecution on the basis of the
claims of the main request. Oral proceedings were
requested if the main request was not allowable.

VI. The board then cancelled the oral proceedings.

VII. The main and sole request comprises 18 claims, of which
independent claims 1, 15 and 17 read as follows:

"1. A method for reversible staining of cells
comprising the steps:
(a) providing a sample comprising a cell having a
receptor molecule on the surface of the cell,
(b) contacting said cell with
   (i) at least one receptor ligand capable of binding
to said receptor molecule wherein said at least one
receptor ligand is conjugated to at least one first
partner of a binding pair consisting of at least
one first partner and at least one second partner,
wherein the receptor ligand and the receptor
molecule exhibit a low-affinity interaction; the
dissociation constant Kd of which is in the range
of 10^{-2} to 10^{-7} M;
(ii) at least one second partner of said binding
pair that has at least two binding sites for said
first partner; and
(iii) a detectable label bound to or capable of binding to the at least one receptor ligand (i) and/or the at least one second partner (ii); wherein the first partner and the second partner of the binding pair are capable of forming a reversible bond, wherein the reversible bond has a $K_d$ between $10^{-5}$ and $10^{-13}$ M, and wherein an aggregate comprising at least two receptor ligands (i), at least one second partner (ii) and at least one detectable label (iii) is bound via the receptor molecule to said cell, thereby reversibly staining said cell,
(c) optionally separating said stained cell from other components of said sample and
(d) optionally removing said staining from said cell by disrupting the reversible bond between the first partner and the second partner."

"15. Use of the method of any one of claims 1-14 for the isolation of a specific cell population."

"17. Reagent kit for reversible staining of a cell having a receptor molecule on the surface of the cell comprising
(i) at least one receptor ligand capable of binding to a receptor molecule on a cell wherein said at least one receptor ligand is conjugated to at least one first partner of a binding pair consisting of at least one first partner and at least one second partner, wherein the receptor ligand and the receptor molecule exhibit a low-affinity interaction, the dissociation constant $K_d$ of which is in the range of $10^{-2}$ to $10^{-7}$ M,
(ii) at least one second partner of said binding pair, wherein said second partner has at least two binding sites for said first partner, wherein the first partner and the second partner are capable of forming a
reversible bond, the dissociation constant $K_d$ of which is in the range of $10^{-5}$ and $10^{-13}$ M and (iii) a detectable label bound or capable of binding to the at least one receptor ligand (i) and/or the at least one second partner (ii)."

VIII. The appellant's arguments, in so far as they are relevant to the present decision, may be summarised as follows:

Claim 1 was not to be interpreted as a method for isolating cells. Rather, it was directed to a method for reversible staining of cells, using reversibly multimerised "low-affinity" receptor ligands, that could be practised without cell isolation. The staining of the cells was reversible because, as recited in claim 1, the bond between the first partner and the second partner was reversible with a $K_d$ between $10^{-5}$ and $10^{-13}$ M, the staining being removed by disrupting this particular reversible bond. Reversible complexes were formed by making use of this reversible bond between the first partner and the second partner. Disruption of these reversible complexes was carried out in the claimed method only after the staining and, for example, after the subsequent separation of the stained cells from unstained cells as defined in the optional step (c). Also, the experimental section of the application showed that the staining of the invention could be carried out with or without removal of the staining (e.g. Example "Development of a reversible MHC multimer staining" starting on page 27, line 25, and Figures 5 and 6 of the application as filed). Accordingly, there was clear and unambiguous support in the application as filed for the optional nature of removing the staining by disrupting the
reversible bond between the first partner and the second partner.

IX. The appellant requested in writing that the appealed decision be set aside and the case be remitted to the examining division for further prosecution on the basis of the claims of the main request filed with its letter of 31 October 2019.

Reasons for the Decision

1. The appeal is admissible.

2. Main request

The main request was filed after the oral proceedings had been arranged. The board considers the new request a legitimate attempt to address the appealed decision and the objections raised by the board in its communications. Moreover, the amendments do not raise any new issues and advance procedural economy. Therefore, the board decided to admit the new request into the proceedings, pursuant to Article 13(1) and (3) RPBA 2007.

2.1 Article 123(2) EPC

2.1.1 The only objection raised by the examining division under Article 123(2) EPC concerned the subject-matter of claim 14 of the then main request and auxiliary request 1, and claim 13 of the then auxiliary request 2. Such subject-matter is not claimed in the present main request; hence the objection no longer applies.
2.1.2 Moreover, the board comes to the conclusion that the claims of the main request have a basis in the application as filed.

2.1.3 Claim 1 of the main request differs from claim 1 as originally filed in that the following amendments have been made (insertions being indicated by underlining and deletions by strikethrough):

"1. A method for reversible staining of cells comprising the steps:
(a) providing a sample comprising a cell having a receptor molecule on the surface of the cell,
(b) contacting said cell with
   (i) at least one component specifically receptor ligand capable of binding to said receptor molecule wherein said at least one component receptor ligand is conjugated to at least one first partner of a binding complex pair consisting of at least one first partner and at least one second partner, wherein the component receptor ligand and the receptor molecule exhibit a low-affinity interaction; the dissociation constant \( K_d \) of which is in the range of \( 10^{-2} \) to \( 10^{-7} \) M;
   (ii) at least one further second partner of said binding complex pair having that has at least two binding sites for said first partner and
   (iii) a detectable label bound to or capable of binding to the at least one receptor ligand (i) and/or the at least one second partner (ii), wherein the first partner(s) and the further second partner(s) of the binding complex pair are capable of forming a reversible bond, wherein the reversible bond has a \( K_d \) between \( 10^{-5} \) and \( 10^{-13} \) M, and wherein an aggregate comprising at least two components receptor ligands (i), at least one component second partner (ii)
and at least one component detectable label (iii) is bound via the receptor molecule to said cell, wherein said cell is stained thereby reversibly staining said cell, (c) optionally separating said stained cell from other components of said sample and (d) optionally removing said staining from said cell by disrupting the reversible bond."

2.1.4 The amendments have a basis in the following passages of the application as filed:
- "on the surface of the cell", page 6, lines 4 to 6, and page 7, lines 1 to 8;
- "receptor ligand capable of binding to said receptor molecule", page 6, lines 4 to 6;
- "binding pair consisting of at least one first partner and at least one second partner", page 7, lines 26 to 28;
- "the dissociation constant of which is in the range of 10⁻² to 10⁻⁷ M", page 6, lines 4 to 8;
- "at least one second partner of said binding pair that has at least two binding sites for said first partner", page 7, lines 26 to 32;
- "wherein the reversible bond has a K_d between 10⁻⁵ and 10⁻¹³ M", page 8, lines 1 to 4.

2.1.5 As to the further claims, the basis in the application as filed is as follows:
- claim 2: claim 2 as originally filed, rendered dependent on claim 1;
- claims 3 to 10: claims 3 to 10 as originally filed, with amendments to claims 7, 9 and 10 to bring them into line with claim 1;
- claim 11: paragraph bridging pages 13 and 14, and page 13, lines 2 to 5;
- claim 12: original claim 11;
- claim 13: page 11, lines 1 to 8;
- claim 14: original claim 12, amended in line with claim 1; the feature "removal of the staining" of original claim 12 was replaced by "disruption of the reversible bond between the first and second partner", an amendment which finds its basis in original claim 1, for example, which states that the staining is removed by disrupting the reversible bond;
- claim 15: original claim 13, with the deletion of the redundant feature;
- claim 16: original claim 14;
- claim 17: original claim 19, amended in line with claim 1;
- claim 18: original claim 23, amended in line with claim 1.

2.1.6 Hence, the board considers that all the claims of the main request have a basis in the application as filed. The requirements of Article 123(2) EPC are thus fulfilled by the main request.

2.2 Article 84 EPC

2.2.1 In the appealed decision, the examining division considered that claim 1 lacked essential features and was therefore unclear because the destaining step (d) was optional. According to the examining division, this step was mandatory in view of claim 1 being directed to a method for reversible staining, this feature being "a functional feature defining the mandatory method step of removing a stain (see Guidelines F-IV.4.13, para. 3)" (decision, section 3.1.2, page 3). That this was an essential feature was supported by the following passages of the application: page 7, lines 30 to 33; page 8, lines 24 to 26; page 9, lines 17 to 20; page
11, lines 6 to 11; page 13, lines 29 to 31; page 20, lines 6 to 8; and by Annex 1 ("diagram illustrating the method of the invention") submitted during the oral proceedings. Moreover, the examining division considered that reversible staining of the target cell being carried out at low temperatures (page 8, second paragraph of the description) was also an essential feature. Its absence in the claim led to a further lack of clarity and support.

2.2.2 The board disagrees with these conclusions of the examining division and instead is convinced by the appellant's arguments that neither the step of stain removal nor the performance of the method at low temperatures is an essential feature of the claimed method, for the reasons explained below.

2.2.3 The claims are directed to methods for reversible staining of cells. Such methods thus have the aim of staining the cells in such a way that the staining can be removed afterwards; the removal of the stain, however, is not necessarily a step of the staining method, as is apparent from the application read as a whole.

2.2.4 None of the passages cited by the examining division teaches that the stain removal step is an essential feature of the method; they teach only that staining has to be reversible, a feature which is already part of the claim (e.g. in the preamble), as a limiting feature.

Page 7, lines 30 to 32, reads: "The bond between the first and the second partner should be reversible, i.e. the bond should be capable of being disrupted under conditions suitable for carrying out the claimed
method". The next sentence then teaches: "Preferably the reversible bond has a $K_d$ between $10^{-2}$ and $10^{-13}$ M, more preferably between $10^{-3}$ and $10^{-10}$ M and most preferably between $10^{-5}$ and $10^{-8}$ M as determined under appropriate conditions".

The passage on page 8, lines 24 to 26, reads: "The removal of the staining preferably occurs by targeted disruption of the reversible bond between the first and the second partner of the binding complex". A similar statement is repeated on page 9, lines 17 to 25, explaining that removal of the staining (or of any reagent bound to the target cell) is achieved by disruption of the reversible bond: "The removal of the staining from the target cell by disruption of the reversible bond between the first and the second partner results in a loss of the cooperative bond between at least two low-affinity ligands and receptor molecules on the target cell. (...) This results in a complete removal of any reagent bound to the target cell, because the bond between the receptor-binding component and the receptor on the target cell is a low-affinity interaction".

The passage on page 11, lines 6 to 9, teaches how the binding complexes can be disrupted in particular embodiments making use of the calmodulin system. The passage on page 13, lines 29 to 31, which also refers to particular embodiments making use of the streptavidin-biotin system, reads: "As one key element of the present invention is the fact that the reversible bond can be disrupted in a targeted manner ...", thus making it clear that the stain can be removed but does not necessarily have to be.
Finally, the passage on page 20, lines 6 to 8, which is the legend to Figure 2, reads: "By targeted disruption of peptide/MHC multimer binding to the TCR molecules the staining is removed resulting in a purified T cell population having substantially unaltered characteristics (invention)". This passage describes the advantage of the invention's method of reversible staining: it makes it possible to purify cells with unaltered characteristics, since the staining can be removed. The same is also shown in Annex 1 submitted during the oral proceedings before the examining division. The present claims, however, are not directed to a method for the purification of cells, in which case the step of stain removal might have been essential, but instead to a method for reversible staining only, i.e. a method of cell staining wherein the staining can be removed afterwards.

2.2.5 Since the aim of the claimed method is to achieve reversible staining of the cells, the essential features of the method are simply those that are required to achieve that aim, i.e those that allow the staining to be reversible. Said features, which have to do with the use of complexes of two partners linked by a "low-affinity", reversible bond, as apparent from the passages of the application cited above, are part of the claim.

2.2.6 Finally, the passage on page 8, second paragraph, of the application teaches: "It is a further important feature of the present invention that the staining of the target cell, (....), and the subsequent optional steps, namely the isolation and purification of the stained target cell, (....), and the removal of the staining may be carried out at low temperatures, i.e. at temperatures where substantially no activation and/
or signaling events occur, which might result in an alteration of the target cell(...)" (emphasis added by the board). This passage clearly teaches that the "isolation and purification of the stained target cell" as well as the "removal of the staining" are "subsequent optional steps" and thus not essential features of the claimed method. Moreover, it does not state that the method has to be carried out at low temperatures, but that it should be possible to carry out the method at low temperatures. So, again, this feature is not an essential feature of the claimed method.

2.3 Article 83 EPC

2.3.1 As regards Article 83 EPC, the examining division raised objections to claim 1 of the then pending auxiliary request 2. This claim included features which are no longer part of the claimed subject-matter, and the objection of the examining division was directed to this particular embodiment. Hence, the objection no longer applies.

2.3.2 The requirements of Article 83 EPC are considered fulfilled.

3. Remittal for further prosecution

3.1 Under Article 111(1) EPC, the board, following the examination as to the allowability of the appeal, shall decide on the appeal and may either exercise any power within the competence of the department which was responsible for the appealed decision or remit the case to that department for further prosecution.
3.2 The appealed decision was based solely on Articles 123(2), 84 and 83 EPC. Hence, essential questions regarding the patentability of the claimed subject-matter have not yet been examined or decided upon by the examining division. In its communication of 14 December 2018, the board expressed the intention to remit the case should the appellant's appeal prove successful. Relying on this expectation, the appellant has requested that the board remit the case and has not provided any reasoning regarding the novelty and inventive step of the subject-matter of the main request. The board considers these circumstances to be reasons to remit the case to the examining division for further examination of the remaining EPC requirements.
Order

For these reasons it is decided that:

1. The decision is set aside.

2. The case is remitted to the examining division for further prosecution on the basis of the claims of the main request filed with the letter of 31 October 2019.

The Registrar: 

The Chairman:

M. Schalow

A. Lindner

Decision electronically authenticated