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Case Number: T 0506/04 - 3.3.08

Application Number: 93902851.0

Publication Number: 0618925

IPC: C07H 21/04

Language of the proceedings: EN

Title of invention:
Antisense oligonucleotides

Patentee:
ISIS PHARMACEUTICALS, INC.

Opponents:
Vernalis(R & D) Limited
Santaris Pharma A/S

Headword:
RNase H gapmers/ISIS

Relevant legal provisions:
EPC Art. 56, 83

Keyword:
"Main request and first auxiliary request - inventive step (no)"
"Second auxiliary request - inventive step (yes), sufficiency of disclosure (yes)"

Decisions cited:
T 0939/92

Catchword:
-

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Case Number: T 0506/04 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 5 December 2006

Appellant: (Patent Proprietor)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 13 January 2004 revoking European patent No. 0618925 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Heath

Summary of Facts and Submissions

I. European patent no. 0 618 925 was granted on the basis of European patent application No. 93 902 851.0 (published as WO 93/13121) and was opposed by two opponents on the grounds of Articles 100(a),(b),(c) EPC. The patent was revoked by the opposition division and the reasons given therefor were that the main and first auxiliary requests contravened Article 54 EPC and that the second auxiliary request contravened Article 56 EPC.

II. The patentee (appellant) lodged an appeal and, with the statement setting out the grounds of appeal, filed a new main request and auxiliary requests I to VI.

III. Opponents 01 and 02 (respondents I and II) replied to the appellant's statement of grounds of appeal.

IV. In a letter dated 10 August 2005, the appellant commented on the respondents' submissions and filed a new main request and new auxiliary requests I to V in replacement of the previous requests.

V. With the summons to the oral proceedings, the board sent a communication to the parties under Article 11(1) of the Rules of Procedure of the Boards of Appeal (RPBA) (OJ EPO 2003, 89), wherein they were informed of the board's preliminary opinion on the relevant issues.

VI. The appellant and the respondents replied to this communication and the former filed new auxiliary requests II and IV in replacement of the corresponding previous requests.

VII. Oral proceedings took place on 5 December 2006.

VIII. Claim 1 of the **main request** read as follows:

"1. An oligonucleotide comprising a sequence of nucleotide units capable of specifically hybridizing to a strand of nucleic acid, wherein:

at least one of said nucleotide units is a nucleotide having a nuclease resistant internucleotide linkage to increase nuclease resistance of said oligonucleotide;

a plurality of said nucleotide units bear a 2' substituent group that increases binding affinity of said oligonucleotide to said strand of nucleic acid, said substituent-bearing nucleotides being divided into a first nucleotide unit sub-sequence and a second nucleotide unit sub-sequence; and

at least 5 of said nucleotide units have 2'-deoxy-erythro-pentofuranosyl sugar moieties, said 2'-deoxy-erythro-pentofuranosyl nucleotide units being consecutively located in said sequence of nucleotide units and positioned within the oligonucleotide between the first nucleotide unit sub-sequence and the second nucleotide unit sub-sequence."

IX. The **auxiliary request I** differed from the main request by the length of the nucleotide units having 2'-deoxy-erythro-pentofuranosyl sugar moieties, which instead of "at least 5" read "at least 7".

X. Claim 1 of **auxiliary request II** read as follows:

"1. An oligonucleotide comprising a sequence of phosphorothioate nucleotides capable of specifically hybridizing to a strand of nucleic acid, wherein:

a plurality of said nucleotides bear a 2' substituent group that increases binding affinity of said oligonucleotide to said strand of nucleic acid, said substituent-bearing nucleotides being divided into a first nucleotide unit sub-sequence and a second nucleotide unit sub-sequence; and

at least 5 of said nucleotides have 2'-deoxy-erythro-pentofuranosyl sugar moieties, said 2'-deoxy-erythro-pentofuranosyl nucleotide units being consecutively located in said sequence of nucleotide units and positioned within the oligonucleotide between the first nucleotide unit sub-sequence and the second nucleotide unit sub-sequence."

Claims 2 and 3 related to embodiments of claim 1 which further defined the 2'-substituent group. Claim 4 was concerned with an oligonucleotide as in claim 1 with specific 2' substituents. Claim 5 was directed to an oligonucleotide according to any one of claims 1 to 4 for use in medicine and claims 6 and 7 to the use of an oligonucleotide according to any one of claims 1 to 4 in the preparation of a composition for the treatment of a disease.

XI. The following documents are cited in the present decision:

D1: E. Uhlmann and A. Peyman, Chemical Reviews, Vol. 90, No. 4, pages 544 to 584, June 1990;

D2: S. Shibahara et al., Nucleic Acid Research, Vol. 15, No. 11, pages 4403 to 4415, 1987;

D3: S. Shibahara et al., Nucleic Acid Research, Vol. 17, No. 1, pages 239 to 252, 1989;

D4: R.S. Quartin et al., Nucleic Acid Research, Vol. 17, No. 18, pages 7253 to 7262, 1989;

D8: WO-A-91/12323 (publication date 22 August 1991);

D9: H. Inoue et al., Vol. 215, No. 2, pages 327 to 330, 1987;

D11: WO-A-91/10671 (publication date 25 July 1991);

D14: S. Agrawal et al., Proc. Natl. Acad. Sci. USA, Vol. 87, pages 1401 to 1405, 1990;

E3: B.P. Monia et al., J. Biol. Chem., Vol. 268, No. 19, pages 14514 to 14522, 1993;

XII. The appellant's arguments insofar as relevant to the present proceedings may be summarized as follows:

Main request
Article 56 EPC

Since the prospects of antisense inhibition at the priority date of the opposed patent were rather low, the selection of RNase H cleavage for this purpose represented already in itself a non-obvious step. Although there was a lot of information on nucleotide

modifications (*inter alia* document D1), the specific claimed subject-matter was not suggested in the prior art and it could be achieved only with hindsight.

Document D8, which was not concerned with antisense inhibition but with the production of a modified protein, disclosed the cleavage of a target nucleotide sequence and the ligation of this cleaved target sequence by a ligase. It did not merely aim at cutting a target sequence but at cutting out (excising and, optionally, replacing) and ligating the cleaved sequence. The document did not suggest that, once the target sequence had been cleaved, it was essential to leave the resulting gap open for further degradation of the target nucleotide. However, this degradation was essential for achieving antisense inhibition. Document D8 and the claimed subject-matter had thus different aims that gave rise to different structural designing parameters. The features of the claimed subject-matter could not be envisaged in the context of document D8, which required a very specific RNase H cleavage of the target nucleotide sequence and to keep the resulting gap small in order to satisfy the steric requirements of a ligase. This latter requirement was not important in the opposed patent, since only the RNase H cleavage was essential for the degradation of the target sequence. No constraints other than the ones of the RNase H cleavage were associated with the claimed subject-matter.

If, however, document D8 was taken as closest prior art, the technical problem to be solved was the provision of improved gapmers for therapeutic purposes. This problem was solved by the claimed gapmers. Figure 1 of the

opposed patent showed the advantageous effects of these modified gapmers achieved by the presence of 2' substituent groups and of at least one internucleotide phosphorothioate (PS) linkage. The patent provided guidance to select the essential elements and to obtain these improved gapmers for therapeutic purposes.

The modifications suggested in document D8 were all and always made on the internucleotide linkage. There was no indication to modify the disclosed gapmers at the 2' position. The presence of an hydroxyl group in the deoxynucleotides could not be taken as an indication to introduce other substituents at this 2' position. Deoxynucleotides and ribonucleotides were identified in document D8 as naturally-occurring nucleotides and no emphasis was attached to the hydroxyl group at the 2' position. An interpretation other than this one was the result of hindsight and was not supported by document D8. In the light of the different structural designing requirements referred to above, the selection of bulky substituents at the 2' position (with possible detrimental effects on the activity of a ligase) was not obvious. Nor was it made obvious by other prior art.

Document D1, a general review, referred to four important features for obtaining antisense inhibition and further stated that the development of antisense oligonucleotides involved walking a tightrope, i.e. the positive modification of one of these features could result in an overall negative effect. Although 2' substituents were described as improving the resistance to nucleases, the introduction of these groups could also result in a disadvantageous binding affinity and specificity. Since resistance to nucleases was not

addressed in document D8, there was no motivation to carry out such a modification let alone in the flanking regions of the disclosed gapmers. However, even if it was contemplated, there was no reason to expect that it would lead to any improvement. On the contrary, the presence of bulky substituents at the 2' position could impair the binding affinity and become a hindrance for ligase activity. In fact, there was no evidence on file showing that this modification was compatible with the activity of a ligase.

There was post-published evidence on file showing that the authors of document D8 considered the therapeutic effects obtained with 2' substituents as surprising, although they had been aware of other prior art using this modification for a different purpose (production of universal restriction endonucleases, document D9). It was only with hindsight that document D9 could be read in combination with document D8 and thereby one could consider that this combination made gapmers with 2' substituents available for therapeutic purposes.

This was even more so since the claimed subject-matter comprised a combination of two modifications, namely 2' substituents and at least one PS linkage. The latter was not evident from document D8 which referred to the stability of a gapmer with mixed phosphate backbone, methyl phosphonate (PC) and phosphorodiester (PO) linkages (PC/PO/PC) and to the lower efficiency of PS-oligomers in comparison to PO-oligomers. There was therefore no reason to exchange the PO linkages for PS linkages. Nor could such a motivation be derived from document D1, which only referred to the disadvantages of PS in comparison to PO or PC, such as a lower

stability, a loss of specificity (resulting in non-specific RNase H cleavage and production of multiple falling-off fragments) and a lower penetration through (cellular) membranes. Whereas the former two features were critical for ligase activity and the production of altered proteins, the latter feature was critical for therapeutic purposes. Thus, document D1 did not render the claimed subject-matter obvious in the context of document D8. Nor was it rendered obvious by other prior art, in particular documents D2 and D9 (cited in document D1), which were both interested in having a narrow gap (a very specific RNase H cleavage) and did not give any motivation for exchanging the PO linkages for PS linkages.

Auxiliary request I
Article 56 EPC

If document D8 was taken as closest prior art, the technical problem to be solved remained the same as for the main request and it was solved by the claimed subject-matter. Documents D8 and D14 showed that (for the production of a desired altered protein) the best results were obtained using a gapmer with an internal segment of 6 nucleotides (6-gap). No motivation could be derived from these documents for enlarging this internal gap. On the contrary, there was an incentive to keep it as small as possible so as to maintain the RNase H sterically constrained and avoid it to cleave the target sequence at other non-specific sites (which, after ligation, could result in non-desired altered proteins). Both documents showed that a 6-gap was large enough for a RNase H to cleave a target sequence and short enough for the enzyme to be constrained. Moreover,

no other prior art on file provided such a motivation for enlarging this internal 6-gap, since a specific cleavage of the target sequence was always considered to be of advantage, if not essential (universal restriction endonucleases, documents D2 and D9).

For the opposed patent, however, the specificity of the RNase H cleavage was not relevant, since the cleaved target sequence was destroyed. No advantages were associated therefore with the maintenance of steric constraints on the RNase H. On the contrary, if no constraints were present, the RNase H cleaved at more sites and thereby, further advantageous degradation of the target sequence followed. This was shown by the opposed patent, which disclosed an optimal internal gap of at least 7 nucleotides, and it was also supported by post-published evidence on file (document E3).

Auxiliary request II
Article 56 EPC

The claimed subject-matter comprised "*a sequence of phosphorothioate nucleotides*" with no internucleotide linkages other than PS linkages. The presence of additional 5' and 3' flanking sequences with other internucleotide linkages did not prevent the 2' substituted PS gapmer (the gist of the invention) to achieve the advantageous inhibitory effects shown in the opposed patent. Starting from document D8 as closest prior art, the technical problem was the same as for the main request. The claimed 2' substituted PS gapmers were not a mere alternative to the gapmers disclosed in document D8 but improved gapmers for (antisense) therapeutic purposes.

As argued for the main request, the introduction of 2' substituents or of a complete PS backbone, let alone a combination of both modifications, was not obvious from document D8. There was no reference in this document to nuclease resistance, binding efficiency or cellular membrane penetration. Moreover, PS linkages were disclosed as having a lower stability than PO linkages, and, at the flanking regions, PC linkages had a better binding efficiency than PS linkages. This was in line with other prior art on file (document D1), which referred to PS linkages as providing a lower binding efficiency and specificity as well as a poor cellular membrane penetration.

Thus, even if admitting that the introduction of 2' substituents was obvious from document D8 (which was not), there was nothing in that document nor in the prior art to motivate the skilled person to add another modification, namely PS linkages at both the internal segment and flanking regions. This was all the more so since these PS linkages were considered particularly disadvantageous for therapeutic purposes.

Article 83 EPC

The opposed patent made available the inventive concept of the invention and provided a list of possible 2' substituents. These substituents were easy to test with methods and means known to the skilled person. Once the concept and guidance were available, optimization of the invention (best modes) under different conditions fell within the normal competence of the skilled person as shown by post-published document E3. No evidence was

on file raising serious doubts on the therapeutic efficiency of the 2' modified PS gapmers of the patent. Nor could those doubts be credibly raised for the 2' substituents known from the prior art. It did not amount to undue burden and it did not require any inventive skill to test those substituents and verify the results (binding efficiency and antisense inhibition) obtained.

Document D11 was not relevant since it did not disclose any gapmer with a combination of 2' substituents and PS linkages. Table II of document E3 showed an improved efficiency of binding for all 2' substituents and, except for one of them (O-pentyl), improvement in the antisense inhibition was always measured. Respondents' assessments were merely speculative and had no support in the prior art.

XIII. The respondents' arguments insofar as relevant to the present proceedings may be summarized as follows:

Main request
Article 56 EPC

The closest prior art document D8 disclosed gapmers of mixed phosphate backbone comprising an internal segment (with internucleotide (PO, PS) linkages capable of activating a RNase H to a target nucleotide sequence) and 5' and 3' flanking regions (with internucleotide (PC) linkages that were not capable to activate a RNase H). Table 2 showed a gapmer (M) with an internal segment of 6 nucleotides (6-gap). Thus, document D8 disclosed the same basic concept, effects and purpose as the opposed patent. The flanking sequences were

defined as "*deoxyribonucleotides or ribonucleotides and their modifications*", thereby providing a motivation to look for those modifications.

Starting from this closest prior art, the technical problem to be solved was the provision of alternative (modified) gapmers. The claimed subject-matter, however, did not solve this problem. Firstly, the possible presence of PO linkages within the claimed gapmers rendered them non-resistant to nucleases and therefore, highly unstable. Secondly, the scope of claim 1 was so broad that it embraced a large number of non-functional gapmers. The alleged effect was not credible for all the alternatives claimed (T 939/92, OJ EPO 1996, 309).

The modification of the gapmers disclosed in document D8 at the 2' position was obvious in the light of the prior art. Firstly, document D8 itself already disclosed a modification at this position, since the flanking sequences of the gapmers were referred to therein as admitting ribonucleotides as well as deoxyribonucleotides. The latter had a modification (hydroxyl group) at the 2' position with respect to the former. Thus, there was a clear indication to modify the gapmers at this 2' position. This was all the more so since modifications at the 2' position were known to provide important advantages, such as improved thermal stability and binding efficiency (document D1, a review article representing the common general knowledge).

Document D8 referred to the further (optional) ligation of the cleaved target nucleotide but there was no reference to any requirement associated with the (optional) action of a ligase. Nor was any evidence on

file showing that this enzyme imposed any particular constraint other than the ones known for the RNase H. Document D14 (same authors and gapmers as for document D8) referred to RNase H antisense inhibition and there was no reference to a ligase let alone to constraints associated therewith. None of these documents supported a bias or prejudice against the use of a ligase in the presence of modifications at the 2' position. There was no indication in the prior art that could lead the skilled person to perceive this modification as being disadvantageous when using a ligase. In fact, document D14 explicitly referred to document D9, which was only concerned with 2' modified gapmers. The opposed patent did not exclude the use of the claimed subject-matter as substrate for a ligase and it did not show that a modification at the 2' position of a gapmer introduced any hindrance for a ligase activity.

Internucleotide PS linkages could not be seen as disadvantageous, since document D8 explicitly referred to their use. The alleged disadvantages of PS linkages were due to the nature of the nucleotide used (inosine homo-oligomers) but not to the PS linkages per se.

Auxiliary request I
Article 56 EPC

The closest prior art and the technical problem to be solved remained the same as the main request as well as the reasons for which the claimed subject-matter was not considered to solve this problem. The patent showed gapmers with internal segments longer than 7 nucleotides (9-gap) and for which the results obtained were worse than the ones obtained with shorter length.

The claims embraced subject-matter that did not provide any improvement and they embraced non-functional embodiments (gapmers with long internal segments).

There was nothing in document D8 identifying the (optimal) 6-gap as an essential requirement of the disclosed gapmers. On the contrary, gapmers with shorter (2, 4 nucleotides) internal segments were described as eliciting RNase H cleavage (albeit at lower levels) and Figure 1 depicted a gapmer with an internal segment of 8 nucleotides (8-gap). Document D8 taught that the length of the internal gap had to be long enough for a RNase H to cleave a target nucleotide but it could be different depending on the system and conditions used. The design of a gap length optimal for the (human, E. coli) system used was a matter of routine that did not require any inventive skill.

Document D8 was silent on the specificity of the RNase H, which was only required to cleave anywhere within the internal segment of the disclosed gapmers. There was no other requirement associated with the length of the internal segment. In certain cases, the excision of multiple or large fragments from a target nucleotide sequence could be of interest and it could be easily achieved by multiple, unspecific RNase H cleavages of this target sequence. Document D8 did not show any bias against the lengthening of the internal segment (longer than 6 nucleotides). There was also evidence on file showing that, irrespective of what use was made of the disclosed gapmers (site-specific cleavage or antisense inhibition of a target sequence), the requirements associated with the length of the internal segment were the same (document E3).

Auxiliary request II
Article 56 EPC

The closest prior art and the technical problem to be solved remained the same as for the main request. Claim 1 of this second auxiliary request still comprised a large number of non-functional gapmers which did not solve this problem (cf. T 939/92, *supra*). The reason therefor was attributed to the absence of limitations on three of the features characterizing the claimed gapmers, namely the length of the internal segment, the complete length of these gapmers and the non-exclusion of PO linkages in the flanking regions.

Figure 1 of the opposed patent showed that for a 17-gapmer with PS linkages, 2'-O-methyl substituents in the flanking sequences and an internal segment of 9 nucleotides (9-gap) (oligo 3984 in Table 1), the antisense inhibition obtained was similar to the one obtained with a control oligonucleotide (oligo 2570). Significant inhibition was only obtained with 17-gapmers of a 5-gap or 7-gap. Post-published evidence on file also referred to the importance of the gap length and gap position within the flanking sequences (document E3). However, these essential features were not present in the claims. Nor did they define a complete length for the claimed gapmers. Thus, they comprised very short gapmers (7-gapmer) with short internal segments and flanking sequences that were known to be unspecific and unsuitable for the (therapeutic) purpose of the opposed patent. Moreover, the claimed gapmers did not exclude the presence of additional nucleotides with PO linkages in their

flanking sequences. However, these PO linkages made these gapmers highly unstable (nuclease degradation) and rendered them unsuitable for therapeutic purposes.

Moreover, in the light of the prior art, the claimed subject-matter was also obvious. Document D1 (by explicitly referring to document D3) disclosed the advantages of combining internucleotide PS linkages with 2'-O-methyl modifications. There was no evidence on file showing that the claimed PS gapmers were advantageous over gapmers with mixed phosphate backbone (PC linkages in flanking regions and PS or PO linkages in internal segment). Thus, the claimed subject-matter was merely an obvious alternative - among all the readily available workshop alternatives suggested in document D1 - of the gapmers disclosed in document D8.

Article 83 EPC

Document D1 acknowledged that the synthesis of functional antisense oligonucleotides involved walking a tightrope since the introduction of a modification with a positive effect on one property could result, however, in negative effects on other properties. The claimed subject-matter relied only on mere functional definitions of the desired features but there was no indication on how to achieve them. Nor was any further guidance provided by the opposed patent as a whole, which disclosed a single very specific example (17-gapmer with 2'-O-methyl substituents). This was not enough for the skilled person to achieve the whole broad area covered by the claims without undue burden.

The opposed patent disclosed only 17-gapmers and failed to provide enough information on the means required to achieve appropriate stability and binding efficiency as well as the desired specific antisense inhibition when using small gapmers (7-gapmer). The prior art, however, showed that these short gapmers could never be used for therapeutic purposes since they were highly unstable (at human body temperature) and totally unspecific (huge size of human genome).

Evidence was on file showing that the mere reference in the claims to a 2' substituent with increased binding affinity and the mere recitation of known 2' substituents from the prior art was not enough for achieving antisense inhibition without undue burden. Post-published document D11 showed that none of the known 2' substituents provided an increased binding efficiency for a 21-oligomer (Table 2). Whereas no effect was shown for a 2'-fluoro substituent in this 21-oligomer, the same substituent showed very different effects on the binding efficiency of oligonucleotides of different composition (Table 3). Likewise, Table II of post-published document E3 showed that for a 17-gapmer with PS linkages and an internal segment with 7 nucleotides (7-gap), introduction of a 2'-O-pentyl substituent provided only a slightly higher stability (binding efficiency) and no improvement in the antisense inhibition when compared with a control (deoxy)oligonucleotide. The desired antisense inhibition was achieved only at random and not directly as a result of the combination of the two defined modifications (2' substituents and internucleotide PS linkages). According to the established case law, a reasonable amount of trial and error was permissible

but the patent had to contain adequate instructions that would lead the skilled person directly towards success after the evaluation of initial failures. These instructions were not given in the opposed patent.

XIV. The appellant (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of either the main request or auxiliary request I, both filed on 10 August 2005, or auxiliary request II filed on 3 November 2006, or auxiliary request III filed on 10 August 2005, or auxiliary request IV filed on 3 November 2006, or auxiliary request V filed on 10 August 2005.

XV. The respondents (opponents) requested that the appeal be dismissed.

Reasons for the Decision

Main request, first and second auxiliary requests
Articles 123(2)(3), 84 and 54 EPC

1. No formal objections have been brought forward by the respondents with regard to Articles 123(2),(3), 84 and 54 EPC for the main request and for the first and second auxiliary requests. Nor does the board see any reason to do so. Therefore, the requirements of these articles are considered to be met.

Main request
Article 56 EPC

The claimed subject-matter

2. The opposed patent refers to two antisense mechanisms for disrupting the function of cellular nucleic acids and for treating thereby diseases characterized by the undesired production of a protein, namely a first mechanism based on hybridization to a targeted RNA (hybridization arrest) and a second mechanism based on the enzymatic cleavage of a targeted RNA by intracellular RNase H. The opposed patent claims in general terms an oligonucleotide (gapmer) to be used in this second approach, which is characterized by the combination of three features, namely i) the ability to elicit RNase H (by using 2'-deoxy-erythro-pentafuranosyl sugar moieties that result in the formation of DNA-RNA duplexes), ii) the presence of nuclease resistant (PS, phosphorothioate) internucleotide linkages so as to survive in a cell for a time sufficient to activate RNase H, and iii) an improved hybridization (by using 2' substituent groups) to the targeted RNA. At least 5 2'-deoxy-erythro-pentafuranosyl sugar moieties (defining the internal gap) are placed within a first and second flanking nucleotide subsequences containing a plurality of 2' substituent groups. These oligonucleotides are said to activate a RNase H enzyme upon hybridization to a targeted RNA while concurrently having an increased resistance to cellular nucleases and improved hybridization properties (binding affinity).

The closest prior art

3. Several criteria have been defined by the Boards of Appeal for the selection of the closest prior art. In particular, it has been defined as being a document that discloses subject-matter conceived for the same purpose as the claimed invention and which has the most relevant technical features in common or else as being a document that constitutes the most promising starting point for an obvious development leading to the claimed invention (cf. "Case Law of the Boards of Appeal of the EPO", 4th edition 2001, I.D.3 and I.D.3.5, pages 102 and 104). In any case, the Boards have also established that it must be avoided to interpret the prior art being influenced by the problem solved by the invention if this problem is neither mentioned nor even suggested in this prior art (ex post facto analysis) (cf. "Case Law", *supra*, I.D.6.1, page 116).

4. Document D8 is considered to represent the closest prior art since it discloses mixed phosphate backbone oligonucleotides comprising an internal segment of deoxynucleotides (eliciting RNase H) flanked on each side by sequences of modified deoxyribonucleotides or ribonucleotides unable to activate RNase H. The length of the internal segment is functionally defined as being "*capable of activating RNase H*" (cf. page 9, lines 7 to 13) and a pentadecamer having six consecutive (6-gapmer) deoxynucleotides with phosphodiester (PO) internucleotide linkages - and flanking sequences with methyl phosphonate (PC) linkages - is referred to as eliciting "*complete RNase H cleavage of the substrate RNA*" and as being the best oligomer in the exemplified conditions (HeLa

nuclear extracts) (cf. page 25, lines 23 to 28, oligomer M in Table 2). Document D8 also refers to the importance of the total length of the (gapmers) oligonucleotides for hybridization to the target RNA segment (cf. *inter alia* page 5, line 25 to page 6, line 1 and page 9, lines 2 to 7) as well as to the advantages of an increased hybrid stability (by additional complementary nucleotides in the flanking regions) (cf. page 25, line 28 to page 26, line 6). The internal deoxynucleotide segment is defined as including "*two or more phosphodiester linkages, which may be unmodified or modified*" (cf. page 6, lines 6 to 9), in particular covering "*any phosphate modification capable of activating RNase H, such as phosphorothioates*" (cf. page 9, lines 14 to 17). The flanking nucleotide sequences "*can be deoxyribonucleotides ... or... ribonucleotides and their modifications*" as long as they are not able to activate RNase H (cf. page 6, lines 9 to 18 and page 9, lines 17 to 24).

5. The appellant has argued that document D8 cannot be considered the closest prior art since it is not conceived for the same purpose as the opposed patent, namely antisense inhibition, but for site-directed alteration of mRNA molecules for the production of modified proteins (cf. Section XII *supra*).

The board observes that document D8 contemplates two different embodiments. A first one, wherein a selected nucleotide sequence of a target mRNA molecule is solely removed (excised), and a second embodiment, wherein the removed sequence is replaced by another (desired) nucleotide sequence (cf. *inter alia* page 2, lines 14 to

26, page 3, lines 20 to 31 and page 7, lines 1 to 23). The first step, i.e. the cleavage of a target RNA by RNase H, is shared by both embodiments. Document D8 discloses the essential requirements as well as detailed instructions for carrying out the first cleavage step, which is also the only one exemplified in the document. However, apart from very general references to (appropriate endogenous) ligases, there is no other information on the ligation step (cf. *inter alia* page 10, lines 4 to 8, page 15, lines 16 to 21). Although document D8 refers to the alteration of proteins, the gist of its disclosure is the cleavage of a target RNA by RNase H, which is also the gist of the claimed subject-matter. Therefore, the board considers that document D8 has the same immediate purpose as the patent in suit and that it is also the most appropriate closest prior art. In this respect, it is also noted that the use of gapmers for RNase H cleavage of a target RNA in order to obtain antisense inhibition was already described in the prior art (cf. document D14) and thus, it cannot be seen *per se* as a significant contribution.

6. The board also considers that, although not exemplified, document D8 as a whole makes available to the skilled person an oligonucleotide (gapmer) with an internal deoxynucleotide segment capable of activating a RNase H and having more than five nucleotide units connected by phosphorothioates (PS) - an internucleotide linkage known to be nuclease resistant (cf. page 548, paragraph bridging left- and right-hand columns in document D1, a review article reflecting the common general knowledge of the skilled person, "Case Law", *supra*, II.A.2(a), page 145) - and flanking regions which are unable to

activate a RNase H. Thus, the sole structural difference between the claimed oligonucleotides and the ones of document D8 is the presence in the flanking regions of 2' substituent groups that increase the binding affinity of the oligonucleotide to the target nucleic acid.

The technical problem to be solved

7. Starting from this closest prior art, the technical problem underlying the patent in suit is considered to be the provision of alternative oligonucleotides (gapmers) to be used for RNase H cleavage of a target RNA.

8. The examples of the patent in suit show that at least part of the claimed subject-matter provides a true solution to this technical problem. In the light of the conclusion achieved below on obviousness, the board does not consider it necessary to assess at this point whether or not this solution is achieved throughout the whole scope of the claims.

Obviousness of the claimed solution

9. As stated at the end of point 4 above, document D8 refers several times to possible modifications of the flanking nucleotide sequences and, although only internucleotide bridging phosphates are explicitly mentioned (methyl phosphonates, phosphoromorpholidates, etc.), other appropriate modifications (i.e. unable to activate RNase H) are not excluded. Thus, document D8 itself provides the skilled person with a motivation to look for modifications.

10. Contrary to the respondents' position (cf. Section XIII *supra*), the board does not consider that the mention in document D8 of deoxyribonucleotides (with an hydroxyl group at 2' position in comparison to ribonucleotides) provides an explicit hint to modify the 2' position of the flanking nucleotide sequences. Nowhere in document D8 are those deoxyribonucleotides disclosed as modified ribonucleotides. Both nucleotides are disclosed at the same level, i.e. as naturally-occurring nucleotides, and when modifications are referred to, this is always made in connection with both types of nucleotides, such as "*may be deoxyribonucleotide or ribonucleotide sequences and is modified*", "*can be deoxribonucleotides ... or can be ribonucleotides and their modifications*" (cf. page 6, lines 9 to 11 and page 9, lines 17 to 20).

11. Document D1, which represents the common general knowledge of the skilled person (cf. point 6 *supra*), reviews the RNase H mechanism and refers to the possible use of "*suitably modified or chimeric oligodeoxynucleotides/oligoribonucleotides*" for inducing "*a predictable site-specific cleavage of RNA*" with cross-references to the gapmers of documents D2 and D9, which both have 2'-O-methylribonucleotides as flanking nucleotide sequences (cf. page 572, right-hand column to page 573, left-hand column). No inventive skill is required in order to combine these 2' substituted flanking nucleotide sequences with an internal segment as described in document D8, i.e. with PS internucleotide bridging phosphates (cf. point 6 *supra*). Thereby the claimed subject-matter is achieved in a straightforward and obvious manner.

Reasonable expectation of success

12. According to the established case law of the Boards of Appeal, when a suggested approach is obvious to the skilled person, it is still necessary to assess whether or not a reasonable expectation of success is also given (cf. "Case Law", *supra*, I.D.6.2, page 117). Two main reasons have been put forward against the presence of this reasonable expectation of success (cf. Section XII *supra*), namely i) the structural changes caused by the introduction of 2' substituents and the resulting implications for the hybridization with the target molecule and for the action of a ligase (hindered by steric impediments), and ii) the known disadvantages of using phosphorothioates.

13. The quoted documents D2 and D9 disclose the formation of stable duplexes of gapmers having 2'-O-methylribonucleotides in their flanking nucleotide sequences with complementary nucleotides, and their use for directing the RNase H site-cleavage of these complementary molecules (cf. page 4403, last paragraph of document D2 and page 327, right-hand column of document D9). There is no reference to these 2' substituents as causing any particular steric problem or hindrance for the desired hybridization, which is also in line with the known advantageous (thermal stability) properties of these 2' substituents (cf. page 558, paragraph bridging right- and left-hand columns of document D1).

14. Both documents D2 and D9 are silent on the actual fate of the (RNase H) cleaved target nucleotide, which might

well be used to insert an heterologous sequence (as it is sometimes the case for nucleotides specifically cleaved by using known restriction endonucleases) or else partially degraded and replaced by an heterologous sequence. There is no prior art on file suggesting, let alone showing, that the 2' substituents might represent a relevant (steric) hindrance for such a purpose. Nor is this suggestion to be drawn from document D8 itself, since - as outlined above (cf. point 5 *supra*) - this document is completely silent on the requirements needed for a ligase to act. Moreover, document D8 itself also contemplates the use of other possible bulky substituents, such as methyl phosphonates and phosphoramidates, although admittedly in a different position.

15. Although the low stability of phosphorothioate hybrids was already known in the prior art (cf. page 572, right-hand column, last paragraph in document D1) and also acknowledged in the examples of document D8 (cf. page 23, line 14 to page 24, line 6), the fact remains that phosphorothioates are explicitly given in document D8 as an appropriate alternative for the internal segment of the disclosed gapmers (cf. page 9, lines 14 to 17). Although they have some disadvantageous properties, the use of these internucleotide linkages might well be, under certain conditions, of advantage due, among others, to their stability to nucleases and retention of solubility in water (cf. page 548, paragraph bridging right- and left-hand columns in document D1). Therefore, the use of phosphorothioates linkages in the internal segment of the gapmers would not be disregarded by the skilled person and it

represents a serious alternative to the phosphodiester linkages also indicated in document D8.

16. It follows from the above that the positive expectations of the skilled person based on the combination of the disclosure of document D8 with the 2' modifications known from the prior art would not have been lessened by any of the problems alleged by the appellant. Therefore, the board considers that the claimed subject-matter does not fulfil the requirements of Article 56 EPC.

Auxiliary request I
Article 56 EPC

17. Since the sole difference between this first auxiliary request and the main request is the length of the nucleotide units having 2'-deoxy-erythro-pentofuranosyl sugar moieties (cf. Section IX *supra*), both the closest prior art and the technical problem to be solved are considered to be the same as for the main request (cf. points 3 to 7 *supra*). Likewise, the examples of the opposed patent show that at least part of the claimed subject-matter provides a true solution to the technical problem. However, this solution - as shown below - is considered to be obvious. Therefore, and as for the main request, the board refrains at this point from assessing whether or not this solution is achieved throughout the whole scope of the claims.

18. Document D8 acknowledges that "*the internal segment must be of sufficient length ... to be capable of activating RNase H*" and refers to a minimum length of two bases from studies of the prior art using RNase H

from E. coli (cf. page 9, lines 7 to 13). A 15-gapmer with PC linkages within the flanking sequences and an internal segment containing six consecutive PO linkages elicits complete RNase H cleavage of the substrate RNA when using human RNase H from HeLa cell nuclear extracts (cf. page 25, lines 20 to 28). The presence of different optimal cleavage conditions and particular requirements for RNase H enzymes of different sources (human and E. coli) is already reported in the prior art (cf. paragraph bridging pages 1404 and 1405 of document D14), which also refers to the length of the internal segment of similar gapmers as being the result of a compromise between the ability to elicit the RNase H enzyme (long enough) and the resistance to other nucleases (short enough) (cf. page 7260, last full paragraph in document D4). Thus, nothing of inventive significance can be seen in the determination of the optimal length of the internal segment of a gapmer, which under certain conditions might well be of 7 nucleotide units or even longer. In fact, Figure 1 of document D8 already discloses a gapmer with an internal segment longer than 7 nucleotide units (8-gapmer). The fact that it is not exemplified and no results are reported cannot bring to a different conclusion.

19. For the sake of completeness, it is noted that document D8 does not refer to any particular requirement for a ligase to act (cf. point 5 *supra*) and therefore, the appellant's argument that a short internal segment would be considered essential for the purpose of document D8 cannot be followed (cf. Section XII *supra*). The only steric constraint directly derivable from document D8 is the one required for RNase H cleavage. There is no other information so as to assess whether a

short (rigid) or else a long (flexible) internal segment is of any advantage to the action of a ligase. Nor is such information derivable from the prior art and/or the evidence on file.

20. Thus, the claimed subject-matter does not fulfil the requirements of Article 56 EPC.

Auxiliary request II
Article 56 EPC

21. The (gapmer) oligonucleotide claimed in this request comprises a sequence of nucleotides which is entirely phosphorothiolated (PS), a plurality of the nucleotides in the flanking subsequences bearing a 2' substituent group and the internal sequence of at least 5 nucleotides between the two subsequences having 2'-deoxy-erythro-pentofuranosyl sugar moieties (cf. Section X *supra*).

22. In the light of this subject-matter, the board considers that both the closest prior art, document D8, and the technical problem to be solved, namely the provision of alternative gapmers, are the same as for the main request (cf. points 3 to 7 *supra*). This technical problem is considered to be solved by the claimed subject-matter. It has been argued, however, that this solution is not achieved throughout the whole scope of the claims since there is no limitation for some of the features characterizing this subject-matter, in particular the length of the internal segment and of the complete (gapmer) oligonucleotide and the possible presence of PO linkages in the flanking sequences (cf. Section XIII *supra*).

Scope of the claims

23. As stated above for the first auxiliary request (cf. point 18 *supra*), the determination of the optimal length of the internal segment of a gapmer (for a particular RNase H in a particular system) is described in the prior art, which refers to a compromise between the ability to elicit the particular RNase H enzyme and the resistance to other nucleases present in the particular system used. The opposed patent shows that, when using RNase H (human HeLa nuclear extracts) and the exemplified 17-gapmers, the greatest inhibition is found with an internal segment of 7 nucleotides long, whereas worse results are achieved with an internal segment of 9 nucleotides (cf. paragraph [0091] and Figure 1). Likewise, the requirements to determine the appropriate length of antisense oligonucleotides (for a particular target nucleotide within a particular system, such as the human genome) are also common general knowledge, such as the ability to hybridize specifically with the target sequence, the passage through cellular membrane, etc. (cf. *inter alia* page 544, right-hand column, lines 26 to 30 and page 576, right-hand column, full paragraph in document D1), and they all lead to a range which corresponds to the one indicated in paragraph [0061] of the opposed patent, i.e. "*from about 10 to about 30 nucleotide or nucleobase subunits*" (cf. page 10 of the patent).

24. Thus, both the length of the internal segment and of the complete gapmer might be selected in accordance with the specific RNase H and the particular system used, such as an *in vitro* system without presence of

other nucleases and with few sequences other than the target nucleotide sequence. Since document D8 also contemplates the use of the disclosed gapmers in such cell-free systems (cf. page 4, lines 6 to 9), the actual scope of the present claims provides valid alternative gapmers that might be successfully used in those systems as well.

25. Although oligonucleotides comprising nucleotide sequences with PO linkages flanking a "*sequence of phosphorothioate nucleotides*" as defined in the claims are not explicitly disclosed in the opposed patent, they are, however, embraced by the claims and it has been argued that they do not solve the technical problem (cf. Section XIII *supra*). The presence of additional (modified or unmodified) nucleotides might certainly influence, under certain conditions and depending on the actual nature of these nucleotides, the overall properties of the claimed oligonucleotides, such as the stability to nucleases, the penetration through (cellular) membranes, the water solubility, etc. Nevertheless the fact remains that there is no evidence on file showing that the presence of these additional flanking sequences with PO linkages has a negative or detrimental effect on the "*sequence of phosphorothioate nucleotides*" in the sense that they prevent or hinder the internal segment to elicit the RNase H enzyme and the cleavage of a target nucleotide sequence. In the absence of this evidence, the board considers this argument as a mere assumption without proper basis.

26. Thus, in the light of the technical contribution of the opposed patent to the prior art, the board considers the scope of the claims to be justified.

Non-obviousness of the claimed subject-matter

27. Document D8 refers to the use of PS linkages only and exclusively for the internal segment since these linkages are able to elicit RNase H, an essential property which is to be strictly avoided in the flanking sequences (cf. *inter alia* page 6, lines 14 to 18). Thus, although in the light of document D8 the modification of the flanking sequences by introduction of 2' substituents has been considered to be obvious (cf. point 11 *supra*), the additional introduction of PS linkages in the flanking sequences is not derivable from document D8 nor from any other prior art document on file. There is no motivation in this prior art for adding yet one further modification (PS linkages) to the obvious one (2' substituents).

28. It has been argued by the respondents that document D1 discloses the stabilization of 2'-O-methyloligoribonucleotides (by a combination with phosphorothioate residues) which is necessary because of their partial instability to nucleases (cf. paragraph bridging pages 566 and 567 and page 567, left-hand column, first full paragraph in document D1). In this context reference was made also to document D3, which compares the (HIV) antisense inhibitory effect of several oligo(2'-O-methyl)ribonucleotides. Based on the results achieved with a patched derivative (HSOS-2) and a derivative with a full-length phosphorothioate backbone (HS-2b), it is concluded, however, that "*all their thiophosphate linkages are not necessary for this kind of oligomers to exhibit anti-HIV activity*" (cf.

page 245, Figure 1 and page 247, full paragraph in document D3).

29. The board notes that this reference to document D3 is found when describing the general properties of antisense oligonucleotides and that, in this context, reference is also made to the advantageous (nuclease) stability of other phosphate backbones, such as methylphosphonates and phosphoroamidates (cf. page 566, right-hand column, document D1), which are also cited in document D8 (cf. page 6, lines 11 to 14). There is, however, no reference in document D1 to the relevance of this disclosure when addressing the specific RNase H mechanism (cf. page 571, document D1) nor there is in document D3 any indication on the relevance of these studies for gapmers involving the RNase H mechanism (although these is an explicit reference to these gapmers in document D3, cf. paragraph bridging pages 4403 and 4404). There is no indication about the possible (conformational and steric) effects of this combined modification (PS linkage and 2' substituents) on the internal segment (with only PS linkages) of the gapmer and its ability to elicit the activity of RNase H. In the light thereof, the board is convinced that hindsight is required to combine all these disclosures so as to achieve the specific claimed subject-matter.

30. Thus, the claimed subject-matter is not obvious from the prior art and therefore, fulfils the requirements of Article 56 EPC.

Article 83 EPC

31. It is established case law that the skilled person when considering a claim should rule out interpretations which are illogical or which do not make technical sense. The claims must be interpreted in a technically sensible manner taking into account the whole disclosure of the patent, which itself must be construed by a mind willing to understand not a mind desirous of misunderstanding (cf. "Case Law", *supra*, II.B.4.1, page 168).

32. Although the claims embrace a minimal PS 7-gapmer (a 5-gap internal segment with one nucleotide in the 5' and the 3' flanking sequences) and there is no upper limitation for the length of the flanking regions and the internal segment, the board considers that the disclosure of the opposed patent as a whole and the relevant common general knowledge provide sufficient and appropriate guidance as to how to obtain gapmers suitable for each (*in vitro* or *in vivo*) system used and for every desired purpose (cf. point 23 *supra*). No technical problems should be encountered in the synthesis of these gapmers or in the measurement of their antisense inhibitory effect. Nor have the respondents challenged this or provided any evidence in this respect.

33. Although the position of a 2' modified nucleotide within an oligonucleotide sequence, the overall base composition of this sequence and, in particular, the (sterically bulky) nature of the 2' substituents, might strongly influence the actual contribution of these 2' substituents to the thermal stability (binding affinity)

of the oligonucleotide sequence, it is acknowledged in the art that 2' substituents generally increase the thermal stability of an oligonucleotide sequence (cf. page 558, paragraph bridging left- and right-hand columns in document D1). On the contrary, the presence of phosphorothioate linkages are generally acknowledged as decreasing thermal stability (cf. page 562, right-hand column, second paragraph in document D1). The teachings of document D11 referred to by the respondents do not go much beyond the ones of this common general knowledge since this document only discloses the effects of (more or less sterically bulky) 2' substituents or of PS linkages on the thermal stability of several oligonucleotide sequences of a very particular composition (cf. pages 123 and 125, Tables 2 and 3 of document D11). Nevertheless, there is no gapmer exemplified in document D11 comprising the combined double modification (PS linkages and 2' substituents) of the claimed subject-matter. Nor is it possible to derive directly therefrom any meaningful information on the (positive, negative or random) influence of different (sterically bulky) 2' substituents on the thermal stability of gapmers with only PS linkages.

34. In fact, Table II on page 14521 of document E3 (post-published document, cited as expert opinion) shows that all four disclosed 2' substituents (when introduced in the flanking sequences of a PS 17-gapmer with an internal segment of 7 nucleotides, 7-gap) result, as expected, in a significant increase of the thermal stability. Apart from 2'-O-pentyl, the introduction of all other 2' substituents results in a significant increase of the antisense inhibition under

the particular RNase H system used and the specific experimental conditions disclosed. For the 2'-O-pentyl substituent the very same degree of inhibition as for the control deoxyoligomer sequence is reported.

35. Thus, document E3 demonstrates that with common general knowledge and following the guidance of the opposed patent, the provision of gapmers alternative to the ones disclosed in document D8 does not involve undue burden and is within the grasp of the skilled person. This is all the more so, since the technical problem to be solved is the less ambitious one of providing only alternative gapmers (not improved ones with increased antisense inhibitory effect) (cf. point 22 *supra*) and the description of the opposed patent gives sufficient information to remedy an occasional failure with a reasonable amount of trial and error (cf. "Case Law", *supra*, II.A.4, page 148).

36. Thus, the requirements of Article 83 EPC are considered to be fulfilled.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remanded back to the previous instance with the order to uphold the patent in the form of the auxiliary request II as filed with letter of 3 November 2006 and a description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski L. Galligani