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Datasheet for the decision of 6 December 2012

Case Number: T 1958/09 - 3.3.08

Application Number: 96920386.8

Publication Number: 832291

IPC: C12Q 1/68

Language of the proceedings: EN

Title of invention:
Enzyme linked oligonucleotide assays (Elonas)

Applicant:
GILEAD SCIENCES, INC.

Headword:
Elonas/GILEAD

Relevant legal provisions:
EPC Art. 56

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

Decisions cited:
-

Catchword:
-

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Case Number: T 1958/09 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 6 December 2012

Applicant: GILEAD SCIENCES, INC.
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Decision under appeal: **Decision of the Examining Division of the European Patent Office of 14 May 2009 refusing European patent application No. 96920386.8 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: M. Wieser
Members: T. J. H. Mennessier
J. Geschwind

Summary of Facts and Submissions

I. The applicant (appellant) lodged an appeal against the decision of the Examining Division dated 14 May 2009, whereby the European patent application No. 96 920 386.8 with publication number 0 832 291 was refused. The application, entitled "*Enzyme linked oligonucleotide assays (ELONAS)*", originated from an international application published as WO 96/40991.

II. The decision was based on the main request and auxiliary requests 1 to 3 all filed with the letter of 6 February 2009. All four requests were refused for reasons of non-compliance with the requirements of Article 56 EPC.

III. Under cover of a letter of 23 September 2009, the appellant filed a statement setting out the grounds of appeal.

IV. On 1 August 2012, the Board issued a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), sent together with the summons to oral proceedings, in which it expressed its provisional, non-binding views.

V. On 6 November 2012, in reply to the Board's communication, the appellant filed additional submissions which were accompanied by a new main request and three new auxiliary requests to replace the previous requests.

VI. At the oral proceedings which took place on 6 December 2012, the appellant withdraw the requests filed on 6 November 2012 and submitted a new main request.

VII. The new main request consisted of 10 claims, claim 1 of which read:

"1. A method for detecting the presence of a target molecule in a substance which may contain said target molecule, wherein the target is a protein but is not a nucleic acid binding protein, the method comprising:

(a) exposing a substance which may contain said target molecule to capture molecules capable of binding to said target molecule and forming capture molecule:target molecule complexes, wherein the capture molecules are bound to a solid carrier;

(b) removing the remainder of said substance from the capture molecule:target molecule complexes;

(c) adding to said capture molecule:target molecule complexes detector molecules capable of binding to said target molecule; and

(d) detecting the capture molecule:target molecule:detector molecule complexes;

wherein said capture molecule is a nucleic acid ligand which binds specifically to said target molecule and which is identified by a SELEX method comprising

i) contacting the candidate mixture with said target molecule, wherein nucleic acids having an increased affinity to said target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

ii) partitioning the increase affinity nucleic acids from the remainder of the candidate mixture;

iii) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids; and

iv) identifying said nucleic acid ligand."

Claims 2 to 10 were directed to preferred embodiments of the method of claim 1.

VIII. The following documents are referred to in the present decision:

(D1) F. T. Vertosick and R. H. Kelly, Journal of Immunological Methods, Vol. 102, 1987, pages 15 to 21

(D2) A. Ait Kaci and J.C. Monier, Pathologie-Biologie, Vol. 30, No. 2, February 1982, pages 73 to 78

(D8) D. Jellinek et al., Biochemistry, Vol. 33, 1994, pages 10450 to 10456

(D11) US-A-5,270,163 (granted on 14 December 1993)

(D12) WO 91/19813 (published on 26 December 1991)

(D13) WO 94/04548 (published on 3 March 1994)

(D16) "Molecular Analysis and Genome Discovery", Edited by Ralph Rapley and Stuart Harbron, Wiley, 2004, pages 231 to 232

Declaration of N. Janjic dated 5 November 2012

IX. The submissions made by the appellant, in so far as they are relevant to the present decision, may be summarised as follows:

Document D13, which was considered to represent the closest prior art, described a method for determining a target in a sample using, as a capture molecule, a nucleic acid ligand having attached thereto a label generating a detectable signal. The capture molecule:target molecule complex was directly detected.

In view of document D13, the technical problem was defined as the provision of an alternative method to detect the presence of a target molecule in a substance.

Document D13 did not contain any information concerning the use of nucleic acid ligands in so-called "indirect detection" methods. No consideration was given to the use of nucleic acid ligands bound to a solid carrier in a sandwich assay having separate capture and detector molecules that both bind the target.

Documents D11 and D12 expressed vague hope only concerning the possible use of nucleic acid ligands in conventional antibody-based diagnostics.

On the contrary, the skilled person was aware, that the bringing together of a plurality of nucleic acid ligands at the surface of a solid carrier would create a cloud of negative charges which would be the cause for non-specific binding of the target. He/she would therefore have anticipated that these non-specific binding interactions had made nucleic acid ligands, when used as

capture molecules in a molecular sandwich-type detection assay, ineffective for the detection of protein targets.

The performance of native double-stranded DNA, or denatured single-stranded DNA, as described in documents D1 and D2, did not have any predictive value for the performance to be expected by nucleic acid ligands. Document D16 supported the problem identified in document D1 that non-specific binding was likely to be a real problem.

Proximity to non-aqueous, generally hydrophobic surfaces such as polystyrene had to be expected to have a denaturing effect on nucleic acid ligands. Moreover, single-stranded nucleic acids were known to be susceptible to aggregation at high concentrations. Therefore, a major concern with the use of nucleic acid ligands bound to a solid support in sandwich-type formats was the potential loss of their conformational integrity, which would prevent high affinity binding.

Furthermore, at the filing date a large number of nucleic acid ligands had been showed to exhibit biphasic binding, which was attributed to the ligand being able to adopt two conformations, one where high affinity and specificity target binding were possible and one where this was not the case. This was illustrated in document D8.

In view of the fact that everything that might change the conformation of the ligand, such as possible denaturing effects of the solid carrier or aggregation of ligands on the solid carrier, inevitably had a negative effect on the usefulness of the ligand for an

analytical detection method, the skilled person would have had no reasonable expectation of success that a method as the one according to present claim 1 could solve the underlying technical problem.

X. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 to 10 of the main request filed at the oral proceedings.

Reasons for the Decision

Admissibility of the main request

1. The main request was filed at the oral proceedings held on 6 December 2012. The claims of this request differ from the claims of auxiliary request 2 considered by the Examining Division in the decision under appeal only in so far as they respond to some objections under Article 84 EPC, raised for the first time by the Board in its communication dated 1 August 2012. The Board, therefore, exercising its discretion according to Article 13(1) (RPBA), decides to admit this request into the procedure.

Article 123(2) EPC

2. Auxiliary request 2 before the Examining Division had been found to meet the requirements of Article 123(2) EPC (see point (C)(1) on page 10 of the decision under appeal). The Board has no reason to depart from this finding. The claims of the present new main request

differ from the claims of this former auxiliary request 2 only in three points.

3. Firstly, the term "or a polynucleotide" in line three of claim 1 has been deleted. Secondly, claim 1 has been amended by specifying that the nucleic acid ligand is identified by a SELEX method, (as disclosed in examples 1 to 3 of the application as published), and by introducing the technical features (i) to (iv) from previous claim 12, which was dependent on any one of previous claims 1 to 11, which characterise such a method. Thirdly, previous claims 9 and 10 have been deleted and previous claims 11 and 12 have been renumbered as claims 9 and 10.

4. These amendments do not extend beyond the content of the application as filed. The new main request complies with the requirements of Article 123(2) EPC.

Articles 84 and 54 EPC

5. The Board is satisfied that, owing to the amendments carried out (see point (3) above), the main request complies with the requirements of Article 84 EPC.

6. The method according to claim 1 is not disclosed in any of the prior art documents on file and is therefore novel within the meaning of Article 54 EPC (see also point (C)(2) on page 10 of the decision under appeal).

Article 56 EPC

7. Document D13 represents the closest state of the art. It describes a method for determining the presence or

the amount of a target in a sample using a nucleic acid ligand, also referred to as an aptamer, as a capture molecule, having attached a label, which ligand is capable of binding said target under specific conditions. Thereby a complex is formed between said aptamer and said target (see pages 8 to 9 and claim 14). Coupling the aptamer to a solid support is mentioned on page 13, lines 16 to 18, as an option. The only assay formats referred to in the document rely on the formation of an 'oligonucleotide:target' complex (see the sentence bridging pages 13 and 14), including direct and competitive formats (page 14, lines 2 to 5).

8. In the light of the disclosure in document D13, the technical problem underlying the present application is the provision of an alternative method for detecting a target molecule using a nucleic acid ligand. As a solution to this problem the application proposes the method according to claim 1 which features an assay based on a sandwich format involving the formation of 'capture molecule:target molecule:detector molecule' complexes, wherein the capture molecules are nucleic acid ligands bound to a solid carrier, which ligands are prepared according to a procedure known as the SELEX procedure. Considering the disclosure in the experimental part of the description, which reports three sandwich-type-linked oligonucleotide assays (VEGF-, hCG- and hTSH-ELONAs), the Board is convinced that the technical problem has credibly been solved by the claimed method.

9. It remains to be answered whether, starting from the method of document D13 and in view of the prior art

documents on file, the skilled person would have arrived at the claimed solution in an obvious way.

10. The skilled person knew from document D16 that aptamers, being oligonucleotides, i.e. small molecules (see for example the H-42 RNA ligand as referred to on page 18, lines 12 to 15 in the application), when used in proximity to non-aqueous, generally hydrophobic surfaces such as polystyrene (i.e. when being immobilised to such surface), were at risk of being denatured and, thereby, of loosing their conformation integrity. Moreover, the cloud of negative charges created by a plurality of nucleic acid ligands brought together at the surface of the solid carrier was also expected to result in non-specific binding between the ligand and a potential target molecule. Furthermore, as credibly argued in the declaration of N. Janjic (see point 6 thereof), the skilled person would not have ignored that aptamers were susceptible to aggregate at high concentrations when fixed at the surface of the solid support because of their propensity to form intermolecular base pairs which could compromise the binding to a target molecules.

11. Documents D11 and D12, members of the same patent family as document D13, which disclosed the SELEX method (acronym for **S**ystematic **E**volution of **L**igands by **Ex**ponential Enrichment), only expressed hope that nucleic acid ligands could be useful in conventional antibody-based diagnostics (D11, column 8, lines 63 to 66; D12, page 16, lines 2 to 13). This vague information would not have dispelled the skilled person's doubts based on the credible arguments listed in point (10) above.

12. Documents D1 and D2 report of the use of surface-immobilised native or denaturated DNAs for capturing DNA-binding antibodies. Contrary to aptamers, such DNAs are large molecules, which when fixed to a solid support in their native form maintain their conformational integrity. If they are denaturated their binding to proteins such as antibodies does not require that any distinct conformational integrity be maintained (see point 5 of the declaration of N. Janjic).

13. Finally, the disclosure in document D8 (see page 10451, hand-right column), saying that aptamers may exhibit biphasic binding, which is consistent with the simultaneous existence of correctly folded and incorrectly folded conformations under the same solution conditions, would have been regarded by the skilled person as a further pointer preventing him/her to consider the use of aptamers bound to a solid carrier as capture molecules in a sandwich-type format.

14. All the above considerations are signals that the skilled person trying to solve the underlying technical problem and starting from the disclosure in document D13 had no reasonable expectation of success to arrive at the solution disclosed in present claim 1.

15. Thus, the subject-matter of claim 1, as well as of dependent claims 2 to 10, involves an inventive step. Therefore, the main request complies with the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the Examining Division with the order to grant a patent on the basis of claims 1 to 10 of the main request filed at the oral proceedings and the description yet to be adapted thereto.

The Registrar The Chairman

A. Wolinski M. Wieser