Datasheet for the decision of 31 July 2006

Case Number: T 0273/05 - 3.3.08
Application Number: 95902511.5
Publication Number: 0728206
IPC: C12N 15/52

Language of the proceedings: EN

Title of invention: Alteration of sequence of a target molecule

Applicant: RIBOZYME PHARMACEUTICALS, INC.

Opponent: -

Headword: Trans-splicing method/RIBOZYME

Relevant legal provisions: EPC Art. 56

Keyword: "Main and auxiliary requests - inventive step (no)"

Decisions cited: -

Catchword: -
Case Number: T 0273/05 - 3.3.08

DECISION
of the Technical Board of Appeal 3.3.08
of 31 July 2006

Appellant: RIBOZYME PHARMACEUTICALS, INC.
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Decision under appeal: Decision of the Examining Division of the European Patent Office posted 19 October 2004 refusing European application No. 95902511.5 pursuant to Article 97(1) EPC.

Composition of the Board:
Chairman: L. Galligani
Members: M. R. Vega Laso
C. Rennie-Smith
Summary of Facts and Submissions

I.

The applicant (appellant) lodged an appeal against the decision of the examining division posted on 19 October 2004, whereby the European patent application No. 95 902 511.5 (published as WO 95/13379) was refused pursuant to Article 97(1) EPC. The refusal was based on the finding that, having regard to the disclosure of document D1 (see Section IX, infra) supplemented with the common general knowledge of the skilled person at the priority date, the subject-matter of both the main request (claims 1 to 5 as filed on 25 February 2003) and the auxiliary request (claims 1 to 5 as filed on 17 September 2004) lacked an inventive step (Article 56 EPC).

II.

Claim 1 of the main request read as follows:

"1. Method for splicing a target RNA molecule comprising a mutant beta-globin nucleotide sequence within a cell in culture with a separate RNA molecule comprising a wild type beta-globin nucleotide sequence, wherein protein product of said target RNA molecule comprising a mutant beta-globin nucleotide sequence is deleterious to the cell in which it is located, and wherein said separate RNA molecule is adapted to form a target RNA molecule with the wild type beta-globin nucleotide sequence in place of mutant beta-globin nucleotide sequence when spliced with at least a part of said target RNA molecule comprising a mutant beta-globin nucleotide sequence, such that the protein product of the target RNA molecule with the wild type beta-globin nucleotide sequence is beneficial to the survival of the cell, comprising the step of:
contacting said target RNA molecule comprising a mutant beta-globin nucleotide sequence with a catalytic RNA molecule, wherein said catalytic RNA molecule comprises said separate RNA molecule comprising a wild type beta-globin nucleotide sequence, under conditions in which at least a portion of said separate RNA molecule is spliced with at least a portion of said target RNA molecule comprising a mutant beta-globin nucleotide sequence to form said target RNA molecule with the wild type beta-globin nucleotide sequence in place of mutant beta-globin nucleotide sequence when spliced with at least a part of said target RNA molecule comprising a mutant beta-globin nucleotide sequence."

Dependent claims 2 to 5 concerned various embodiments of the method of claim 1.

III. Claims 1 to 5 of the auxiliary request differed from the corresponding claims of the main request only in that the words "mutant beta-globin" were replaced by "sickle cell beta-globin".

IV. In its statement setting out the grounds of appeal, the appellant maintained the claim requests on the basis of which the application had been refused, and put forward arguments in support of an inventive step. As a subsidiary request, the appellant requested oral proceedings under Article 116 EPC.

V. The examining division did not rectify its decision and, pursuant to Article 109(2) EPC, remitted the appeal to the boards of appeal.
VI. The appellant was summoned to oral proceedings. In a communication under Article 11(1) of the Rules of Procedure of the Boards of Appeal ("RPBA") sent with the summons, the board drew attention to some of the matters to be discussed during the oral proceedings and in particular to issues in connection with Article 56 EPC.

VII. In response to this communication, the appellant informed the board of its intention not to attend the scheduled oral proceedings. No substantive reply to the issues raised by the board was filed.

VIII. At oral proceedings, which were held on 31 July 2006, the appellant was not present.

IX. The following document is cited in the present decision:


X. The arguments put forward by the appellant in writing may be summarised as follows:

Main request

The invention was directed to the repair of a mutant beta-globin RNA sequence via trans-splicing and the restoration and maintenance of the regulated expression of a wild-type RNA sequence in a given host cell. The claimed method provided an alternative to standard gene therapy and allowed the repair of a mutant RNA sequence to obtain a wild-type RNA sequence "in context", i.e. under the control of its natural regulatory sequence.
Document D1 disclosed in a general manner trans-splicing ribozymes being capable of splicing a new 3' exon sequence into a given target RNA sequence, in particular in order to destroy an existing target RNA sequence or to express in an heterologous manner any new sequence under the control of the regulatory elements of the target RNA. Nowhere in document D1 was there any teaching or suggestion pertinent to the use of trans-splicing ribozymes as a means to repair a genetic defect in a host cell so that the wild-type protein was expressed, nor was there any suggestion or teaching of the specific mechanisms to carry out the repair as taught in the application at issue.

The examining division defined the technical contribution to the state of the art by the disclosure of the application as the selection of a different target RNA, namely a mutant beta-globin RNA, among a large number of RNAs that could serve as a target for trans-splicing. However, the examining division failed to appreciate that the mere replacement of one target RNA sequence for another based on the disclosure of document D1 would not lead to the repair of a mutant RNA sequence. Most of the key technical features of the invention lay not in the choice of a target RNA, but in the choice of a separate nucleic acid to be spliced into the target and in the design of the catalytic RNA molecule which directed and aligned the splicing. Thus, more fundamental than the choice of a defective sequence as a target was the methodology by which the catalytic RNA "transformed" the target into the wild-type sequence.
There were significant conceptual as well as mechanistic differences between the claimed subject-matter and the disclosure of D1. The teachings of this document did not allow the replacement of a cleaved target RNA with a similar 3' exon, but only with an exon that was derived from a different RNA sequence. In contrast, the application disclosed - for example in Figure 7 - a trans-splicing ribozyme which could react with a target RNA and splice on a sequence that was very similar to the wild-type target sequence.

It was an essential feature of the invention that, after splicing, the corrected RNA target sequence was capable of being translated in the correct reading frame resulting in a functional (restored) wild-type protein. Such an "in frame fusion" of the 3' exon, or the generation of a single splicing product were not mandatory when using the technology of D1. This issue became particularly apparent in view of Figure 4 of D1, which illustrated the splicing of the diphteria A-chain RNA sequence into two different splice sites of the cucumber mosaic virus coat protein RNA resulting in proteins of different lengths. The terms "accurate" and "effective" in D1 were erroneously interpreted by the examining division to mean "in frame". However, "in frame" trans-splicing to generate a translatable transcript (lacZ mRNA) was achieved for the first time in the application.

Document D1 did not give the skilled person any hint that would prompt him/her to adapt the teaching of this document to the restoration of the regulated production of wild-type beta-globin by trans-splicing. Moreover, even if the skilled person could have considered trying
such an approach, he/she would not have expected a priori that trans-splicing would work when using beta-globin mRNA as a target, due to the extremely long half-life of this transcript. Thus, having regard to the prior art on file, the claimed subject-matter involved an inventive step.

Auxiliary request

The claims of the auxiliary request concerned the repair of the sickle cell beta-globin RNA sequence characterised by an A-T transversion in the sixth codon of the gene. At the priority date of the application, no curative treatment existed for sickle cell anaemia due to the highly regulated expression of the beta-globin gene, which was difficult to recapitulate faithfully after gene transfer. Based on the teaching of the application, an improved and refined strategy had been developed by the applicant to convert sickle cell beta-globin transcripts into RNAs encoding its foetal counterpart gamma-globin.

XI. The appellant requested in writing that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 to 5 of the main request as filed on 25 February 2003, or of claims 1 to 5 of the auxiliary request as filed on 17 September 2004 and re-filed with the statement of grounds of appeal.
Reasons for the Decision

Main request - Inventive step (Article 56 EPC)

1. The issue to be decided in the present case is whether or not the examining division was correct in deciding that, having regard to the disclosure of document D1, the claimed subject-matter, in particular the subject-matter of claim 1, does not involve an inventive step.

2. The disclosure of the present application relates to the alteration of the sequence of a target RNA molecule by use of a splicing reaction in vivo or in vitro (cf. page 10, lines 33 to 35). In one embodiment of the disclosed splicing method, the target molecule is contacted with a catalytic molecule (ribozyme) which includes a separate nucleic acid molecule (cf. page 11, lines 9 to 11). Trans-splicing of the separate nucleic acid molecule with the target molecule results in an altered RNA molecule that encodes a chimeric protein with advantageous features (cf. page 11, lines 5 to 8). The target molecule can be any desired molecule with which a splicing reaction can occur (cf. page 11, lines 19 to 20), and the resulting chimeric molecule is either one which may occur in nature but is not present prior to the splicing reaction, or a completely novel structure which does not occur in nature but which is useful in gene therapeutic treatment of an organism (cf. page 13, lines 3 to 7).

3. Whereas claim 1 as originally filed was directed to the disclosed trans-splicing method defined in a general manner, amended claim 1 of the main request as presently on file is limited to a method for trans-
splicing a mutant beta-globin RNA molecule (the target RNA) with a wild-type beta-globin sequence (the separate molecule) attached to a catalytic RNA (ribozyme), the trans-splicing resulting in the replacement of the mutant sequence by the wild-type sequence.

4. Document D1 is considered to be the closest prior art. This document teaches a method for in vitro or in vivo trans-splicing a desired genetic sequence with a target RNA sequence, by contacting the target RNA with a catalytic chimeric RNA molecule capable of efficiently splicing the desired sequence to link it to or insert it into any chosen target RNA sequence in a highly precise manner (cf. page 9, lines 1 to 10, and page 15, lines 3 to 17). Catalytic chimeric RNA molecules ("trans-splicing ribozymes") may be designed which trans-splice essentially any RNA sequence onto any target RNA (cf. page 24, lines 1 to 3). The trans-splicing method disclosed in document D1 is applied in particular to the genetic modification of crop plants (cf. page 11, lines 14 to 20). On page 25, lines 5 to 15 the use of trans-splicing for the provision of a genetic trait to be selectively expressed under the same conditions as the target RNA is explicitly mentioned. Furthermore, it is apparent from the embodiment described on page 11, lines 21 to 27 that, when the disclosed trans-splicing method is applied to a plant cell infected with a pathogen, a target molecule deleterious to the cell (ie the pathogen genome as such or RNA transcripts required for infection) is transformed in a chimeric RNA molecule beneficial for the survival of the cell, as the pathogen is destroyed or inhibited. In Example 1 of
document D1, the construction and use of a ribozyme capable of splicing a sequence encoding beta-galactosidase (LacZ) alpha-peptide to a site in the 5' coding sequence of the chloramphenicol acetyl transferase gene is described. Example 2 concerns the design of a trans-splicing ribozyme that provides plant virus resistance.

5. It is apparent from a comparison of the disclosure of the present application and document D1 (cf. points 2 and 4 above) that the general teaching of the present application, namely a targeted trans-splicing method for the alteration of a given target RNA molecule, is the same as taught in the prior document. The subject-matter of present claim 1, however, differs from the disclosure of D1 in the choice of a specific target RNA molecule, i.e. a mutant beta-globin RNA.

6. Thus, the objective technical problem to be solved in view of document D1 can be defined as the application of the targeted trans-splicing method disclosed in the prior art to a further target RNA sequence. The solution proposed is the method of claim 1, wherein mutant beta-globin RNA is chosen as a target.

7. In the decision under appeal, the examining division held that it would have been obvious for the skilled person to try to apply the targeted trans-splicing method of D1 to any defective gene, in particular to a mutant beta-globin gene. This view is shared by the board. As mentioned above (cf. point 4), document D1 indicates that catalytic chimeric RNA molecules which are capable of trans-splicing essentially any RNA sequence onto any target RNA can be designed
(cf. page 24, lines 1 to 3), and that the choice of both the target RNA and the desired sequence to be trans-spliced into the target RNA will reflect the desired purpose of the trans-splicing reaction (cf. page 24, lines 18 to 19; and page 26, lines 12 to 15).

8. Whereas it is true that document D1 does not give to the skilled person a hint towards the specific choice of a mutant beta-globin RNA as a target RNA, this choice, which in the absence of any technical details or experimental support in the application (see below) appears to be the sole technical contribution to the state of the art by the application, is, in the board's view, arbitrary and determined only by the actual need to delimit the claimed subject-matter against the prior art in the examination procedure.

9. The appellant contended that most of the key technical features of the invention lay not in the choice of the target RNA sequence, but in the choice of the separate nucleic acid to be spliced into the target, and in the design of the catalytic RNA molecule which directs and aligns the splicing. The board, however, notes that the application contains no specific technical details and/or experimental evidence whatsoever for the replacement of a mutant beta-globin sequence by a wild-type beta-globin sequence, either with respect to the choice of the separate nucleic acid or the design of the catalytic RNA molecule. The sole passage dealing specifically with the repair of defective transcripts, in particular of mutant beta-globin RNA is found in the first full paragraph of page 21 of the application. This passage reads:
"Targeted trans-splicing can potentially repair or correct globin transcripts that are either truncated or contain point mutations. In the process, the cellular expression pattern of theses genes is maintained (Fig. 7)."

10. The passage itself merely contains a suggestion of a possible application of a targeted trans-splicing method as known from the art and also described in the application, but no specific technical guidance therefor. Figure 7, which is referred to in the passage, represents schematically the mechanism of targeted trans-splicing applied to a mutant beta-globin sequence, but provides no technical details concerning the design of the required catalytic RNA molecule beyond what was already known from document D1.

11. The person skilled in the art may learn from the diagram in Figure 7 that the catalytic molecule must contain, besides the catalytic portion as such (ie the ribozyme), a complementary RNA sequence for targeting the ribozyme to hybridize to the targeted RNA, and the desired genetic sequence (ie the wild-type beta-globin sequence) to be trans-spliced. It may also be apparent to the skilled person that the splice site within the target RNA must be chosen such as to allow the desired sequence (ie the wild-type sequence) to be inserted into the target molecule and the mutated sequence to be excised. Hence, except for the use of a mutant and wild-type beta-globin as target and desired genetic sequence, respectively, the whole guidance provided by Figure 7 can be unmistakably taken from the disclosure of document D1, in particular from the strategy and the
general guidelines for the engineering of suitable ribozymes for trans-splicing (cf. page 24, lines 6 to 17, and passage starting on page 18, line 29).

12. Contrary to the appellant's allegation, the board is unable to see any conceptual or mechanistic differences between the method disclosed in the application and the method described in document D1, that might hinder the skilled person from replacing a mutated portion of a defective RNA molecule by a similar sequence lacking the mutation, following the technical guidance provided in the prior art document. In this respect, it is worth stressing that neither the description nor the examples of the present application disclose any technical details concerning the design of the required catalytic RNA molecule that have not already been disclosed in D1.

13. As a matter of fact, the examples of the application do not relate to the replacement of a given mutant sequence by the corresponding wild-type sequence, let alone to the replacement of a mutant beta-globin sequence by the wild-type sequence. Example 1 of the application describes an in-frame fusion of a truncated 39 nucleotide LacZ transcript (the target molecule) to a fragment of 200 nucleotides in length of the alpha-complement of the LacZ gene (the separate molecule) using a Tetrahymena ribozyme to splice the separate molecule onto the target molecule (cf. Figure 2b). In example 2, a transcript containing the first 1106 nucleotides of the LacZ coding sequence as well as signals for in vitro translation (the target molecule) is contacted with a Tetrahymena ribozyme attached to the last 1987 nucleotides of the LacZ coding sequence (the separate molecule). Trans-splicing of the separate
molecular onto the target molecule results in a 1074 nucleotide fusion product encoding the entire LacZ coding sequence. Thus, both examples of the application concern splicing together two different portions of a lacZ transcript, rather than "transforming" a mutant target sequence into a similar wild-type sequence, what the appellant alleges to be the technical teaching of the application.

14. The board cannot accept the appellant's argument that in-frame fusion and generation of a single splicing product are not disclosed in D1 as mandatory. What is relevant in the framework of assessing inventive step is whether the skilled person could learn from document D1 that in cases when a specific protein product is desired as a result of the trans-splicing event, it is necessary to maintain the amino acid reading frame in the resulting fusion. This is clearly disclosed on page 26, lines 25 to 28 of document D1. Furthermore, it is immaterial whether according to D1 the generation of a single splicing product is mandatory or not, as long as the skilled person is able to derive this teaching from D1. It should be noted that Figure 4 of D1 represents only a specific embodiment of the teaching of this document.

15. Summarizing the above: no technical details or examples for either the specific method claimed or the technical effect alleged to be achieved by this method (ie repair or correction of a mutated sequence) are provided in the application. Thus, it cannot be said that the actual technical contribution to the state of the art by the disclosure of the application consists in providing experimental support for a particular
application of the general trans-splicing method known from the prior art. Rather, the technical contribution of the invention as claimed consists in the mere suggestion to apply a general technique known from the prior art to a specific target sequence arbitrarily chosen.

16. The board shares also the view of the examining division that the skilled person would not expect any complications when applying the trans-splicing method described in D1 to a mutant beta-globin sequence in order to replace the mutated sequence by a wild-type sequence. The appellant has failed to demonstrate plausibly that the skilled person would in fact have encountered such difficulties.

17. Consequently, the board concludes that the subject-matter of claim 1 of the main request does not involve an inventive step. Since the additional features specified in the dependent claims 2 to 5 are apparent to the skilled person from the disclosure of D1, also the subject-matter of these claims lacks an inventive step.

Auxiliary request - Inventive step (Article 56 EPC)

18. In the auxiliary request, the claims have been limited to a method for trans-splicing sickle cell beta-globin RNA with wild-type beta-globin RNA using a chimeric ribozyme. This amendment finds its basis on page 29, line 27 of the application, where particular diseases are mentioned as potentially treatable by using targeted trans-splicing to correct defective RNAs.
19. The reasons put forward above for claim 1 of the main request apply equally, mutatis mutandis, to claim 1 of the auxiliary request. Firstly, the RNA sequence encoding sickle cell beta-globin is only one of many possible defective sequences which the skilled person would consider as target for trans-splicing according to the general method disclosed in D1. Secondly, no reasons have been put forward by the appellant as to why the skilled person would have been prevented from doing so in the expectation of objective difficulties. And thirdly, as for claim 1 of the main request, no technical details or experimental support whatsoever are provided in the application for the repair of this particular target sequence by trans-splicing.

20. Thus, the auxiliary request does not fulfil the requirement of Article 56 EPC either.

Article 113(1) EPC

21. The reasons given by the board in the present decision were apparent from the communication sent in preparation for the oral proceedings, and the appellant was given the opportunity to file observations in writing and to put forward its counter-arguments during oral proceedings under Article 116 EPC. Nevertheless, the appellant chose not to file a reply to the board's communication and not to attend oral proceedings. The provisions of Article 113(1) EPC are complied with (see also Article 11(3) RPBA).
Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:    The Chairman:

A. Wolinski     L. Galligani