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Datasheet for the decision of 15 February 2017

Case Number: T 0754/11 - 3.3.08

Application Number: 01985833.1

Publication Number: 1407044

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
RNA INTERFERENCE MEDIATING SMALL RNA MOLECULES

Patent Proprietor:
Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.
Europäisches Laboratorium für Molekularbiologie

Opponents:
Sirna Therapeutics
Pfizer, Inc.
Roques, Sarah Elizabeth
BASF SE
Silence Therapeutics AG

Headword:
RNA interference, 3' overhang 19-23 nt dsRNA/MAX-PLANCK-GzFdW, ELMB

Relevant legal provisions:
EPC Art. 123(2), 83, 54, 56
RPBA Art. 13(1), 13(3)

Keyword:
Main request - requirements of the EPC met (yes)

Decisions cited:
G 0001/03, T 0609/02, T 0544/12

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern
Boards of Appeal
Chambres de recours

European Patent Office
D-80298 MUNICH
GERMANY
Tel. +49 (0) 89 2399-0
Fax +49 (0) 89 2399-4465

Case Number: T 0754/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 15 February 2017

Appellant II: (Opponent 2)	Pfizer, Inc. 235 East 42nd Street New York, NY 10017-5755 (US)
Representative:	Pfizer European Patent Department 23-25 avenue du Docteur Lannelongue 75668 Paris Cedex 14 (FR)
Appellant IV: (Opponent 4)	BASF SE 67056 Ludwigshafen (DE)
Representative:	Neuefeind, Regina Maiwald Patentanwalts GmbH Elisenhof Elisenstrasse 3 80335 München (DE)
Appellant V: (Opponent 5)	Silence Therapeutics AG Robert-Rössle-Strasse 10 13125 Berlin (DE)
Representative:	Stratagem IPM Limited Meridian Court Comberton Road Toft, Cambridge CB23 2RY (GB)
Respondent:	Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.

(Patent Proprietor 1)	Hofgartenstrasse 8 80539 München (DE)
Respondent: (Patent Proprietor 2)	Europäisches Laboratorium für Molekularbiologie Meyerhofstrasse 1 D-69117 Heidelberg (DE)
Representative:	Weiss, Wolfgang Jellbauer, Stephan Weickmann & Weickmann PartmbB Postfach 860 820 81635 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 15 February 2011 concerning maintenance of the European Patent No. 1407044 in amended form.**

Composition of the Board:

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

Summary of Facts and Submissions

I. European patent No. 1 407 044, entitled "*RNA interference mediating small RNA molecules*", is based on European patent application No. 01 985 833.1, originally published as International patent application WO 02/44321 (hereinafter "*the patent application*"). Five oppositions were filed against the patent and, at the end of the opposition proceedings, the opposition division decided that the patent could be maintained in amended form on the basis of claims 1 to 22 of the main request filed on 26 March 2009 and a description adapted thereto.

II. All five opponents filed an appeal against the decision of the opposition division. All opponents/appellants requested that the decision under appeal be set aside and the patent be revoked. In a letter dated 5 March 2014, appellant I (SIRNA THERAPEUTICS, INC) withdrew its opposition. In a letter dated 29 April 2016, appellant III (ROQUES, Sarah Elizabeth) withdrew its opposition.

III. The patent proprietors (respondents) filed a reply to the statements of grounds of appeal. They requested that the appeals be dismissed or, in the alternative, that the decision under appeal be set aside and the patent be maintained on the basis of one of auxiliary requests I to IV, all filed with the statement setting out the response of the patent proprietors.

IV. The parties were summoned to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, informed them of the preliminary, non-

binding opinion of the board on some of the issues of the appeal proceedings.

V. With their reply to the board's communication, the respondents filed a new main request and new auxiliary requests I and II.

VI. Appellants II, IV and V, without making any substantive submission, informed the board that they were not attending the oral proceedings.

VII. Oral proceedings were held on 15 February 2017, in the presence of the respondents.

VIII. Claims 1 and 9 of the main request read as follows:

"1. Isolated double-stranded RNA molecule, wherein each RNA strand has a length from 19 to 23 nucleotides and wherein at least one strand has a 3'-overhang from 1 to 3 nucleotides, wherein said RNA molecule is capable of target-specific RNA interference.

9. An in vitro method of mediating target-specific RNA interferences in a eukaryotic cell, comprising the steps:

(a) contacting said cell with the double-stranded RNA molecule of any one of claims 1 to 8 under conditions wherein target-specific RNA interferences can occur, and

(b) mediating a target-specific RNA interference effected by the double-stranded RNA towards a target nucleic acid having a sequence portion

substantially corresponding to the double-stranded RNA."

Claims 2-8 and 10-12 refer to specific embodiments of claims 1 and 9, respectively. Claims 13, 15 and 16 refer to the use of a double-stranded RNA (dsRNA) molecule of any of the claims 1-8 for the manufacture of a medicament for modulating the function of a pathogen-associated gene (claim 13), a tumor-associated gene (claim 15), or an autoimmune disease associated gene (claim 16). Claim 14 refers to a specific embodiment of claim 13.

IX. The following documents are cited in this decision:

D1: B.L. Bass, Cell, 28 April 2000, Vol. 101, pages 235 to 238;

D2: WO 01/75164 (publication date: 11 October 2001);

D2a: US Application No 60/193,594, filed on 30 March 2000, first priority document for D2;

D3: P.D. Zamore *et al.*, Cell, 31 March 2000, Vol. 101, pages 25 to 33;

D4: S.M. Hammond *et al.*, Nature, 16 March 2000, Vol. 404, pages 293 to 296;

D11: S. Parrish *et al.*, Molecular Cell, November 2000, Vol. 6, pages 1077 to 1087;

D13: D. Yang *et al.*, Current Biol., September 2000, Vol. 10, pages 1191 to 1200;

D36: T. Nguyen *et al.*, Current Opinion in Molecular Therapeutics, 2008, Vol. 10, Nr. 2, pages 158 to 167;

D37: A. de Fougerolles and T. Novobrantseva, Current Opinion in Pharmacology, 2008, Vol. 8, pages 280 to 285;

D39: F. Czauderna *et al.*, Nucleic Acids Research, 2003, Vol. 31, No. 11, pages 2705 to 2716;

D40: J.G. Doench *et al.*, Genes & Dev., 2003, Vol. 17, pages 438 to 442;

D47: D.S. Schwarz *et al.*, Molecular Cell, September 2002, Vol. 10, pages 537 to 548;

D58: Declaration by T. Tuschl, signed on 21 January 2009;

D64: X. Liu and M.A. Gorovsky, Nucleic Acids Research, 1993, Vol. 21, No. 21, pages 4954 to 4960;

D65: W.P. Wahls, PCR Methods and Applications, 1994, Vol. 3, pages 272 to 277;

D66: B.C. Schaefer, Analytical Biochemistry, 1995, Vol. 227, pages 255 to 273.

X. The written submissions of the appellants, as far as relevant for the present decision, can be summarized as follows:

Article 123(2) EPC

The patent application did not disclose an *in vitro* method of mediating target-specific RNA interference (RNAi) in a eukaryotic cell as defined in claim 9. In the decision under appeal, the opposition division stated that the feature "in a eukaryotic cell" in claim 9 had a basis on page 8, line 14 of the patent application, which was found in the paragraph reading:

"*The method of the invention may be used for determining the function of a gene in a cell or an organism or even for modulating the function of a gene in a cell or an organism, being capable of mediating RNA interference. The cell is preferably a eukaryotic cell or a cell line...*" (underlined by the board).

In the description of the patent application, the term "method of the invention" appeared only in this paragraph. However, several methods were disclosed and claimed in the patent application, namely a method for preparing a dsRNA molecule (original claim 13) and a method of mediating target-specific nucleic acid modifications in a cell or an organism (original claim 16). Since the term “method of the invention” was not clearly and unambiguously defined in the patent application, the subject matter of claim 9 was not clearly and unambiguously disclosed in the patent application.

Moreover, the term "eukaryotic cell" appeared in the cited paragraph only in connection with a distinct use of “the method of the invention”. A method as such and the use of such a method were however not identical and covered different aspects of the alleged invention. This conclusion was supported by the presence of original claims 20 and 21 which related to the use of the method of any one of original claims 16 to 19.

Therefore, the disclosure that the cell could be a eukaryotic cell was not generally applicable to each and every aspect of the alleged invention but only to a particular use of "the method of the invention".

The medical uses referred to in claims 13 to 16 were not directly and unambiguously disclosed in the patent application. Original claim 16 in the patent application related only to a method of mediating target-specific modifications in a cell or an organism but not to any medical use of the claimed dsRNA molecules.

Article 83 EPC

The teaching of the patent application was not sufficient to perform the claimed invention readily across the entire breadth of the claims. The patent application neither sufficiently disclosed the minimal structural requirements which an isolated dsRNA molecule according to claim 1 had to fulfil in order to be "*capable of target-specific RNA interference*", nor the reaction conditions under which RNAi took place.

In particular, the teaching of the patent application was not enabling with regard to the length requirements of the claimed isolated dsRNA molecule. If, for instance, both strands were designed with 19 nucleotides (nt) sequences and 3' overhangs of 3 nt each, the double-stranded structure of such an isolated dsRNA molecule would consist of 16 base pairs (bp) only. However, according to post-published document D39, a double-stranded structure of less than 18 bp was not suitable to mediate RNAi.

Moreover, the teaching of the patent application was also insufficient in respect of the allowable chemical modifications of the nucleotides within the claimed isolated dsRNA molecule. From either document D39 or document D40, it could be taken that a dsRNA molecule having (a) mismatch(es) and a bulge, respectively, was not effective in mediating RNAi. From document D47, it was evident that the 5' end of a dsRNA molecule mediating RNAi had to be phosphorylated. However, none of these teachings were provided by the disclosure of the patent application.

As for the therapeutic use claims 13 to 16, the post-published document D37 showed that, although RNAi was acknowledged as having revolutionized the biological sciences, significant steps were still required with respect to its use in therapy.

Article 54 EPC

The meaning of the term "*isolated nucleic acid*" had to be seen in the context of the technical area of the patent. In the field of naturally occurring products including biologicals, the term was used to delimit claimed compounds from those (forms of the) compounds available in nature. In the field of RNA biochemistry, the term "*isolated RNA molecule*" meant RNA molecules obtained as the result of an isolation or purification procedure. In this context, however, "*isolated*" did not mean "*isolated to homogeneity*" and it could not be construed to mean an RNA species having a unique sequence. The claimed "*isolated dsRNA molecule*" could also comprise mixtures of several RNA species. Because of this understanding, the specific definition given to the term "*isolated dsRNA molecule*" by the opposition division was wrong.

Document D1 anticipated the subject-matter of claims 1 and 2 of the main request. Each strand of the dsRNA molecule shown in Figure 1 of this document had a length of 23 nt, and each of the two strands had a 3'-overhang of 2 nt. The author of document D1 referred to the experimental set up described in document D3 and recognized that it produced 21-23 nt dsRNA molecules with 3'-overhangs of 2 nt. According to the model shown in Figure 1 of document D1, the enzyme producing these dsRNA molecules was the RNAse III. In the decision under appeal, the opposition division ignored the fact that the author of document D1 also recognized that these dsRNA molecules could be isolated and that they were capable of triggering RNAi. This was clearly stated on page 238, penultimate paragraph of document D1, by explicit reference to the results described in document D3.

Document D2 belonged to the state of the art under Article 54(3) EPC to the extent that it was entitled to claim priority from its first priority application (document D2a). The "*isolated dsRNA molecules*" of claims 1 to 8 were anticipated by document D2 which described *inter alia* the isolation of 21-23 nt fragments obtained by incubating 500 bp dsRNA with a soluble extract from Drosophila embryo (page 40, Example 3). These fragments were isolated and shown to mediate RNAi (Figure 12). Even though D2 did not explicitly mention that the 21-23 nt fragments obtained in Example 3 had 3'-overhangs, this was inevitably the case. As evidenced by prior art on file, it was well known in the art that the direct products of treating long dsRNA molecules with Drosophila embryo lysate were 21-23 nt dsRNAs with 3'-overhangs. The additional features of claims 3 to 8 were also disclosed in

document D2 (and document D2a). Moreover, as stated on page 4 of document D2 (and the corresponding paragraph on page 3 of document D2a), the isolated RNA could be used for mediating RNAi in a cell. Because the open language used in claims 9 to 12 did not restrict the claimed methods to the use of a single species of RNA molecule, the subject matter of these claims was also anticipated by document D2.

Both documents, D3 and D13, reported studies on the mechanism of dsRNA to direct sequence-specific degradation of mRNA through RNAi. Whilst in document D3 long dsRNAs were processed to 21-23 nt dsRNA species upon incubation in Drosophila embryo lysate (e.g. Figure 3), the long dsRNAs described in document D13 were converted into 21-23 nt dsRNAs that mediated RNAi (e.g. page 1191, abstract) by injection into Drosophila embryo cells and further extraction (page 1200, left-hand column under the heading "*Stability of mRNA and dsRNA*"; page S1 of "*Supplementary material*", right-hand column). The 21-23 nt dsRNA fragments of document D3 were thus obtained by the same process as described in Example 1 of the patent (paragraph [0034], "*Experimental Procedures*" referring to the lysate preparation as described in document D3). Following incubation in Drosophila lysate, the 21-23 nt dsRNAs were deproteinized, run on a gel, isolated from said gel and redissolved in water. This process inevitably yielded 21-23 nt dsRNAs having 3'-overhangs as confirmed by prior art on file. Likewise, it was also derivable from this prior art that the long dsRNAs in document D13 were processed to 21-23 nt dsRNAs having two 3'-overhangs on each strand. Thus, both documents D3 and D13 anticipated the subject-matter of claim 1 of the main request.

Since the wording of claim 1 did not require the presence of a single molecular species nor the absence of molecules with different structures, also document D4, describing the separation of RNA molecules from other components of a Drosophila lysate, deprived claim 1 of novelty.

Article 56 EPC

Document D1 represented the closest state of the art. In the decision under appeal, the opposition division stated that document D1 disclosed mixtures of dsRNAs with 3' overhangs of 2 nt (cf. page 33, point 7.4.1 of the decision under appeal). Hence, the only difference between the disclosure of document D1 and the subject matter of claim 1 was that the claimed dsRNA molecule had a unique sequence. The synthesis and the use of individual short dsRNA molecules for the induction of RNAi had however already been disclosed in documents D11 and D13, and producing a dsRNA molecule as claimed with a unique sequence did not require any inventive skills.

Based on the structure of the short dsRNA predicted in document D1, it would also have been obvious to synthesize and test them. In a "try and see approach", the skilled person would have planned experiments to test molecules with 3' overhangs and blunt ended molecules side by side.

Moreover, the person skilled in the art could have further isolated and characterized the short 21 to 23 nt dsRNAs described in documents D1 and D3. Contrary to the respondents' allegations, no inventive skills were needed to clone the short dsRNA fragments from a Drosophila embryo extract. The use of T4 RNA ligase for

the cloning of dsRNAs was well known, as shown in documents D64 to D66.

The subject-matter of claim 1 was moreover obvious when using document D13 as the closest state of the art in combination with document D1. Document D13 stated that there was strong correlative evidence that the 21 to 23 nt RNAs were the active agents *in vivo*, and document D1 suggested that the short dsRNAs were generated by RNAse III leaving 3' overhangs of 2 bases.

XI. The arguments of the respondents, as far as relevant for the present decision, can be summarized as follows:

Admission of new requests

The new requests were filed in reply to the board's communication pursuant to Article 15(1) RPBA. In these requests, certain claims of the previous requests were deleted.

Article 123(2) EPC

The method of claim 9 was disclosed at page 7, line 23 to page 8, line 2 of the patent application. This embodiment referred to a cell in general and was not confined to the mentioned possible uses. The subsequent paragraphs provided a discussion of a number of features recited in this embodiment (such as contacting step, possible applications of the method, the cell, the organism and the target gene), wherein in relation to each of said features preferred options were disclosed. These preferred options were independent of each other.

Regarding the subject-matter of claims 13 to 16, page 8, line 30 of the patent application specified that "*the dsRNA is usually administered as a pharmaceutical composition*". This was reflected by the Swiss-type format of the claims. The disclosure of an "organism" and, as an alternative, of a "cell" emphasized that both *in vitro* and *in vivo* applications were distinctly envisaged in the patent application. Moreover, the passage on page 8, lines 25 to 28, explicitly acknowledged that therapeutic benefits in the field of medicine or veterinary medicine could be obtained, and at page 8, lines 20 to 23, the disease-relevant target genes recited in claims 16 to 19 were also disclosed.

Article 83 EPC

The examples of the patent application provided evidence that various short dsRNAs with at least one 3'-overhang were effective triggers of RNAi. The patent application did not deal with the optimization of delivery or with an invention which would be specific for only a few target genes.

Page 8, first full paragraph, to page 9, first full paragraph, of the patent application provided sufficient teaching for the formulation and delivery of the claimed active agents for medical purposes. Document D37, while indicating that there was room for improvement in terms of delivery, did not contain any evidence of complete failure. In particular, it was stated in the "Conclusions" that "*some success has been achieved with direct instillation of saline-formulated siRNA*". Such simple formulation and route of delivery was well within the skilled person's capability.

Concerning the alleged lack of disclosure of suitable reaction conditions, section 1.1.1 of Example 1 of the patent application disclosed means and methods to perform *in vitro* RNAi and further referred to pertinent prior art publications. As regards RNAi to be effected in cells or organisms, the respective reaction conditions were those existing in said cells and organisms and were inherent to the respective systems used. A variety of mammalian cell lines had been successfully tested as disclosed in section 2.1.2 of Example 2 of the patent application and in the data shown in Figures 9 and 10. Cell culture conditions were also described therein.

Article 54 EPC

The subject-matter of the main request was disclosed in the first priority document EP 00 126 325.0 and, thus, had an effective date of 1 December 2000.

The term "*isolated*" in claim 1 indicated that a composition of matter was provided which previously existed embedded in its natural environment, but was unknown as such. The claimed dsRNA molecule was in a purified form, i.e. separated from other (RNA) molecular species having a different structure, such as length, sequence, etc., and from single-stranded RNA (ssRNA) molecules. Furthermore, the term "*isolated*" was used in conjunction with the term "*dsRNA molecule*" in singular and thus, the isolated molecule had to be a single molecular species.

The cited prior art on file used long and/or blunt-ended dsRNAs to trigger RNAi and, inside the RNAi machinery, short dsRNAs were envisaged only as possible intermediate products. Their structure was only the

subject of speculations as evidenced *inter alia* by the discussion provided in document D1. Prior to the disclosure of the patent, a short interfering RNA (siRNA) molecule having the structure defined in claim 1 of the main request had never been made available in "*isolated*" form.

Document D1 was a review article of scientific literature on RNAi but itself did not contain any experimental data. The RNAi models discussed in this document were not limited to the one depicted in Figure 1. As regards the involvement of RNAse III, it was explicitly stated in the section "*Concluding Remarks*" that the basic mechanism proposed in Figure 1 could be catalyzed by other proteins. As a consequence, the skilled person, desiring to follow the disclosure of document D1, would neither have known which of these various alternatives were reliable, nor how to choose between them, nor what the practical significance of any small dsRNA postulated in this document was. Moreover, the dsRNAs depicted in Figure 1 was stated to remain stably bound to a protein (RNAi nuclease) and thus, it fell outside the scope of claim 1 of the main request.

Claim 1 of document D2 was directed to "*isolated RNA of from about 21 to about 23 nucleotides that mediates RNA interference*", wherein the term "*RNA*" included both dsRNA and ssRNA. Document D2 contained neither an explicit nor an implicit disclosure of a dsRNA molecule comprising at least one strand with a 3'-overhang from 1 to 3 nt. A long dsRNA molecule was processed using a Drosophila lysate system in order to obtain 21-23 nt RNAs. The result was a mixture of RNAs that was further isolated by size separation. The denaturing gel system used for this isolation led to strand separation of

dsRNA molecules and thus, after this step, it was impossible to reconstitute a dsRNA molecule according to claim 1. Figure 4A of the patent showed that the complexity of sequences obtained from gel purification was such that any annealing or re-hybridisation of strands following elution from such a gel would not even recover the original strand pairings. Due to the presence of a large number of overlapping but distinct strands, the mixture would be further diversified by re-combination of different fully or partially complementary single strands. Document D2 only described the contacting of a cell with a crude mixture of RNAs which was different from, and therefore not affecting novelty of, the method of claim 9.

Document D3 described that a long (500 bp) dsRNA (mRNA template) was processed to short 21-23 nt RNA molecules in the presence of an *in vitro* Drosophila lysate system. These short RNA molecules were isolated by polyacrylamide gel electrophoresis and, when added back to a new RNAi reaction, they were capable of causing sequence-specific RNAi. Document D3 did not however describe an isolated dsRNA molecule with overhangs according to claim 1. On the contrary, the model shown in Figure 7 of this document depicted only short blunt-ended dsRNAs.

According to document D4, Drosophila cells contained a nuclease activity that specifically degraded transcripts homologous to transfected dsRNA. This enzyme contained an essential RNA component (page 293, left column, first paragraph). After partial purification, the nuclease co-fractionated with an about 25 nt RNA species, which was identified by means of denaturing gel electrophoresis. The authors of document D4 could not however determine whether the 25

nt RNA was present in the nuclease complex in double-stranded or single-stranded form. Moreover, document D4 did not describe an isolated dsRNA molecule with at least one 3'-overhang as required by claim 1 of the main request.

Document D13 described that processed small dsRNAs could mediate sequence-specific mRNA degradation during RNAi in Drosophila embryos. Long dsRNA molecules were processed to 21-23 nt species in a Drosophila embryo extract (page 1195, Figure 4). The processed RNA fragments obtained after denaturing gel electrophoresis and shown in Figure 4 were however single stranded. According to a model for RNAi shown in Figure 8, the dsRNA template was bound and cleaved by a dsRNA-specific nuclease to generate 21-23 bp products. These products remained incorporated in a multi-protein RNAi nuclease and effected cleavage of the target mRNA. This model neither disclosed short dsRNA molecules in isolated form nor any 3'-overhangs, but showed only blunt ends.

Article 56 EPC

The closest state of the art, document D11, described that a 26 bp dsRNA molecule with blunt ends was capable of inducing RNAi. However, an 81 bp dsRNA was significantly more active than the 26 bp dsRNA. According to the legend to Figure 1B, the 81 bp dsRNA had a 5' overhang. Starting from this document, the technical problem to be solved consisted in the provision of an improved agent for the induction of RNAi. The dsRNA molecules defined by claim 1 solved this problem as demonstrated by Figures 5A and 5B and paragraph [0052] of the patent. The 3' overhangs were clearly beneficial and needed. If anything, document

D11 pointed the skilled person in a different direction, namely to the use of longer molecules with a 5' overhang.

Moreover, when discussing the results obtained in document D11 in the light of those reported in the prior art, in particular documents D1, D3 and D4, the authors of document D11 considered a possible direct participation of the short dsRNAs in RNAi, but they stated that there were also alternative hypotheses to explain these results, namely that the short dsRNAs had no role in RNAi but simply reflected exposure of the dsRNA trigger to abundant RNAse III or that these short dsRNA were only by-products of RNAi. The claimed solution was thus not obvious.

The model for RNAi shown in Figure 1 of document D1 was speculative as made evident by the cautionary statements present in the written section of this document, such as, for instance, that any 23-mer cleavage product of an RNAse III might only be an intermediate product which was further trimmed to 21 to 22-mers with blunt ends by single strand specific nucleases (page 237, left-hand column), that 100 bp dsRNAs were needed for efficient inhibition of gene expression (page 238, second but last paragraph), or that "*there are still many mysteries about the mechanism of PTGS*" (page 238, last paragraph). Thus, document D1 presented several options that the skilled person could have pursued.

Document D3, which was mentioned in document D1, described a mixture of short dsRNAs comprising short endogenous RNAs present in Drosophila embryo extracts. The proposed model for RNAi shown in Figure 7 depicted blunt ended dsRNAs. The cloning of the short RNA

fragments required new methods which went beyond the conventional methods as explained in expert declaration D58.

XII. The appellants requested that the decision under appeal be set aside and the patent be revoked.

XIII. The respondents requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request, or alternatively upon the basis of one of Auxiliary Requests I or II, all filed under cover of a letter dated 13 January 2017, or alternatively upon the basis of one of Auxiliary Requests III or IV, filed as a Main Request under cover of a letter dated 26 March 2009, and filed as Auxiliary Request II under cover of a letter dated 10 November 2011, respectively.

Reasons for the Decision

1. In their statements setting out the grounds of appeal, the appellants raised objections under Articles 123, 54, 56 and 83 EPC. As far as objections raised in opposition under Articles 53(c) and 57 EPC are concerned, no arguments have been provided why the decision under appeal was not correct. The appeal procedure is therefore limited to re-examination of the objections raised under Articles 123, 83, 54 and 56 EPC.

Article 113(1) EPC - right to be heard

2. As announced in writing, the three remaining appellants did not attend the scheduled oral proceedings. Nor did they file substantive arguments in reply to the board's communication pursuant to Article 15(1) RPBA (cf. point

VI, above). By neither replying to the board's communication in substance nor attending the oral proceedings, the appellants effectively chose not to avail themselves of the opportunity to comment or present their observations on the board's opinion (Article 113(1) EPC). The appellants have relied on their written cases as presented in their statements of grounds of appeal. Moreover, as a consequence of appellants' course of action, there are no submissions from the appellants on file as regards the admissibility and compliance with the EPC of the main request and auxiliary requests I and II filed by the respondents in reply to the board's communication (cf. point V, above). Notwithstanding this fact, some of the objections raised by the appellants against former requests apply also to the present main request and therefore, they have been considered by the board when arriving at a decision on the appeal.

Admission of the main request

3. The main request submitted under cover of a letter dated 13 January 2017 differs from the main request previously on file by the deletion of former claims 9 to 11, and 20 to 22. The respondent deleted these claims in reply to the board's communication annexed to the summons to oral proceedings. The amendments are thus a straightforward reply to the provisional, non-binding opinion of the board expressed in this communication. The deletions reduce the complexity of the case. Therefore, the board, in the exercise of its discretion, decides to admit the main request into the appeal procedure (Article 114(2) EPC in conjunction with Article 13(1) and (3) RPBA).

Article 123(2) EPC

4. Concerning the subject-matter of claim 9 of the main request, the appellants argued that the patent application did not disclose an *in vitro* method of mediating target-specific RNAi **in a eukaryotic cell** (cf. point X, above).

5. Except for the feature "in a eukaryotic cell", the paragraph bridging pages 7 and 8 of the patent application explicitly discloses a method of mediating target-specific RNAi in a cell with all the features of the method of claim 9. The subsequent paragraph discloses preferred ways of contacting a cell with a dsRNA molecule (step (a) of claim 9). The next paragraph refers to "*the method of the invention*" (page 8, lines 11 to 18) and first discloses possible uses of this method (lines 11 to 13) and then preferred cell types, wherein line 14 states: "*The cell is preferably a eukaryotic cell ...*".

6. In the context of page 8, this reference to "*the method of the invention*" is clearly and unambiguously understood to be only the method of mediating target-specific RNAi disclosed in the paragraph bridging pages 7 and 8 of the patent application. The disclosure on page 8 merely provides further definitions for a number of features of this method of mediating target-specific RNAi. Moreover, when viewed in the whole context of page 8, the preferred cell type mentioned in the sentence beginning at line 14 is not limited to the specific use referred to in the immediately preceding sentence, (i.e. the use of the method for determining or modulating the function of a gene in a cell or an organism), but applies also to the method itself, thus disclosing the subject-matter of claim 9 in a direct and unambiguous manner.

7. The board arrives at the same conclusion even if the cell type mentioned in the sentence starting at line 14 of page 8 is considered to be preferred or limited to the specific use referred to in the immediately preceding sentence. If the method of mediating target-specific RNAi is used for determining or modulating the function of a gene in a eukaryotic cell, it is still the method itself which comprises, in this case as step (a), the contacting of a eukaryotic cell with the dsRNA. The method as such is thus carried out in a eukaryotic cell.

8. Therefore, the subject-matter of claim 9 of the main request is directly and unambiguously disclosed on pages 7 and 8 of the patent application.

9. The appellants also argued that the patent application did not directly and unambiguously disclose the medical uses of claims 13 to 16 of the main request (cf. point X, above).

10. As discussed above, pages 7 and 8 of the patent application disclose a method of mediating RNAi (page 7, line 23 to page 8, line 2) and the use of the method for determining or modulating the function of a gene in a cell or an organism (page 8, lines 11 to 13). Furthermore, the target gene to which the RNA molecule of the invention (dsRNA) is directed "*may be a pathogen-associated gene, e.g. a viral gene, a tumor-associated gene or an autoimmune disease-associated gene*" (page 8, lines 20 to 23). The target gene may also be a heterologous gene expressed in a recombinant cell or a genetically altered organism (page 8, lines 23 to 25). The following sentence, (page 8, lines 25 to 28) reads: "*By determinating or modulating,*

particularly, inhibiting the function of ***such a gene*** *valuable information and* ***therapeutic benefits*** *[...] in the medicine or veterinary medicine field may be obtained*" (emphasis added by the board). In other words, therapeutic benefits may be obtained from the determination or modulation of the function of a pathogen-associated (tumor or autoimmune disease-associated) gene *via* the administration of a dsRNA. In the board's view, this implies, directly and unambiguously, the use of said dsRNA according to claims 13 to 16 of the main request. Therefore, the subject matter of these claims is directly and unambiguously derivable from the disclosure on pages 7 and 8 of the patent application.

11. Thus, the main request does not contravene Article 123(2) EPC.

Article 83 EPC

12. Objections under sufficiency of disclosure and under inventive step were raised by the appellants arguing that the teaching of the patent did not allow the skilled person to practice the invention over the whole breadth of the claims. The appellants did not contest that the patent application provides guidance how to prepare some dsRNA molecules according to the claims. They argued however that the teaching was not sufficient in respect of the minimal structural requirements, such as dsRNA length, degree of sequence identity with target mRNA, modifications of the 5' and 3' ends, insertion of nucleotide analogues, etc., which a dsRNA molecule according to claim 1 has to fulfill in order to be "*capable of target-specific RNA interference*", or in respect of the reaction conditions under which RNAi takes place. Post-published documents

D37, D39, D40 and D47 were cited in this context (cf. point X, above).

13. Claim 1 of the main request contains the functional limitation: "*wherein said RNA molecule is capable of target-specific RNA interference*". Since this effect is a technical feature of the claim, the question of whether the invention can be performed across the entire scope of the claim is one of sufficiency of disclosure (Article 83 EPC; cf. G 1/03, OJ EPO 2004, 413, point 2.5.2 of the "Reasons for the Decision").

14. According to the case law established by the Boards of Appeal (cf. *inter alia*, T 544/12 of 22 November 2013, point 4.2 of the "Reasons for the Decision"), there is no requirement for an explicit disclosure of all the possible structural modifications in order to meet the requirements of Article 83 EPC. The fact that some embodiments may not work is not detrimental to the allowability of the claims, as long as the patent provides sufficient guidance, in the form of suitable tests which allow the skilled person to readily test and identify the working embodiments.

15. The patent application discloses assay systems based on Drosophila cell extracts (Example 1), and Drosophila and mammalian (including human) cell cultures (Example 2). Assays to study structural features (length requirements, the insertion of modified nucleotide analogues, sequence specificity (sequence identity with a target sequence)) are disclosed in Example 3. Finally, Example 3 contains "*The siRNA user guide*" (pages 49 to 51 of the patent application) which gives general guidance on how to construct dsRNA molecules. The examples of the patent also disclose suitable reaction conditions under which RNAi occurs

and, wherein the RNAi reaction takes place in cell culture (Example 2) or in a cellular extract (Examples 1 and 3).

16. Document D37 reviews the treatment of lung diseases with siRNAs. Simple saline formulations have been used successfully but also formulations containing PEI, liposomes or surfactants (cf. page 282, Table 1). As summarized in Figure 2, the development of an RNAi therapeutic involves the selection of lead candidates based on bioinformatics followed by testing of potential candidates in a variety of *in vitro* assays. Once potential lead candidates have been identified in the *in vitro* assays, stabilisation by chemical modifications followed by further *in vitro* tests is performed. Finally, an effective and safe delivery formulation is sought. This document teaches thus that the efficiency of dsRNA mediated RNAi may be improved by the introduction of chemical modifications.In the board's view, such a teaching does not support the conclusion that dsRNA without such modifications is not functional.

17. Document D39 describes the use of dsRNAs without 3' overhangs. Molecules with internal 2'-O-methyl modifications were protected against serum-derived nucleases (cf. page 2710, left-hand column). It was found that duplexes of 19 nt in length were highly efficient, whereas duplexes of only 17 nt were dramatically less efficient (cf. page 2707, right-hand column). Based on these results, the appellants argued that two RNA molecules of 19 nt, each having a 3' overhang of 3 nt and thus forming a duplex of only 16 nt, were not capable of target specific RNAi (cf. point X, above).

18. The question is whether this argument is substantiated by the evidence on file.

19. Example 1 and Figures 5A and 5B of the patent application demonstrate that a dsRNA molecule with a duplex of 18 bp was capable of RNAi (duplex 3, albeit with a 3' overhang of 4 nt; see page 32, last paragraph and paragraph bridging pages 33 to 34 of the patent application).

Document D39 shows that a duplex molecule of 17 nt is dramatically reduced in its silencing activity compared to a molecule with a duplex structure of 19 nt. As demonstrated in Figure 3A, a molecule with a duplex structure of 17 nt (duplex 5A/5B), especially when used at increased concentrations, is however not completely inactive.

As for duplexes of 16 nt, there is no conclusive evidence on file to demonstrate that such a molecule is indeed completely inactive.

Moreover, even if there was evidence of a non-working embodiment, this would not render the claim non-compliant with Article 83 EPC, as long as there are a large number of conceivable alternatives and the patent contains sufficient information on the relevant criteria for finding appropriate alternatives over the claimed range with reasonable effort (cf. G 1/03, *supra*, point 2.5.2 of the "Reasons for the Decision").

Due to the chemical nature of RNA molecules, large numbers of alternative molecules with a duplex structure of 16 nt are easily conceivable, and as stated in point 15, above, the patent provides guidance how to functionally assay dsRNA molecules in respect of

length requirements, sequence specificity or the insertion of modified nucleotides, i.e. the patent provides the criteria necessary for identifying appropriate alternatives.

20. The appellants' arguments based on document D39 therefore fail to convince the board.

21. Document D40 states that mismatches in the middle of the duplex structure render the dsRNA molecule incapable of RNAi. The patent application provides some guidance about tests for tolerable mismatches on pages 48 to 49, point 3.2.7 of the patent application. In the board's view, the mere fact that some of the dsRNA molecules described in document D40 are not capable of inducing RNAi is not enough evidence to support the appellants' general objection.

22. Document D47 describes the necessity to have phosphate groups at the 5' end of the dsRNA molecules. However, it also describes that a missing 5' end phosphate group is added in Drosophila embryo extracts by an endogenous kinase activity, and that the same happens in mammalian cells (cf. page 539, left-hand column, first full paragraph). In other words, a dsRNA molecule according to claim 1 without 5' end phosphate groups is nevertheless capable of RNAi in cultured cells or cellular extracts.

23. The board considers that the evidence on file is therefore not sufficient to allow the conclusion that the skilled person cannot readily perform the claimed invention across essentially the entire breadth of the claims.

24. Concerning the medical use claims 13 to 16 of the main request, the board arrives at the same conclusion.

25. Claims 13 to 16 refer to the use of the dsRNA molecule of claims 1 to 8 for the manufacture of a medicament for modulating the function of certain disease-associated genes (cf. point VIII, above). By reference to claims 1 to 8, the term "modulating" is limited to mediating target-specific RNAi, i.e. to the degradation of the targeted disease-specific RNAs. As concluded above, target-specific RNAi by the claimed dsRNAs is sufficiently disclosed in the patent. The selection of suitable target genes represents no undue burden since the skilled person can easily identify numerous genes whose over-expression is associated with the specific pathological conditions mentioned in claims 13 to 16. As stated by the opposition division (cf. page 41, point 8.12.9 *et seq.* of the decision under appeal), the provision of experimental evidence for the claimed medical uses is not necessary as long as the underlying physiological mechanisms make such use plausible (cf. *inter alia*, T 609/02 of 27 October 2004, point 9 of the "Reasons for the Decision"). Target-specific RNA degradation is sufficiently disclosed in Examples 1 to 3 of the patent application to make also credible the target-specific degradation of the disease-associated RNAs mentioned in claims 13 to 16. Furthermore, page 8, line 30, to page 9, line 26, provide guidance on how to prepare a composition for therapeutic applications. Finally, there is also ample post-published evidence on file to confirm this conclusion (cf. *inter alia*, page 159, Table 1 of document D36). This evidence can be taken into account because it only supports the findings and disclosure of the patent (cf. T 609/02, *supra*).

26. The requirements of Article 83 EPC are thus fulfilled.

Article 54 EPC

27. An important issue in the decision under appeal was the interpretation of the feature "*isolated double-stranded RNA molecule*" as used in claim 1.

28. According to the appellants, there was no reason for this feature to be narrowly interpreted. The term "*isolated*" included dsRNA molecules "*isolated*" from a cell as well as from a cell extract, cell proteins, etc. In the technical field of RNA biochemistry, the term "*isolated RNA molecule*" was therefore understood as encompassing compositions comprising mixtures of RNA molecules with distinct sequences (cf. point X, above).

29. The board, however, does not agree with the appellants' interpretation. The teaching of the patent and the understanding of a skilled person in the field of RNAi at the time of filing the patent application, was that long dsRNA was processed to 21-23 nt fragments which probably served as guide RNAs (cf. paragraph [0005] of the patent and references cited therein). The skilled person knew from document D3 that in Drosophila embryo extracts long dsRNA was processed to 21-23 nt RNAs with distinct sequences.

30. The appellants referred also to document D2 in order to further illustrate their argument. Document D2 is cited as relevant for the assessment of novelty under Article 54(3) EPC and is relevant only as far as it discloses subject matter already disclosed in its priority application US 60/193,594 (document D2a). The appellants submitted that, in document D2/D2a, the term "isolated RNA" was used as a synonym for purified RNA

and was used to designate a plurality of dsRNA molecules capable of mediating RNAi (cf. point X, above).

31. The board does not agree with the appellants' interpretation of this document. In document D2/D2a, the preparations obtained by incubating long dsRNA in Drosophila embryo extracts are called "*combinations*" which can be used to further isolate the 21-23 nt RNAs (plural) by gel electrophoresis, size exclusion chromatography, gradient centrifugation, or affinity chromatography with an antibody to the protein having bound the 21-23 nt RNAs (cf. page 16, lines 1 to 10 of document D2; page 11, lines 1 to 8 of document D2a). The skilled person knows that all these methods separate the 21-23 nt RNAs from, for instance, unprocessed RNA and RNAs of different lengths, and/or from other components of the embryo lysate. They do not provide, however, for a separation of the 21-23 nt RNAs according to their sequence. The skilled person does not designate or refer to such a preparation as an "*isolated double-stranded RNA molecule*" (singular) because, evidently, the composition comprises several structurally distinct RNA molecules of 21-23 nt in length. This interpretation is not in conflict with how the term "*isolated molecule*" is understood in general. Nor is it in conflict with the statements on page 2 of document D2/D2a that "*the present invention relates to isolated RNA molecules*", that the terms "*RNA, RNA molecule(s), RNA segment(s) and RNA fragment(s) are used interchangeably to refer to RNA that mediates RNA interference*" (in this document) and that "*[t]hese terms include [...] isolated RNA (partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA) [...]*." The fact that the afore-mentioned terms include isolated RNA does not mean that they are

synonymous terms for "*isolated RNA*" but rather that they have a broader meaning.

32. The feature "*isolated double-stranded RNA molecule*" in claim 1 thus refers to a single molecular species, in the present case to a dsRNA molecule with one distinct sequence, but not to a composition comprising a plurality of dsRNA sequences.

33. It remains to be assessed whether an "*isolated dsRNA molecule*" as defined in claim 1 is disclosed in the prior art.

34. Document D1 is a review article discussing *inter alia* the experimental results disclosed in documents D3 and D4. The author of this document speculates about potential mechanisms for generating dsRNA pieces of about 23 nt in length (cf. page 236, Figure 1) and suggests that these 21-25 nt pieces are generated by "*RNAse III or a highly related enzyme*" (cf. page 236, right-hand column, last paragraph). Therefore, properties of the RNAse III enzymes are incorporated into the model shown in Figure 1, such as "[f]*or example, RNAse III makes staggered cuts that leave 3' overhangs of two base pairs, as shown for the 23-mers of Figure 1*" (cf. page 237, left-hand paragraph, first full paragraph). In the concluding remarks, the author emphasizes that "[a]*lthough the idea that RNAse III-type enzymes are involved in RNAi is compelling, the basic mechanism proposed in Figure 1 could be catalyzed by other proteins*" (cf. page 238, left-hand column, middle of third paragraph). Thus, the presence and the role of any 3' overhangs in the 21-23 nt RNAs is speculative and far from certain.

35. The subject-matter of claim 1 is an "*isolated dsRNA molecule*", i.e. a physical object. The relevant question for the assessment of novelty is therefore whether document D1 discloses the physical isolation of dsRNA with the properties of claim 1. A mere drawing of a hypothetical reaction scheme displaying individual molecules which are moreover bound to an enzyme molecule is not sufficient. Indeed, the complex of RNAi nuclease and dsRNA seems to be disclosed as a highly relevant component in the model of Figure 1 since it is emphasized that the small dsRNA remains stably bound to said RNAi nuclease and that shorter fragments that do not bind well are degraded (cf. page 236, Figure 1, and paragraph bridging pages 236-237). In a possible alternative model, the dsRNA is expected to dissociate completely (cf. page 236, right-hand column, first full paragraph of document D1; see also page 32, Figure 7 of document D3). In any case, document D1 does not go beyond the disclosure of a possible theoretical model without providing any isolated 21-23 nt dsRNAs with 3' overhangs. Moreover, although the possible importance of small dsRNA (as intermediate products) is acknowledged in document D1, reference is also made to particular "*length requirements*" and to the fact that efficient inhibition has been obtained only with long dsRNA (cf. page 238, left-hand column, last full paragraph). For these reasons, the main request is novel over document D1.

36. As already discussed above, document D2/D2a discloses RNA isolates which comprise several dsRNA molecules of distinct sequence but not an isolated dsRNA molecule according to claim 1. These isolates do not fall within the scope of claim 1.

37. Document D3 describes that multiple 21-23 nt RNAs are produced from 501 bp long *Renilla reniformis* luciferase dsRNA and from 505 bp long *Photinus pyralis* luciferase dsRNA (cf. page 27, right-hand column, last paragraph). Also these preparations comprise several dsRNA molecules of distinct sequence but not an "*isolated dsRNA molecule*" with one distinct sequence as required by claim 1.

38. Document D4 describes the fractionation of extracts from Drosophila S2 cells transfected with 540 bp long dsRNA of the Drosophila cyclin E. Fractions displaying nuclease activity contained a family of discrete RNAs of 25 nt in length. As described on page 295 (left-hand column, first full paragraph), the band "*observed on northern blots may represent a family of discrete RNAs* ...". The document does not, however, disclose an "*isolated dsRNA molecule*" with one distinct sequence as required by claim 1.

39. Document D13 describes the processing of long dsRNAs in Drosophila embryos into dsRNA fragments of 21-23 bp in length (cf. page 1194, paragraph bridging left and right-hand columns). These fragments which are monitored on polyacrylamide gels represent a family of discrete RNAs. The document does not, however, disclose an "*isolated dsRNA molecule*" with one distinct sequence as required by claim 1.

40. Therefore, the subject-matter of claim 1 is novel over this prior art.

41. The appellants also raised further novelty objections against claims 9 to 11 in view of document D2 (cf. point X, above).

42. Document D2/D2a discloses methods of mediating RNAi in a cell or organism, e.g. a mammalian cell, in which, in a first embodiment, RNA of about 21-23 nt in length is introduced into a cell or organism (cf. page 4, lines 14 to 17 of document D2; page 3, lines 15 to 17 of document D2a). A skilled person could well understand that a single molecular species of RNA of 21-23 nt in length, i.e. a synthetic RNA, could be used to mediate RNAi. However, the references to RNA on page 4 of this document leave it open whether such a synthetic RNA would be a single-stranded or a double-stranded molecule. Moreover, there is no disclosure that a synthetic RNA should comprise at least one strand having a 3'-overhang of 1 to 3 nt. The first embodiment does not therefore represent an enabling disclosure of the subject-matter of claims 9 to 11.

43. A second embodiment mentioned in the same paragraph of document D2/D2a describes the use of an RNA preparation obtained by the methods disclosed in this document (cf. page 4, line 26 to page 5, line 1 of document D2; page 3, lines 19 to 25 of document D2a). As mentioned in point 31 above, document D2/D2a discloses the production of "*combinations*" comprising mixtures of RNA molecules of 21-23 nt in length. The RNA molecules can then be separated from other components of the combination (cf. page 16, lines 1 to 10, and Example 3 of document D2; page 11, first paragraph, and Example 3 of document D2a). As also mentioned in point 31 above, all the methods disclosed provide an RNA isolate composed of a mixture of RNAs with several distinct sequences but not an individual "*isolated RNA molecule*" as defined in the claims of the main request.

44. The appellants argued that also the use of a composition comprising dsRNAs (plural) for mediating

RNAi, isolated according to Example 3 of document D2/D2a, fell under the terms of claim 9 (cf. point X, above).

45. Step (a) of claim 9 of the main request comprises the step of "*contacting said cell with the double-stranded RNA molecule of any one of claims 1 to 8*" (cf. point VIII, above), i.e. the bringing into contact of a cell with an isolated dsRNA molecule. If the cell is contacted with an isolated dsRNA molecule, this dsRNA has to be added as an isolated dsRNA molecule. Such a step is clearly distinct from bringing the cell into contact with a composition comprising a mixture of dsRNAs. Moreover, Example 3 of document D2/D2a further discloses an assay to demonstrate target specific RNAi. This assay is however performed in a cell-free extract which, for this reason alone, cannot anticipate the subject-matter of claim 9 which relates to a method of mediating RNAi in a eukaryotic cell (cf. point VIII, above). Thus, neither the second embodiment mentioned on page 4 of document D2 nor Example 3 anticipate the subject-matter of claims 9 to 11.

46. The claims of the main request therefore meet the requirements of Article 54(2) and 54(3) EPC.

Article 56 EPC

The closest state of the art

47. According to the case law established by the Boards of Appeal, the closest state of the art for assessing inventive step is normally a prior art document disclosing subject-matter 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 (see "Case Law of the Boards of Appeal of the EPO", 8th edition 2016, I.D.3.1 and I.D.3.2, pages 163 to 164).

48. During the opposition procedure, all parties and the opposition division considered document D1 to represent the closest state of the art (cf. page 31, point 7.1 of the decision under appeal). In its communication attached to the summons to oral proceedings (cf. point IV, above), the board, referring *inter alia* to document D11, informed the parties of its provisional opinion that further prior art documents could be regarded as representing the closest state of the art. Thus, the board considered that, at the scheduled oral proceedings, it was first necessary to establish whether document D1 or D11 represented the closest state of the art (cf. points 53 to 58 of the board's communication annexed to the summons). All opponents/ appellants informed the board that they were not attending the oral proceedings but none of them addressed this issue in substance (cf. point VI, above).

49. As mentioned above, document D1 reviews the results presented in documents D3 and D4. It describes the cleavage of longer dsRNA molecules into small pieces of 21 to 23 nt and proposes a model for the mechanism of RNAi (see Figure 1). This model "*predicts that introducing 21- to 25-mer dsRNAs into a cell should trigger gene silencing, but so far all RNAi systems require dsRNAs greater than ~100 base pairs for efficient inhibition of gene expression. (Although possibly the 21- to 23-mer RNAs work in vitro [Zamore et al., 2000])*" (page 238, left-hand column, second but last paragraph).

50. Document D11 describes the use of 26 and 27 nt blunt ended dsRNA molecules to induce RNAi (cf. Figures 1B and 1C). They are structurally closely related to the isolated dsRNA molecule of claim 1. This document also refers to the experiments and results reported in documents D3 and D4 of the present proceedings and discusses potential mechanisms of RNAi action and roles of the about 25 nt RNAs (pages 1084 and 1085). Indeed, document D3 (Figure 3) as well as document D13 (Figure 4) on which the appellant's based further attacks on inventive step, describe the processing of long dsRNAs to 21 to 23 nt RNAs and propose a model for RNAi (Figures 7 and 8 of documents D3 and D13, respectively). Both models foresee a role for the 21 to 23 nt RNAs in RNAi but documents D3 and D13 remain silent about the use of the short 21 to 23 nt RNAs for inducing RNAi.

51. Since both, documents D1 and D11, describe structurally closely related dsRNA molecules but, whilst document D1 merely predicts a possible role of 21- to 25-mer dsRNAs in the induction of target specific RNAi, document D11 actually demonstrates said induction, the board considers document D11 to represent the closest state of the art.

The objective technical problem and the proposed solution

52. Starting from document D11, the objective technical problem to be solved is the provision of an alternative agent/means to induce RNAi. As a solution to this problem, the patent proposes the isolated dsRNA molecule specified in claim 1.

53. As demonstrated by Figure 5 and described in paragraph [0052] of the patent, 21 and 22 nt dsRNAs with overhanging 3' ends of 2 to 4 nt mediate the efficient degradation of target RNA. Thus, the claimed subject-matter solves the objective technical problem.

54. The appellants have also argued that the technical problem is not solved across the entire breadth of the claims (cf. point X, above). As mentioned in point 13 above, since the capability to induce RNAi is a technical feature of the claimed dsRNA molecule, appellants' arguments on this issue concern a question of sufficiency of disclosure, and the board has dealt with it under Article 83 EPC.

55. Thus, the board is satisfied that the underlying technical problem has been solved across the entire breadth of the claims.

Obviousness

56. It remains to be established whether the claimed solution involves an inventive step.

57. Document D11 describes the results obtained with, among others, dsRNA molecules of 26 and 81 nt in length in an RNAi assay in *C. elegans*. A synthetic dsRNA of 26 nt required at least a 250 fold higher concentration than a dsRNA of 81 nt in order to achieve the same interference activity (page 1078, right-hand column, and Figure 1B and 1C). Whilst the dsRNA of 26 nt was blunt ended, the dsRNA of 81 nt had a 4 base 5' extension (cf. legend to Figure 1). In the discussion of these results, the authors of document D11 refer to those reported in documents D1, D3 and D4 of the present proceedings (page 1084, right-hand column,

second full paragraph). In particular, reference is made to document D4 as showing the co-purification of a RNA degrading activity with a fraction comprising 25 bp RNAs, and to documents D1 and D3 as hypothesising a critical role of RNAse III in RNAi. In this context, the authors of document D11 consider it important not to exclude an alternative hypothesis mentioned in these documents, namely that the short RNAs may have no role in RNAi, but simply reflect exposure of the dsRNA trigger to abundant RNAse III.

Thus, document D11 neither suggests nor renders obvious the production of short dsRNAs, let alone shorter than 26 nt. Based on the unfavourable comparison of RNAi activity between the 26-mer and the 81-mer dsRNAs, the skilled person was rather guided towards longer dsRNA molecules. Moreover, in view of the fact that the more active 81-mer dsRNA of document D11 comprised a 5' overhang, the creation of shorter dsRNA molecules with 3' overhangs according to claim 1 was far from obvious. Based on document D11 alone, the claimed solution was not obvious.

58. It remains to be assessed whether this solution is obvious from a combination of document D11 with further prior art on file, in particular documents D1 and D3.

59. As discussed above, document D1 hypothesises about a role of RNAse III type enzymes and discloses a model for the mechanism of mRNA degradation by RNAi. This model might explain the small RNAs of 21 to 23 nt observed in document D3 (page 237, left-hand column, first full paragraph). The author then states: "*The initial cleavage might produce dsRNAs comprised of sense and antisense 23-mers, but the 3' overhangs would be more accessible to single-strand-specific nucleases*

present in the extract, and trimmed to 21 and 22 nucleotide pieces". The hypothetical model of the author is thus used to explain the length of the short dsRNAs but the 3' overhangs - hypothetically produced by the RNAse III - are only an intermediate product, not necessary for RNAi. Concerning the idea that introducing 21- to 25-mer dsRNAs into a cell should trigger gene silencing, the author observes that "*so far all RNAi systems require dsRNAs greater than ~100 base pairs for efficient inhibition of gene expression. (Although possibly the 21- to 23-mer RNAs work in vitro [Zamore et al., 2000])*" (page 238, second but last paragraph). In view of the cautionary statements about the role of RNAse III type enzymes and the length requirements for achieving RNAi, the solution as defined by claim 1 was therefore not obvious when combining the disclosures of documents D11 and D1.

60. It is worth noting here that the board would also arrive at this conclusion if, as argued by the opponents/appellants, document D1 were considered to represent the closest state of the art (cf. point X, above). First, for the reasons explained above, document D1 hypothesised about possible mechanisms of action in a cautious way, leaving several options for the skilled person. Furthermore, even if the statement "*Although possibly the 21- to 23-mer RNAs work in vitro [Zamore et al., 2000]*" were considered to provide a motivation for the skilled person to further concentrate on the 21- to 23-mer RNAs described in *Zamore et al.* (document D3), the skilled person would not have arrived at the claimed solution in an obvious way, because document D3 leaves it open whether the 21- to 23-mer RNA segments produced in the Drosophila embryo extract are present as single or double stranded products (cf. for instance Abstract: "*both strands of*

the dsRNA are processed to RNA segments 21-23 nucleotides in length"; page 26, left-hand column, second but last paragraph: "*The dsRNA but not single-stranded RNA is processed in vitro to a population of 21-23 nt species*"; page 27, right-hand column, last paragraph: "*When incubated in lysate, approximately 15% of the input radioactivity ... appeared in 21 to 23 nt RNA fragments*"; page 29, left-hand column, line 12: "*ATP may not be required for production of the 21-23 nt RNA species*"; page 31, left-hand column, full paragraph: "*We envision that each small RNA fragment ...*"). Moreover, although the model depicted in Figure 7 of document D3 displays short dsRNA fragments, they are displayed with blunt ends. Thus, also when combining document D1 with document D3, there was no guidance leading the skilled person to the dsRNA molecule of claim 1 in an obvious manner.

61. For similar reasons, the argument fails that the claimed solution was obvious when using document D13 as the closest state of the art and combining its disclosure with that of document D1. Document D13 describes the appearance of 21 to 23 nt RNAs after the injection of 800 bp dsRNAs into Drosophila embryos (page 1194, paragraph bridging the left and right-hand columns), and presents a model for RNAi (Figure 8) which displays short but blunt ended RNAs. As explained above, document D1 hypothesised about possible mechanisms of action in a cautious way and left several options for the skilled person. The combination of the teachings of documents D13 and D1 could therefore not guide the skilled person to the dsRNA molecule of claim 1 in an obvious way.

62. The board is also not convinced by the argument that the skilled person could have set out in a straight

forward manner to further purify the 21- to 23-mers from the Drosophila embryo lysate described in document D3 (cf. point X above). The document itself gives no guidance in this respect. Furthermore, the cloning of the short RNA fragments from the Drosophila embryo lysate required more than ordinary skills. As explained by one of the inventors in document D58, conventional cloning via the addition of a poly-A tail led nowhere and it was necessary to develop a cloning protocol involving the use of RNA ligases. Documents D64 to D66, disclosing the use of T4 RNA ligase for the cloning of RNAs, were submitted in order to challenge the statements made in document D58 (cf. page 11 of appellant I's statement of grounds of appeal). However, the RNAs cloned in all these documents are considerably longer than the 21- to 23-mers described in the patent and it was therefore not obvious that the methods disclosed in these documents could simply be used for the cloning of the RNAs from the Drosophila embryo extract. The cloning procedure described in paragraph [0039] of the patent included a sequence of specific steps to avoid circularization, and the use of specific adapter sequences (cf. also point 7.2.2, pages 19 to 22 of the respondents' reply to the appellants' statements of grounds of appeal). Moreover, cloning of the short RNAs resulted in a large, complex and unequally distributed set of short RNAs (cf. Figure 4 of the patent) the analysis of which also required skills beyond those of the average person skilled in the art. The argument that the cloning of the short RNAs from the Drosophila embryo extract was obvious therefore fails.

63. Therefore, the main request meets the requirements of Article 56 EPC.

Adaptation of the description

64. At the oral proceedings, the appellant submitted amended pages 3 to 6 and 21 of the description to bring the patent specification in line with the main request. The board is satisfied that this has been done in agreement with the requirements of the EPC.

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 maintain the patent as amended in the following version:

Description:
Pages 7 - 20 of the patent specification.
Pages 3, 4, 5, 6 and 21 of the amended patent specification received during the oral proceedings before the Board.

Claims:
Claims 1 - 16 of the Main Request filed under cover of a letter dated 13 January 2017.

Drawings:
Figs 1 - 19 of the patent specification.

The Registrar: The Chairman:

A. Wolinski P. Julià

Decision electronically authenticated