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**Datasheet for the decision
of 14 May 2013**

Case Number: T 1434/09 - 3.3.04

Application Number: 01994359.6

Publication Number: 1337547

IPC: C07H 21/04, C12Q 1/68

Language of the proceedings: EN

Title of invention:
Nuclease-based method for detecting and quantitating oligonucleotides

Applicant:
Isis Pharmaceuticals, Inc.

Opponent:
-

Headword:
Oligonucleotides/ISIS PHARMACEUTICALS

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 123(2)

Keyword:
"Main request - added matter (no) - novelty, inventive step, clarity, sufficiency of disclosure (yes) "

Decisions cited:
-

Catchword:
-



Case Number: T 1434/09 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 14 May 2013

Appellant: Isis Pharmaceuticals, Inc.
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Carlsbad, CA 92010 (US)

Representative: Chapman, Desmond Mark
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Decision under appeal: Decision of the Examining Division of the
European Patent Office posted 28 January 2009
refusing European patent application
No. 01994359.6 pursuant to Article 97(2) EPC.

Composition of the Board:

Chairman: C. Rennie-Smith
Members: B. Claes
G. Alt

Summary of Facts and Submissions

I. The appeal was lodged by the applicant (hereinafter "appellant") against the decision of the examining division to refuse European patent application 01994359.6 with the title "*Nuclease-based method for detecting and quantitating oligonucleotides*" which was published as international application WO 02/059137.

II. The examining division decided that the subject matter of claim 1 of the main request filed by the applicant with its letter dated 13 November 2008 and of the auxiliary request filed with its letter dated 12 December 2008 lacked an inventive step (Article 56 EPC).

Independent claim 1 of the main request before the examining division read:

"1. An *ex vivo* method for detecting or quantitating an oligonucleotide in a bodily fluid or extract obtained from a mammal that has been administered the oligonucleotide, wherein said oligonucleotide comprises one or more modifications to the backbone and/or nucleotide bases, comprising the steps of:
contacting said fluid or extract with a probe complementary to said oligonucleotide, wherein said probe comprises a detectable marker and a binding moiety;
placing said fluid or extract in contact with a solid support to which a binding partner of said binding moiety is attached;
contacting said fluid or extract with a single-strand specific nuclease under conditions in which probe which

is not hybridized to said oligonucleotide is degraded;
and
detecting the binding of said oligonucleotide to said
probe by measuring a label associated with said marker,
wherein the presence of said label indicates the
presence of said oligonucleotide bound to said probe on
said solid support and correlates with the amount of
oligonucleotide present in said fluid or extract."

Claims 2 to 10 of the main request were depending on
independent claim 1.

- III. With the statement of the grounds of appeal dated
5 June 2009 the appellant filed a main request
(identical to the main request considered by the
examining division; see section II), four new auxiliary
requests and ten further documents. The appellant
argued in favour of inventive step of the subject-
matter of claim 1 of the main request and of the
auxiliary requests.
- IV. The board summoned oral proceedings to be held on
14 May 2013 and expressed, in a communication pursuant
to Article 15(1) RPBA, its preliminary opinion that,
the subject-matter of claim 1 of the pending requests
lacked an inventive step (Article 56 EPC).
- V. With a letter dated 18 April 2013 the appellant
submitted further arguments.
- VI. With a communication of the registry dated 29 April
2013, the appellant was informed that oral proceedings
would be held as summoned. Subsequently, with a letter
dated 3 May 2013, the appellant withdrew its request

for oral proceedings and announced that it would not be represented at the oral proceedings.

VII. The appellant requested the board in writing by letter dated 3 May 2013 to set aside the decision under appeal and to order the grant of a patent on the basis the claims of the main request as filed with the letter dated 5 June 2009 or the claims of one of the four auxiliary requests filed with the same letter.

VIII. Oral proceedings took place on 14 May 2013 in the absence of the appellant. At the end of the oral proceedings the board gave its decision.

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

D1: W000/56926

D2: Agrawal *et al.* (1991), PNAS USA, Vol 88, pages 7595-7599.

D3: Temsamani *et al.* (1993), Analytical Biochem., Vol. 215, pages 54-58.

D4: De Serres *et al.* (1996), Analytical Biochem., Vol. 233, pages 228-233.

D5: Leeds *et al.* (1996), Analytical Biochem., Vol. 235, pages 36-43.

D6: Leeds *et al.* (1997), Drug Metabolism and Disposition, Vol. 25, No. 8., pages 921-926.

D7: Leeds et al. (1998), Drug Metabolism and Disposition, Vol. 26, No. 7., pages 670-675.

- X. The appellant's arguments, insofar as they are relevant for the present decision can be summarised as follows:

Main request

Construction of claim 1

Claim 1 specified that the method was performed on "a bodily fluid or extract obtained from a mammal that has been administered the oligonucleotide". The definition of "bodily fluid or extract" on page 23, lines 15 to 29 of the application as filed showed that these did not encompass samples of which the preparation involved nucleic acid purification steps.

Inventive step (Article 56 EPC)

Document (D1) did not represent the closest prior art for the claimed invention because it was not directed to a similar purpose or effect nor did it belong to the same or a closely related technical field. Its teaching focussed on methods for detecting sequence polymorphisms using peptide-labelled oligonucleotides and antibody arrays and thus addressed a completely different technical problem to that of the invention, i.e. the hybridization-based methods were intended primarily to discriminate between sample sequences with a single base difference, or between sample sequences with different splice sites.

Any of the prior art documents (D2) to (D7) sought to address the problem of quantitating modified oligonucleotides after *in vivo* administration, which was a specialized field in the art, and described methods specifically designed therefore. Any of them represented therefore a more appropriate and realistic closest prior art.

Document (D4) was a reasonable choice to represent the closest prior art. It disclosed an *ex vivo* method intended for detecting or quantitating an oligonucleotide in a bodily fluid or extract obtained from a mammal that has been administered the oligonucleotide, wherein said oligonucleotide comprised one or more modifications to the backbone and/or nucleotide bases. However, the method did not include any of the method steps of claim 1. The claimed method and the method in document (D4) were therefore entirely different methods, although having the same purpose and addressing the same problem.

The technical effect of the claimed method was that it was simpler and more versatile. In the claimed method the combination of an immobilised probe that itself contained a detectable marker and a single-strand specific nuclease was used to easily and efficiently detect the presence of the oligonucleotide. There was thus no requirement for a competing labelled analog. There was also no requirement for the use of scintillation beads in combination with a competing radiolabelled analog, so that the claimed method could be performed with a solid support in the form of a column and allowed for the use of detectable labels which did not require proximity to an interacting

reporter support. Importantly, the claimed method did not require the synthesis of a new radiolabelled analog sequence for each therapeutic oligonucleotide to be assayed, in addition to a new sense probe sequence (in contrast to document (D4); see page 229, right hand column) as the same single-strand specific nuclease can be used to detect the presence of a therapeutic oligonucleotide irrespective of the sequence of the oligonucleotide because the immobilised probe itself contains the marker.

The objective technical problem solved by the claimed subject-matter vis-à-vis document (D4) was therefore not merely the provision of an alternative method, but the provision of an *improved* method to detect or quantitate administered modified oligonucleotides in plasma. The claimed invention was however also inventive if a less ambitious problem were to be solved such as "the provision of an alternative efficient method to detect or quantitate administered modified oligonucleotides in plasma".

The technical field of document (D1) was not the same as the claimed method. Furthermore, there was no suggestion in document (D1) that the methods disclosed therein might be used to detect or quantitate an oligonucleotide "in a bodily fluid or extract obtained from a mammal that has been administered the oligonucleotide" or to use the methods to detect or quantitate an oligonucleotide that "comprises one or more modifications to the backbone and/or nucleotide bases". It was only possible with an unallowable level of hindsight to suggest that the skilled person starting from document (D4) would have identified

document (D1) when attempting to solve the objective technical problem. Rather, the skilled person would have turned to documents (D2), (D3) or (D5) to (D7), which were all directed to the same technical problem.

Document (D1) was concerned with the discrimination of single nucleotide differences which required complicated methods of a double-labelling system of the probes containing a "first marker (F1)" and a "second marker (F2)" (see page 10, lines 13 to 23; Figures 2 and 3) and requiring two different nuclease cleavage steps (see page 14, lines 16 to 28; page 15, line 31 to page 16, line 8; Figures 2 and 3), and two different label measurement steps. In some embodiments, the methods were yet further complicated by the need to use both a "positive probe" and a "negative probe" (Figure 2). The different types of methods described in detail in document (D1) were all significantly more complicated than the method disclosed in document (D4). In contrast, the discrimination of single nucleotide differences was not a concern in the context of detecting and quantitating modified oligonucleotides after *in vivo* administration, so the skilled person would not have been motivated to try to use the unnecessarily complicated methods as disclosed in document (D1) as a replacement for the method as disclosed in document (D4), or to somehow adapt the methods of document (D1) for detecting and quantitating modified oligonucleotides after *in vivo* administration.

Some of the methods disclosed in document (D1) comprised specific steps which were analogous to steps in the claimed method. However, all of the methods disclosed involved additional steps beyond those in the

claimed method which were essential in the methods of document (D1), to ensure that single nucleotide mismatches could be discriminated, i.e. steps which did not make sense in the context of detecting and quantitating modified oligonucleotides after *in vivo* administration. The skilled person, when combining the teaching of document (D1) with that of document (D4), would not have considered to replace all of the steps of the document (D4) method with a selection of isolated and modified steps from the methods described in document (D1) because this would have required the skilled person to abandon the basic technical teaching of document (D4) and to isolate certain steps from the methods described in document (D1), abandoning other steps and at the same time ignoring certain essential features of the methods disclosed in document (D1).

The combination of the teachings in document (D4) and (D1) therefore did not render the claimed invention obvious to a skilled person. The claimed subject-matter was therefore inventive.

Reasons for the Decision

1. The appeal is admissible.

Main request

Construction of claim 1 and clarity (Article 84 EPC)

2. Claim 1 specifies that the method is performed on "a *bodily fluid or extract obtained from a mammal*". The application as originally filed defines the notion

"bodily fluid or extract" on page 23, in lines 15 to 18 of the application as filed as *"any bodily substance removed from the subject to be screened for the presence of an oligonucleotide"*. On page 23, in lines 18 to 29, the application as originally filed then further exemplifies that *"[w]hile it understood that some portions of the body are not readily assayed as a fluid, procedures to homogenize and prepare liquid samples from those portions are not uncommon, and are well known. The addition of water or saline to body portions which are normally not liquid is within the scope of the present invention, for example, a homogenized sample of a bone suspension, can be assayed by the methods described herein. Thus the bodily fluid and/or extract may be prepared, or may be selected from, but not limited to, the following; tissue, bone or organ samples, serum, saliva, feces [sic], tears, sweat, and samples of blood cells, epithelial cells, and the like."*

3. Taking the cited passages into account, the board construes the notion *"a bodily fluid or extract obtained from a mammal"* in claim 1 to not include such "extracts" which have been prepared by sample purification methods involving nucleic acid purification. This construction is also supported by the examples of the application as originally filed demonstrating that the method of the invention provides the direct detection of modified oligonucleotides in plasma without the need for any intermediate oligonucleotide purification step.

4. In its decision the examining division has not objected to the clarity of claims. The board has no clarity objections either.

Added matter (Article 123(2) EPC) and novelty (Article 54 EPC)

5. In its decision the examining division found that the claims of the main request before it, which is identical to the main request before the board (see section II), complied with the requirements of Article 54 and 123(2) EPC. The board sees no reason to deviate from this finding.

Sufficiency of disclosure (Article 83 EPC)

6. In its decision the examining division has not formulated any objections concerning sufficiency of disclosure. The board has no objections either.

Inventive step

Closest prior art

7. The invention defined in claim 1 concerns the detection or quantitating of an oligonucleotide, which comprises one or more modifications to the backbone and/or nucleotide bases, in a bodily fluid or extract obtained from a mammal that has been administered the oligonucleotide.
8. In assessing whether or not a claimed invention meets the requirements of Article 56 EPC, the boards of appeal apply the "problem and solution" approach, which requires as a first step the identification of the

closest prior art. In accordance with the established case law of the boards of appeal, the closest prior art is a teaching in a document conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, i.e. requiring the minimum of structural modifications to arrive at the claimed invention (see Chapter I.D.3 of the Case Law of the Boards of Appeal of the EPO, 6th Edition, 2010).

9. The examining division, in its decision and as a basis for its finding that the subject-matter of claim 1 lacked inventive step, considered document (D1) to represent the closest prior art. The appellant has however argued that any of the documents (D2) to (D7), which all sought to address the problem of quantitating modified oligonucleotides after *in vivo* administration, constituted a more appropriate selection of a document representing the closest prior art.

9.1 Document (D1) discloses hybridization-based high-throughput PCR-free methods for detecting single nucleotide polymorphisms (SNPs) and other variations in nucleic acid populations or for the analysis of gene expression (see page 2, lines 24 to 30). The method applies peptide-labelled oligonucleotides and antibody arrays intended primarily to discriminate between sample sequences with a single base difference, or between sample sequences with different splice sites.

9.2 Document (D2) describes the use of ³⁵S-labelled phosphorothioate oligonucleotides to enable quantitation of the oligonucleotides after

- administration to mice (see the abstract and Figures 1 and 2).
- 9.3 Document (D3) describes a method in which oligonucleotides in a sample are immobilized on a nylon membrane, hybridized with a radiolabeled complementary oligonucleotide and then the hybridized sequences are imaged using X-ray film.
- 9.4 Document (D4) describes a method in which modified oligonucleotides are hybridized to "a biotinylated sense oligonucleotide to form a double-stranded nucleic acid complex on the surface of scintillation proximity beads derivatized with streptavidin". The modified oligonucleotide levels in blood samples of rhesus monkeys which had been administered the modified oligonucleotide intravenously are determined by competitive hybridisation of unlabeled oligonucleotide and a radiolabeled analog.
- 9.5 Documents (D5) and (D7) teach the use of capillary gel electrophoresis (CGE) to quantitate oligonucleotides in samples after administration to humans and monkeys.
- 9.6 Document (D6) teaches the use of ^{14}C -labelled phosphorothioate oligonucleotides in combination with strong anion exchange HPLC (SAX-HPLC) to enable quantitation of the oligonucleotides after their administration to rabbits (see the abstract and the table and figures on page 923).
10. Accordingly, all the teachings in documents (D2) to (D7) are conceived for the same purpose or aiming at the same objective as the claimed invention defined in

claim 1 (see point 4, above). The teaching in document (D4) is considered, however, as having the most relevant technical features in common with the claimed invention. Therefore, based on the criteria reviewed in point 8, above, the board considers, rather than the disclosure in document (D1), document (D4) to represent the closest prior art.

The objective technical problem to be solved

11. The board concurs with the appellant that the method steps of claim 1 and the method steps as disclosed in document (D4) are entirely different, although the methods as such have the same purpose and address the same problem.
12. The method of document (D4) is a "scintillation proximity competitive hybridisation assay (SP-CHA)" involving the competitive hybridisation of an administered antisense oligonucleotide and a [³H] radiolabelled analog (page 228, right hand column) for a limited amount of biotinylated complementary sense probes, whereby a key feature is the use of the combination of scintillation proximity beads with immobilised probe and a competing radiolabelled analog (page 229, left hand column).
13. It has been argued by the appellant that the technical effect of the difference of the claimed method over the method in document (D4) is that the claimed method was simpler and more versatile. Accordingly, the appellant considered the objective technical problem to be solved by the claimed subject-matter vis-à-vis document (D4) not merely the provision of an alternative method, but

"the provision of an improved method to detect or quantitate administered modified oligonucleotides in plasma".

14. The board considers however that the alleged improvements referred to by the appellant, in the present case, would only become relevant, if the board were to come to the conclusion that the claimed subject-matter were to solve a less ambitious objective technical problem than the one defined by the appellant in an obvious manner. Accordingly, the board will address the objective technical problem of "the provision of an alternative efficient method to detect or quantitate administered modified oligonucleotides in a bodily fluid or extract obtained from a mammal", and if necessary then assess the alleged advantages referred to by the appellant.
15. The board is satisfied that the problem under consideration is solved by the claimed subject matter, in particular in view of the experimental data in the examples.

Obviousness

16. The board is satisfied that document (D4), considered on its own, does not render the subject-matter of claim 1 obvious to a skilled person and notes that, apart from document (D1), none of the other documents presently on file disclose a similar sequence of method steps as recited in claim 1.
17. Accordingly, it needs to be assessed whether or not the skilled person, starting from the teaching in document

- (D4) representing the closest prior art and embarking on solving the objective technical problem would turn to the teaching in document (D1) and arrive in an obvious manner at the claimed invention.
18. As elaborated in point 9.1, above, document (D1) concerns hybridization-based high-throughput PCR-free methods for detecting single nucleotide polymorphisms (SNPs) and other variations in nucleic acid populations or for the analysis of gene expression which are useful to diagnose disorders, determine predisposition to genetic diseases, determine identity or ancestry, or correlate genetic sequences with phenotypic conditions (see page 2, lines 24 to 30). In contrast, the invention defined in claim 1 concerns the detection or quantitating of an oligonucleotide, which comprises one or more modifications to the backbone and/or nucleotide bases, in a bodily fluid or extract obtained from a mammal that has been administered the oligonucleotide.
19. The board notes therefore that, firstly, it could be questioned whether a skilled person working in the technical field of detection and quantitation of administered modified oligonucleotide in bodily fluids or extracts obtained from mammals, a technical field having a therapeutic connotation as confirmed in the application as originally filed (see page 1, line 32 to page 21 and page 2, line 31 to page 3, line 5) would have straightforwardly searched for a teaching in a different, although closely neighbouring technical field having a diagnostic connotation, to find a solution to the problem. In view of the following considerations however, the board considers it not necessary to decide this question.

20. Document (D1) explicitly defines the first step in the disclosed methods to consist of the preparation of target DNA from a biological sample, such as whole blood, plasma, serum, skin, etc. or a non-biological sample such as food, water, etc. in accordance with common knowledge preparation techniques (see page 12, lines 27 to 33). In contrast, as elaborated in points 2 and 3, above, the presently claimed method make no use of "extracts" which have been prepared by sample purification methods involving nucleic acid purification. Document (D1) is silent, whether or not the disclosed method can also be applied directly on a bodily fluid or extract thereof as defined in the present application. The board notes therefore that, secondly, when considering the methods disclosed in document (D1), the skilled person would be taught to start from purified target nucleic acid preparations obtained from bodily fluid or extract thereof contrary to the claimed method.
21. The methods disclosed in document (D1) concern the discrimination of single nucleotide differences. Figure 1 concerns the schematic representation of the "VGMS-PL" method, which is an abbreviation for "ValiGeneSM Mutation Screening, Peptide-Linked" (see page 1, lines 5 to 9); Figure 2 concerns a method for "genotype mapping" and Figure 3 deals with a method for "gene expression analysis". In order to allow discrimination between closely related sequences the methods apply a double-labelling system of the probes containing a "first marker (F1)" and a "second marker (F2)" (see page 10, lines 13 to 23; Figures 2 and 3), require two different nuclease cleavage steps whereby the second

digestion step is with Endonuclease V and SI nuclease and an additional measurement step (R2) which allows single base differences to be discriminated (see page 14, lines 16 to 28; page 15, line 31 to page 16, line 8; Figures 2 and 3). In some embodiments, the methods are further extended by the need for both a "positive probe" and a "negative probe" (Figure 2). The method as claimed requires only a single nuclease digestion step and only a single measurement step. The board notes therefore that each of the methods disclosed in document (D1) includes such steps which are specific for the detection of sequence differences but which are however not of relevance in the context of detecting and quantitating modified oligonucleotides after *in vivo* administration. Accordingly, the board notes, thirdly, that in order to define the specific method steps of claim 1, the skilled person, when assessing the disclosure in document (D1), would have to isolate certain steps from the methods described in document (D1) thereby simultaneously abandoning other steps.

22. In view of the above considerations, the board considers that, starting from the teaching in document (D4) which represents the closest prior art and embarking on solving the defined objective technical problem, the teaching in document (D1) would not lead the skilled person in an obvious manner to the claimed invention. Accordingly the board is satisfied that the subject-matter of claim 1 involves an inventive step (Article 56 EPC). Since claims 2 to 10 of the main request are dependent on claim 1, this finding also applies to the subject-matter of these claims.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to grant a patent on the basis of claims 1 to 10 of the main request filed with the letter of 5 June 2009 and a description and figures to be adapted thereto.

The Registrar

The Chairman

P. Cremona

C. Rennie-Smith