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D E C I S I O N
of 16 December 2003

Case Number: T 1127/00 - 3.3.8

Application Number: 88311816.8

Publication Number: 0321201

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Language of the proceedings: EN

Title of invention:
Ribozymes

Patentee:
GENE SHEARS PTY LIMITED

Opponent:
Ribozyme Pharmaceuticals Incorporated

Headword:
Ribozymes/GENE SHEARS

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 87, 88, 89, 123

Keyword:
"Main request -novelty (no)"
"First auxiliary request - added subject-matter (yes)"
"Second auxiliary request - novelty (yes), inventive step (no)"
"Third auxiliary request - novelty (yes), inventive step (yes), sufficiency of disclosure (yes)"

Decisions cited:
G 0009/91, G 0010/91, G 0002/98, T 0939/92, T 0994/95

Catchword:
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Case Number: T 1127/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 16 December 2003

Appellant: (Opponent)	Ribozyme Pharmaceuticals Incorporated 2950 Wilderness Place Boulder Colorado 80301 (US)
Representative:	VOSSIUS & PARTNER Siebertstrasse 4 D-81675 München (DE)
Respondent: (Proprietor of the patent)	GENE SHEARS PTY LIMITED Administration Building CNR Barry Drive-Clunies Ross Street GPO Box 1600 Canberra, ACT 2601 (AU)
Representative:	Almond-Martin, Carol Ernest Gutmann - Yves Plasseraud S.A. 62 rue de Bonnel F-69448 Lyon Cedex 03 (FR)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted 18 September 2000 concerning maintenance of European patent No. 0321201 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julia
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent No. 0 321 201 with the title "Ribozymes" was granted with 24 claims based on the European patent application No. 88 311 816.8 with priority dates 15 December 1987 (AU 5911/87), 19 August 1988 (AU 9950/88), 9 September 1988 (AU 353/1988), 4 November 1988 (AU 1304/88) and 7 November 1988 (AU 1333/88).

II. A notice of opposition was filed requesting the revocation of the patent under Article 100(a) EPC (lack of novelty and inventive step) and Article 100(b) EPC (insufficiency of disclosure). The opposition division decided to maintain the patent on the basis of the first auxiliary request then on file, whereas the main request was not considered to comply with Article 56 EPC.

III. Notices of appeal were lodged by the patentee (appellant I) and the opponent (appellant II). Each appellant filed additional observations in reply to the statement of Grounds of Appeal of the other appellant.

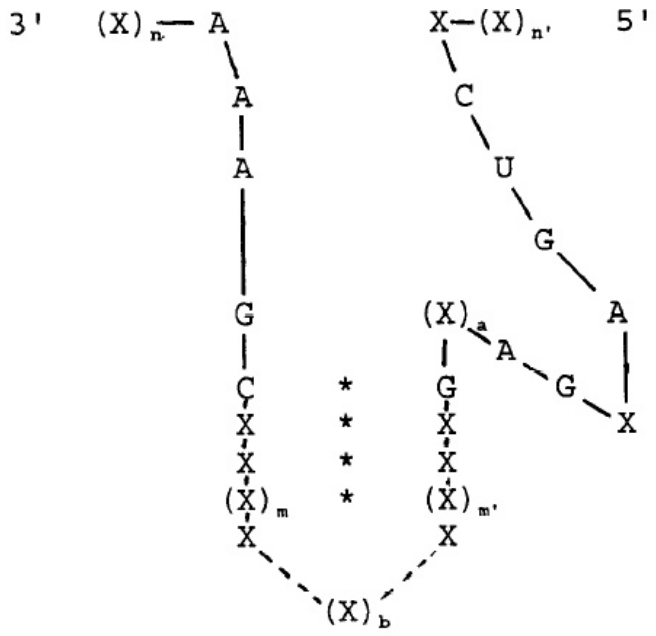
IV. The board summoned the parties to oral proceedings and sent a communication indicating its preliminary opinion.

V. Appellant I filed on 14 November 2003 a new main request and auxiliary requests 1 to 5, and appellant II filed further observations on 12 November 2003. In a subsequent letter, appellant II informed the board of its intention not to attend the oral proceedings.

VI. Oral proceedings took place on 16 December 2003 in the absence of appellant II. During the oral proceedings appellant I filed auxiliary requests 1, 3 and 4 to replace the corresponding auxiliary requests on file.

VII. The **main request** comprised 24 claims which were as the granted claims except that, by adding the sentence "other than a cell in man or animal", claim 16 was intended to exclude in vivo methods. Claim 1 read as follows:

"1. A compound having the formula:



wherein each X represents a ribonucleotide which may be the same or different;

wherein each of $(X)_n$ and $(X)_{n'}$ represents an oligoribonucleotide (a) capable of hybridizing with an RNA target sequence to be cleaved and (b) defined by a predetermined sequence which sequence does not naturally occur covalently bound to the sequences A-A-

A-G-C- and X-C-U-G-A-, respectively, such RNA target sequence not being present within the compound;

wherein each of n and n' represents an integer which defines the number of ribonucleotides in the oligonucleotide with the proviso that the sum of n + n' is sufficient to allow the compound to stably interact with the RNA target sequence through base pairing;

wherein each * represents base pairing between the ribonucleotides located on either side thereof;

wherein each solid line represents a chemical linkage providing covalent bonds between the ribonucleotides located on either side thereof;

wherein a represents an integer which defines a number of ribonucleotides with the proviso that a may be 0 or 1 and if 0, the A located 5' of $(X)_a$ is bonded to the G located 3' of $(X)_a$;

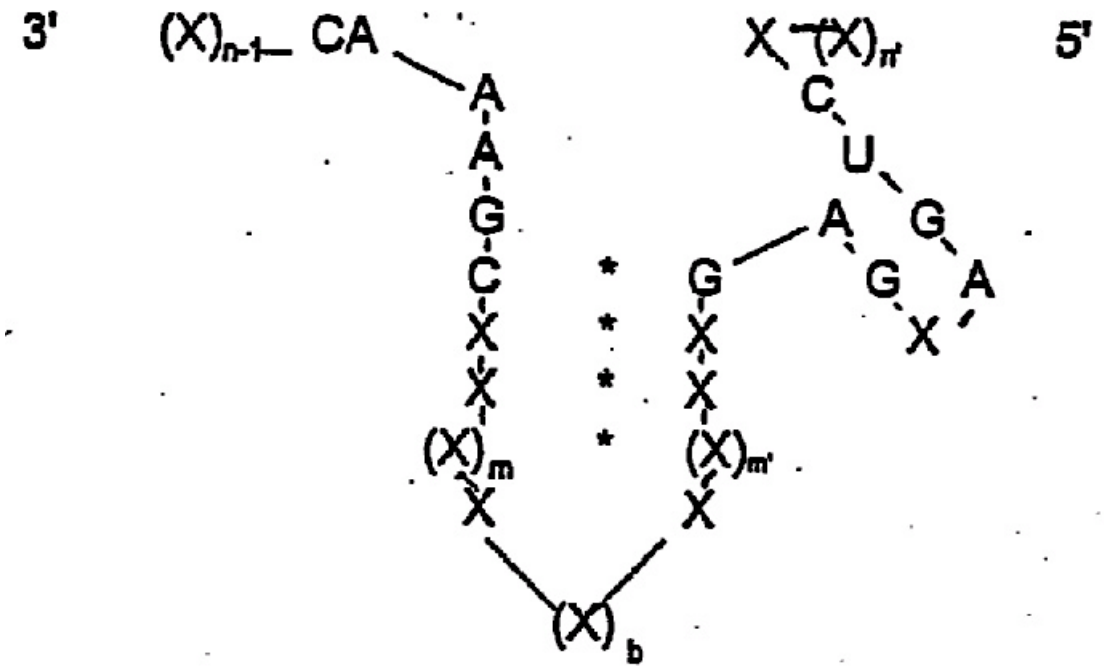
wherein each of m and m' represents an integer which is greater than or equal to 1;

wherein each of dashed lines independently represents either a chemical linkage providing covalent bonds between the ribonucleotides located on either side thereof or the absence of any such chemical linkage, and

wherein $(X)_b$ represents an oligoribonucleotide which may be present or absent with the proviso that b represents an integer which is greater than or equal to 2 if $(X)_b$ is present."

VIII. The **first auxiliary request** comprised the same claims as the main request except for claim 1, which read as claim 1 of the main request but wherein the "3'$(X)_n$ - A -" in the formula had been replaced by "3'$(X)_{n-1}$ - X'A -", defined as "wherein X'A is either UA, or GA or AA".

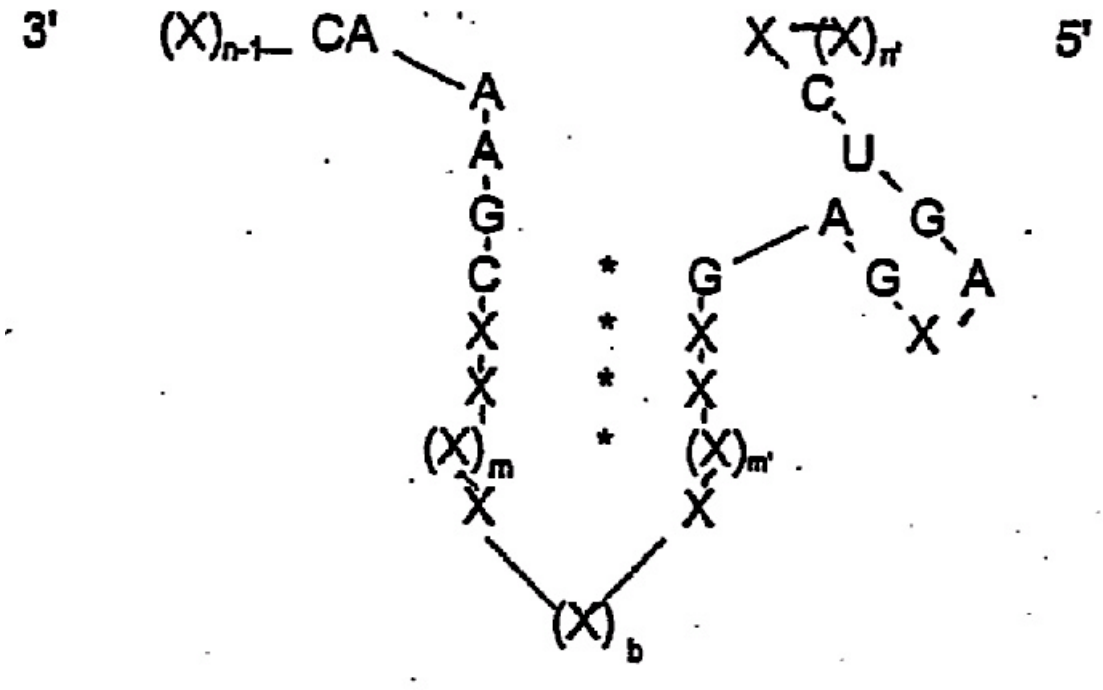
IX. The **second auxiliary request** corresponded to the first auxiliary request upheld by the opposition division. Claim 1 was directed to a compound having the formula:



wherein the wording of the claim was the same as that of claim 1 of the main request, except for: the specification of "C" as ribonucleotide 3' to "A", $(X)_{n-1}$ which was defined as $(X)_n$ in the main request, $(X)_b$ which was defined as an oligoribonucleotide with the proviso that b represents an integer which is greater than or equal to 2, and there were no references to dashed lines.

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

"1. A compound having the formula:



wherein each X represents a ribonucleotide which may be the same or different;

wherein each of $(X)_{n-1}$ and $(X)_{n'}$ represents an oligoribonucleotide (a) capable of hybridizing with an RNA target sequence to be cleaved and (b) defined by a predetermined sequence which sequence does not naturally occur covalently bound to the sequences C-A-A-A-G-C- and X-C-U-G-A-, respectively, such RNA target sequence not being present within the compound;

wherein each of n and n' represents an integer which defines the number of ribonucleotides in the oligonucleotide with the proviso that the sum of n + n' is sufficient to allow the compound to stably interact with the RNA target sequence through base pairing;

wherein each * represents base pairing between the ribonucleotides located on either side thereof;

wherein each solid line represents a chemical linkage providing covalent bonds between the ribonucleotides located on either side thereof;

wherein each of m and m' represents an integer which is equal to 1; and

wherein $(X)_b$ represents an oligoribonucleotide with the proviso that b represents an integer which is equal to 2."

Claims 2 and 3 further defined n and n'. Claim 4 was directed to multimers of the compounds of claims 1 to 3 or of compounds having a formula as defined in **claim 1 of the main request** (cf Section VII *supra*). Claims 5 and 6 were further embodiments concerned with the compounds of any of claims 1 to 4. Claim 7 was concerned with a method for producing the compounds of any of claims 1 to 4. Claims 8 and 9 related to transfer vectors, whereas claims 10 to 13 related to prokaryotic or (plant or animal) eukaryotic host cells.

Claim 14 was directed to a method for inactivating a target RNA in a cell other than a cell in man or animal which comprised contacting the target RNA within the cell with the compound of any of claims 1 to 5 or of compounds having a formula as defined in **claim 1 of the main request** (cf Section VII *supra*). Claims 15 to 19 defined further embodiments of the method of claim 14.

Claims 20 to 22 concerned the use of the compound of any of claims 1 to 5 or of compounds having a formula as defined in **claim 1 of the main request** (cf Section VII *supra*), or of the transfer vectors of claim 8 or of transfer vectors comprising a nucleotide sequence which on transcription gives rise to compounds having a formula as defined in **claim 1 of the main request** (cf Section VII *supra*), in the manufacture of: a medicament for the treatment of a condition associated with a target RNA in man or animals (claim 20), a composition for the inactivation of a target RNA in plants (claim 21) or a medicament for the treatment of a viral disease in man or animals (claim 22).

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

D2: Thesis of A. Jeffries, Univ. Adelaide, November 1986;

D3: A.J. Zaug et al., Nature, Vol. 324, pages 429 to 433, 4 December 1986;

D6: O.C. Uhlenbeck, Nature, Vol. 328, pages 596 to 600, 13 August 1987;

D7: D.H. Dreyfus, Einstein Quart. J. Bio. Med., Vol. 6, pages 92 to 93, June 1988;

D9: J. Haseloff and W.L. Gerlach, Nature, Vol. 334, pages 585 to 591, 18 August 1988;

D12: R. Perriman et al., Gene, Vol. 113, pages 157 to 163, 1992;

D37: Affidavit of Mrs Mary Sheehan dated 28 January 1998;

D42: L. Mazzolini et al., Plant Mol. Biol., Vol. 20, pages 715 to 731, 1992.

XII. For the purpose of discussion the following main features (or groups of features) are identified in the claimed compound (reference being made to claim 1 of the main request):

(A) 3' $(X)_n$ - A -

(B) - G - $(X)_a$ - A -

(C) The base-paired stem (- X - X - $(X)_m$ -) and the associated loop (- X - $(X)_b$ - X -)

(D) The functional features:

(a) each of n and n' represents an integer which defines the number of ribonucleotides in the oligonucleotide,

(b) the sum of n+n' is sufficient to allow the compound to interact stably with the target RNA sequence,

(c) such RNA target not being present within the compound,

(d) $(X)_n$ and $(X)_{n'}$ represents an oligoribonucleotide capable of hybridizing with an RNA target sequence to be cleaved,

(e) $(X)_n$ and $(X)_{n'}$ represents an oligoribonucleotide defined by a predetermined sequence which does not naturally occur.

XIII. Appellant I's arguments in writing and during the oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Main request

Articles 123(2) and 84 EPC

The claims were as granted except for the sentence "other than a cell in man or animal" introduced in claim 16 which did not raise any lack of clarity. Article 100(c) EPC was not mentioned in the notice of opposition and it had not been raised in the opposition proceedings. An objection under Article 123(2) EPC was a fresh ground for opposition and it could not be considered without the approval of the patentee. In the present case, this approval was not given.

Articles 87 to 89 EPC (Claim 1)

The technical feature (A) (cf Section XII *supra*), which was in relation to the target motif of the RNA to be cleaved, had a split priority. In the light of the description, the generic formula of claim 1 could intellectually be separated into several specific subgroups, which were limited alternative subject-

matters enjoying multiple priorities (Article 88(2) EPC). In the words of opinion G 2/98 (OJ EPO 2001, 413), the generic claim was an "OR"-claim. Molecules having XUX as target RNA motif were entitled to the second priority date, whereas molecules having GUX as target RNA motif were entitled to the first priority.

Availability of document D9 before the second priority date.

In its written submissions, the appellant had argued that the nominal publication date of document D9 was not reliable. Evidence from several libraries showed that document D9 was not available before the second priority date. With reference to the jurisprudence of the Boards of Appeal, on the basis of the submitted evidence and on the balance of probabilities, it could not be concluded that document D9 was available on its nominal publication date. However, the matter was not further argued at the oral proceedings.

Article 54 EPC

Document D9 only disclosed the subgroup of ribozymes entitled to the first priority, ie ribozymes having GUX as target RNA sequence. For subject-matter entitled to the first priority, document D9 was not prior art under Article 54(2) EPC.

First auxiliary request

Admissibility

Claim 1, which was the only claim amended with respect to the main request, was directed to ribozymes which

did not have GUX as target RNA sequence. The wording of this claim had been brought into line with that of the application as filed.

Article 123(2) EPC

Page 4, line 3 and lines 43 to 52 of the published version of the application as filed was indicated as a formal basis for claim 1.

Second auxiliary request

Article 123(2) and 84 EPC

This request, which was limited to specific claims of the main request without further amendments, corresponded to the first auxiliary request upheld by the opposition division. As stated in the decision under appeal, no formal objections had been raised by the opponent.

Articles 87 to 89 EPC (Claims 1 to 3)

Claim 1 was limited to ribozymes having GUX as target RNA sequence, ie those disclosed in the first priority document. The technical feature (C) (cf Section XII *supra*), which defined open-ended ranges for both the length of the base-paired stem and the size of the associated loop, had a formal basis on page 5 and Figure 4 of the first priority document, wherein the required minimum length of 4 bases was disclosed and the stem-length was said not to be critical. Similarly, it was taught that the minimum loop-size of 4 bases (Figure 4a) could be varied and Figure 4b showed that the loop was dispensable, ie not critical. These

references were made in the context of the conserved autocatalytic cleavage molecules shown in Figure 2, wherein a stem-length of 5 bases and a loop-size of 6 bases were disclosed. Thus, it was directly derivable from the first priority document that neither the stem-length nor the loop-size were critical.

The technical features (D) (cf Section XII *supra*) were implicitly derivable from the first priority document. Figure 4 and pages 5 and 6, referring to the selection of appropriate complementary base-pairing flanking regions to allow interaction between a ribozyme and its substrate, were indicated as formal basis for the functional wording (a) and (b). Support for the functional wording (c) and (d) were found in Figure 4 and the priority document as a whole, which referred to the separation of enzymatic and substrate activities and it was clearly not concerned with self-cleavage. The functional wording (e) excluded naturally-occurring ribozymes and ribozymes derived from naturally-occurring self-cleavage RNA molecules (Figures 2 and 3). The first priority document was concerned with synthetic ribozymes (page 5). The document as a whole and, in particular, Figure 4 (n+n'=7+7 and n,n'>6) were a formal basis for claims 2 and 3 too.

Article 54 EPC

As document D9 was not prior art under Article 54(2) EPC, the subject-matter of this request was novel.

Article 56 EPC (Claim 1)

If, notwithstanding the submissions on priority, document D9 was considered prior art, the claimed subject-matter involved an inventive step. Starting from document D9, the problem to be solved was the provision of alternative ribozymes. Structural and functional requirements of the ribozyme activity, particularly the implications of possible structural modifications, were unknown and represented an unexplored area. Document D9 disclosed a generic ribozyme with a base-paired stem and an associated loop having, respectively, a specific base composition and a fixed length and size (Figure 3). This region was highly conserved and, even if flexibility was mentioned, it was in the context of the self-cleavage domains of Figure 1a (flexible stem II, 2 to 7 bases). However, this flexibility was not transposed to the generic model of Figure 3 and there was no reason to do so as this model was not directly derived from Figure 1a and, more importantly, the implications of this flexibility on the tertiary folding of the ribozyme were unknown. Document D9 taught that all mutations in the 52-nucleotide sequence (Figure 1b) abolished the activity of the self-cleavage domain. Therefore, even assuming that the alteration of both the stem-length and the loop-size were obvious, in the light of these (mutation) studies and the unknown consequences on the ribozyme structure, there was no reasonable expectation of success. Document D9 referred to document D6 in the context of the self-cleavage RNA molecules of Figure 1 but not for designing new ribozymes. Document D6 disclosed the Symons model for self-cleavage RNA molecules and stated that the

essential features for cleavage were unclear and that the insertion of additional nucleotides could alter the cleavage.

Third auxiliary request

Admissibility

This request was a combination of subject-matter present in other requests on file. The compounds of claims 1 to 3 were those of auxiliary request 5, whereas the other claims essentially corresponded to the claims of the main request.

Articles 123(2) and 84 EPC

The requirements of these Articles were considered to be satisfied for the same reasons put forward in respect of the second auxiliary request. The specific ribozymes of this request had a basis in the whole content of the application as filed.

Articles 87 to 89 EPC

The first priority document disclosed ribozymes having GUX as target RNA sequence, a stem-length and a loop-size of 4 bases each. Feature (D) (cf Section XII *supra*) was implicitly derivable from this document, as argued for the second auxiliary request. For features (A) and (C) (cf Section XII *supra*) of claims concerned with generic ribozymes having XUX as target RNA sequence and open ranges for both the stem-length and loop-size, the relevant arguments were the ones used for the main request and the second auxiliary request, respectively. Feature (B) (cf Section XII *supra*),

concerning $(X)_a$, had a split priority with a=0 entitled to the first priority and a=1 to the fourth one. The paragraph bridging pages 11 and 12 of the first priority document was given as a formal basis for multimers, whereas pages 9 to 12 were a formal basis for in vivo applications.

Article 83 EPC

Examples 8 and 9 of the patent in suit showed the stability and in vivo activity of ribozymes in plant and animal cells. Even if this stability was lower in biological fluids, the patent specification disclosed methods for overcoming this problem, including methods of administration, preparation of derivatives (ribozymes were defined as comprising only RNA or derivatives thereof) and, particularly, the use of a carrier gene modifying a short ribozyme into a long one. Post-published evidence (to be taken as expert documents) showed the feasibility of these teachings, even for short ribozymes. In agreement with the established case law, an occasional failure - eg absence of in vivo activity for short and long ribozymes (document D42) - was not enough to demonstrate that the technical effect could not be achieved within the whole range or without undue burden. Possible reasons for such failure were also indicated, such as the absence of target mRNA.

Article 54 EPC

Document D9 was not prior art for the subject-matter of claims 1 to 3 as these claims were entitled to the first priority. Moreover, document D9 did not disclose

multimers and did not make plausible any in vivo application of ribozymes. Thus, the requirement of novelty was satisfied.

Article 56 EPC (Claims 1 to 3 directed to specific ribozymes)

Document D6, the closest prior art, disclosed an oligonucleotide (01) able to trans-cleave a substrate oligonucleotide (02), wherein both oligonucleotides were derived from self-cleaving RNA molecules. The oligonucleotide 01 had a restricted number of substrates due to the presence of sequence constraints in oligonucleotide 02. Starting from this closest prior art, the problem to be solved was the provision of alternative oligonucleotides able to cleave a wider range of substrate oligonucleotides. Whereas document D6 referred to possible modifications of the disclosed (Symons) model, it was unclear how many - sequence and structural - features of this model were essential for the reaction. Moreover, since activity was not detected for other substrate RNAs, there was no expectation of success. Document D6 (trans-cleavage molecules) could only be combined with document D3 (self-cleavage introns) with hindsight as they concerned different catalytic systems without structural relationship.

Article 56 (Claim 4 directed to multimers)

Figure 4 of document D9, the closest prior art for this aspect of the invention, disclosed three separate ribozymes attached to one RNA target sequence, wherein each one alone was incubated with the RNA substrate (use of a multiplicity of discrete ribozymes). However,

there was no hint to transform these ribozymes into a multimer and it would have required hindsight in order to know whether a plurality of single ribozymes in the same reaction mixture could co-exist without losing their activity.

Article 56 (Claims 14 to 22 directed to methods and uses of ribozymes and multimers thereof)

Although document D9 referred to potential in vitro and in vivo applications of the ribozymes described therein, it did not make any in vivo use plausible as the statements were merely hypothetical and there was absolutely no reasonable expectation of success. Ribozymes were derived from self-cleavage RNA molecules of pathogenic agents and their mechanism of disease was not understood. It was not known whether the self-cleavage mechanism by itself could interfere with the normal RNA mechanisms within the cell and the in vivo specificity of ribozymes was also unknown. Other effects in vivo, apart from the cleavage, could not be excluded. Self-cleavage sequences derived from viroid satellites were only active within specific host cells and the requirements underlying this specificity were not characterized.

XIV. Appellant II's arguments in writing, insofar as they are relevant to the present decision, may be summarized as follows:

Admissibility

No comments were made in writing on the admissibility of the requests filed on 14 November 2003, which

comprised the **main request and second auxiliary request** maintained during the oral proceedings. As for the **first and third auxiliary requests** filed during the oral proceedings, appellant II did not attend those proceedings and therefore expressed no opinion on the matter.

Articles 123(2) and 84 EPC

As for the **main request**, the functional wording of claim 1 had no support in the application as filed. Although this ground had not been raised before, the board had to use its discretion under Article 114(1) EPC and examine whether the requirements of both Articles 123(2) and 84 EPC were satisfied. As for the other **auxiliary requests**, the combination of amended structural features with unamended functional features was not found in the claims as granted. In keeping with decision G 9/91 and opinion G 10/91 (OJ EPO 1993, 408 and 420, respectively), the board had therefore to consider all claims comprising this combination as arising out of an amendment. As the functional wording had no basis in the application as filed, all these claims offended against Article 123(2) EPC.

Articles 87 to 89 EPC

As for the **main request**, the subject-matter of claim 1 was not entitled to the first priority. According to opinion G 2/98 (cf *supra*), multiple priorities for a subject-matter defined by a generic formula were only possible if the subject-matter could be subdivided into a limited number of clearly defined subgroups. This was not the case for the claimed subject-matter which was

defined in terms of a group of functional features - feature D (cf Section XII *supra*) - that could not be subdivided in subgroups in the sense of a clearly defined alternative subject-matter.

As for all requests comprising **feature (C)** (cf Section XII *supra*), ie the **main request** and **first and second auxiliary requests**, this feature was not derivable from the first priority document. A stem-length of 4 bases in Figure 4a and the reference on page 5 to this length, not being critical, could not be seen as a basis for an open range with the selection of 4 bases as a lower limit. Similarly, the minimum loop size shown in Figure 4a constituted no support for an open range. Moreover, **all requests** comprised **feature (D)** (cf Section XII *supra*) which was not found in the first priority document. There was no teaching supporting the selection of specific n and n' integers, and no mention that the length of the hybridizing oligonucleotides was of relevance, let alone that the stable interaction of the ribozymes with the target RNA sequences through base-pairing was a function of the sum of the lengths of the flanking oligonucleotides (functional wording (a) and (b)). The first priority document did not exclude that the ribozyme and the substrate RNA sequence were both part of the same molecule (functional wording (c)). Similarly, no basis was found for the functional wording (e), as nowhere was it stated that the flanking sequences could not be naturally-occurring ones. None of the structural requirements implied by the functional wording (a) to (e) could be directly derived from the first priority document. Lastly, **all requests** comprised multimers of ribozymes and in vivo applications, however, there were

no references to multimers in the first priority document and the in vivo applications mentioned therein were theoretical suggestions without technical support.

Article 83 EPC

The arguments concerning Article 83 EPC were relevant for **all requests**. For a ribozyme to cleave in vitro and in vivo a target RNA sequence, it was essential to retain its primary and secondary structures, to arrive at its target RNA in a cell as well as to be stable within the cellular environment avoiding (nuclease) degradation. The patent in suit failed to disclose how to achieve these requirements and therefore, its contribution was only at a general conceptual level. Sufficiency of disclosure was not supported by a conceptual disclosure, as stated *inter alia* in decision T 994/95 of 18 February 1999 concerned with antisense oligonucleotides. Examples 8 and 9 of the patent in suit showed the activity of ribozymes in a cell culture by expression with carrier genes. However, they were very specific embodiments and the presence of these additional elements and modifications were not reflected in the claims. Post-published documents showed that the activity of ribozymes was essential but not sufficient to ensure the inhibition of a target gene in a cell and that essential modifications were required in order to have activity in vivo. Document D42 showed that, even with a carrier gene, no activity was found in vivo. The technical effect - in vivo cleavage activity - was only credible for a small subgroup of modified ribozymes but not over the whole range claimed. These post-published documents substantiated, in a verifiable manner, serious doubts

as to the sufficiency of disclosure and, in the absence of essential information in the patent in suit, undue burden was placed on the skilled person.

Availability of document D9 before the second priority date

Document D37, an affidavit from the operations editor of document D9, showed that document D9 was theoretically available on its nominal publication date. Evidence was filed showing that document D9 was received and stamped before the second priority date in two technical libraries.

Article 54 EPC

Since none of the requests was entitled to the first priority, **all requests** were anticipated by document D9, which disclosed a subgroup of ribozymes falling within the claimed generic ribozymes, the advantages of using multimers of ribozymes, and in vivo applications.

Article 56 EPC
Claims directed to ribozymes

If notwithstanding the above submissions, the first priority was acknowledged and thus, document D9 was not to be taken into consideration as prior art under Article 54(2) EPC, then the following objections applied.

Document D6 was identified as the closest prior art. This document disclosed the conserved sequences and essential structural requirements for self- and trans-

cleavage activities (Figures 1a and b, respectively). Document D6 differed from the patent in suit only by the presence of a half/half combination of a hammerhead motif instead of the three-quarter/one-quarter combination of the patent (Figure 3 of the patent in suit). Starting from this document, the technical problem to be solved was the provision of a hammerhead (ribozyme) with lower constraints in the target sequence, ie with a wider range of target sequences. Document D6 itself provided incentives for designing a three-quarter/one-quarter ribozyme, since it recognized the importance of trans-cleavage activity for potential biological applications and, thus, made it obvious to separate the cleaving structure from the substrate target RNA sequence so as to reduce the structural constraints in this target sequence. It was also obvious from document D6, which indicated that hairpin loops were not necessary for the activity (Figure 1a), that the modifications required for a three-quarter/one-quarter ribozyme would have no effect on that activity. Document D3 already disclosed the conversion of a self-cleavage reaction (intron splicing) into a trans-cleavage reaction (ribozyme), wherein the conserved sequences responsible for the activity were shifted to (located on) the ribozyme and the target RNA sequence only retained a minimal part of the conserved sequences. Document D3 also showed that active-site mutations could alter substrate specificity (Figure 3). The results disclosed in these documents, in particular document D6, provided the skilled person with a reasonable expectation of success.

Claims directed to multimers

As multimers were not entitled to the first priority, document D9 represented the closest prior art. It disclosed a target RNA sequence comprising multiple ribozyme-cleavage sites wherein each of these sites was a target for a specific ribozyme. It was evident to the skilled person to combine the three separate ribozymes of Figure 4 by extending their hybridizing arms so as to incorporate all three ribozymes into a single catalytic structure, ie a multimer.

Claims directed to methods and uses of ribozymes and multimers thereof

These claims were also not entitled to the first priority date. Thus, document D9 was the closest prior art. This document disclosed potential in vivo applications of ribozymes. In keeping with the established case law, *inter alia* decision T 939/92 (OJ EPO 1996, 309), when the presence of an inventive step was supported by a technical effect, this effect had to be achieved by all the compounds covered by the claims. However, the patent in suit only provided experimental evidence for ribozymes having GUX as target RNA sequence and there was evidence on file showing that an important number of claimed ribozymes were not able to cleavage target RNA sequences (document D12). Similarly, post-published evidence showed that short ribozymes - even with a carrier gene - did not achieve the desired effect (document D42). Thus, the claimed subject-matter did not solve the technical problem and there was no inventive contribution over the prior art.

XV. Appellant I requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request filed on 14 November 2003, or, in the alternative, auxiliary requests 1, 3 or 4 filed during the oral proceedings or 2 or 5 filed on 14 November 2003.

XVI. Appellant II requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Main request
Articles 123(2),(3) and 84 EPC

1. Article 100(c) EPC was not mentioned as a ground for opposition in the Notice of Opposition. Points 4.1 and 5.1 of the Reasons of the decision under appeal state that no objections were raised under this article against the main request (claims as granted) and against the first auxiliary request then before the opposition division. The objection has been raised for the first time in the appeal proceedings and thus, it is a fresh ground for opposition. According to decision G 9/91 and opinion G 10/91 (cf *supra*), a fresh ground for opposition can only be admitted into the appeal proceedings with the approval of the patentee. No approval has been given in the present case and, thus, the ground for opposition cannot be considered by the board.

2. Nevertheless, it remains to be assessed whether any objection arise out of amendments, in which case, according to decision G 9/91 (cf *supra*, point 19 of the Reasons for the decision), such amendments are to be fully examined as to their compatibility with the requirements of the EPC. The only difference between the claims as granted and the main request is the sentence "*other than a cell in man or animal*" introduced in claim 16 in order to exclude in vivo medical methods which are not patentable under Article 52(4) EPC.

3. The application as filed discloses the use of ribozymes in vitro (cf examples 1 to 7 and claim 13 of the application as published) and in vivo (cf examples 8 and 9 and claim 14), wherein in vivo is defined as "*within the cell or cells of an organism*" (cf page 5, line 2). This definition embraces embodiments concerned with cells in culture as well as with cells in an organism. As for the former embodiments, they are exemplified by the inactivation of RNA transcripts in plant cell cultures (cf example 8, protoplasts of Nicotiana) and in animal cell cultures (cf example 9, COS1 cells), whereas for the latter embodiments, the application refers to therapeutic and biological applications (cf page 5, line 11), including the treatment of viral diseases in man, animals and plants (cf page 5, lines 33 to 37 and claims 18 and 19). The amendment introduced in claim 16 makes clear that in respect of human and animal cells only the former embodiments are claimed, ie the use in cultures of human and animal cells.

4. Thus, for this specific limitation a formal basis can be seen in the application as filed (Article 123(2) EPC). Moreover, there is no extension of the scope of the claims as granted (Article 123(3) EPC). The feature is *per se* clear and in the context of claim 16 does not introduce any lack of clarity (Article 84 EPC).

Articles 87 to 89 EPC

5. According to the opinion of the Enlarged Board of Appeal G 2/98 (cf *supra*), the right to priority for the same invention is to be acknowledged only if the skilled person derives the **same subject-matter** of the claim directly and unambiguously, using common general knowledge, from the previous application as a whole. In point 6.7 of the Reasons, reference is made to a memorandum expressing the legislative intent underlying Article 88(2) EPC, second sentence, wherein it is held that the "*use of a generic formula in a claim for which multiple priorities are claimed in accordance with Article 88(2) EPC, second sentence, is perfectly acceptable under Articles 87(1) and 88(3) EPC, provided that it gives rise to the claiming of a limited number of clearly defined alternative subject-matters*".

6. Claim 1 relates to a generic formula which covers a great number of alternative compounds. These result both from the alternatives offered within each of the single main - structural and functional - features A to D (cf Section XII *supra*) and from their different combinations. Feature A, for example, ie the sequence $3'(X)_n$ - A-, covers a number of generic compounds with sequences which are complementary to the target RNA containing the triplet XUX - the so-called target motif.

However, the alternative compounds are not, as such, spelled out in the claim. The fact that they might be intellectually envisaged to fall within the scope of the claim does not make up for a clear and unambiguous presence of these alternatives, individualized as such, in the claim. Claim 1 **does not** embrace a limited number of clearly defined alternative subject-matters in the form of an "OR"-claim which could be split up into groups of different priorities.

7. Thus, claim 1 cannot enjoy the **partial** priority from a priority document, but can only be entitled to the priority date of the document where the said generic formula is for the first time disclosed. This is **not** the first priority document as this discloses only more specific synthetic ribozymes. Although these are covered by the general formula of claim 1, there is in the said priority document no direct and unambiguous disclosure of the broad generic group as represented by that formula. Thus, claim 1 **does not** enjoy the first priority date.

Availability of document D9 before the second priority date

8. Document D9 is an article published in the scientific journal "Nature". The nominal publication date of document D9 - the date printed on it - is 18 August 1988, ie one day before the second priority date of the patent in suit (19 August 1988). Document D37, an affidavit by Mrs Mary Sheehan, Nature's operations editor, asserts that Nature is published on Thursday of each week (18 August 1988 was a Thursday) and that members of the public can purchase a copy of Nature

from the editorial office on and after the Wednesday of each week in which Nature is published. It is also stated that in 1988 copies delivered to the principal London newspapers were embargoed until Thursday, that is the cover date of the issue, and that copies were dispatched to UK subscribers by first class post on the Wednesday and should have been received the following day. This assessment is supported by evidence from two reading rooms - the Holborn and Aldwych reading rooms - of the British Library in London, where copies of the issue of "Nature" with document D9 were stamped on its nominal publication date, ie Thursday 18 August 1988.

9. The evidence put forward by appellant I showing that the issue of "Nature" in question was received in several libraries around the world - Australia, Japan and the United States of America - later than the second priority date and that the date stamp was, in some cases, earlier than the date on which the journal was actually put on library shelves, as well as the fact that no actual purchase of the issue of "Nature" in question had taken place, is not relevant since it does not change the fact that this issue of "Nature" was made available even before its nominal publication date. It is established jurisprudence of the Boards of Appeal (cf "Case Law of the Boards of Appeal of the European Patent Office", 4th edition 2001, I.C.1.6, 42), that the theoretical possibility of having access to information renders it available to the public.

10. Thus, document D9 is state of the art under Article 54(2) EPC for the subject-matter of claim 1 which is not entitled to the first priority.

Article 54 EPC

11. Figure 3 of document D9, a scientific publication from the inventors of the patent in suit, shows a model ribozyme with a generic formula having a consensus sequence derived from naturally-occurring self-cleavage RNA molecules (cf Figure 2). Several specific ribozymes exemplifying the teachings of the document are also disclosed (cf Figure 4). All these ribozymes fall within the scope of the generic compound of claim 1 and, thus, they anticipate the subject-matter of this claim.

12. Therefore, the main request, which contains claim 1, does not fulfil the requirements of Article 54 EPC.

First auxiliary request
Admissibility (Rule 57a EPC)

13. The request, which was filed during the oral proceedings, essentially corresponds to the previous auxiliary request 1 filed on 14 November 2003 but is positively worded, more in agreement with the application as filed. It intends to overcome, by overcoming the priority problem, the objection raised under Article 54 EPC against the main request. Neither the board nor the absent party could be surprised by this request. Thus, it is considered to be admissible under Rule 57a EPC.

Article 123(2) EPC

14. Page 4, lines 43 to 52 of the published application as filed has been given as a formal basis for amended claim 1. Therein the preferred ribozymes are defined

functionally as being those capable of cleaving the target RNA which contains the sequence X^0UY, X^0 being any ribonucleotide and Y being A, C or U. The limitation of Y, which is not base paired, to these three nucleotides is understood as an important requirement of the target triplet, possibly due to the proximity of the third position (Y) to the ribozyme active site, and **excludes Y=G**. However, claim 1 at issue defines the claimed ribozyme **without** reference to the target RNA sequence. The claim refers to the presence of a generic ribozyme doublet X'A which is complementary to the target X^0U doublet. However, taken out of its original context of the target (X^0UY) triplet, the said doublet leaves the third position (Y) of this triplet unrestricted. This means that a subgroup of target triplets is added, namely X^0UG, which is not derivable from the cited passage of the application. Thereby the functional definition of the subject ribozymes is amended to include those capable of cleaving target RNA which contains the sequence X^0UG for which no support is found in the said passage. No other paragraphs have been identified as a formal basis for the introduction of such an individualized generic doublet in the claims.

15. Thus, claim 1 comprises added subject-matter and the request, containing this claim, offends against the requirements of Article 123(2) EPC.

Second auxiliary request
Articles 123(2),(3) and 84 EPC

16. The request essentially corresponds to the main request but without claims 1 and 2. In line with the arguments

followed for the main request (cf points 1 to 4 *supra*), this request is also allowable under these Articles.

Articles 87 to 89 EPC (Claim 1)

17. The compound of claim 1 is characterized by a general formula (cf Section XII *supra*), wherein:

- **feature A** is limited to 3' $(X)_{n-1}$ -CA-, which means a restriction to ribozymes having GUX as target RNA sequence,

- **feature B** is -G-A-,

- **feature C** offers the alternatives wherein n and n' are equal to 1 or greater and b equal to 2 or greater,

- **feature D** is as in the main request.

18. As a compound characterized by **features A and B** is unambiguously derivable from the first priority document, the question arises whether said compound is also characterized by feature D and whether the alternatives in respect of feature C are also offered in combination.

19. As regards the group of features designated as **feature D** (cf Section XII *supra*), the following is observed: Figure 4 of the first priority document discloses a consensus ribozyme having flanking sequences of a specific length. There is no reference to the integers of the functional wording (a) and (b) of feature (D), ie "*n and n' represents an integer*

which defines the number of ribonucleotides" and "*with the proviso that the sum of n + n' is sufficient to allow the compound to stably interact with the RNA target sequence through base pairing*". However, the first and second paragraphs of pages 5 and 6, respectively, refer to an appropriate selection of flanking sequences for an accurate interaction between the ribozyme and its substrate. It is said that "*the extent of base-pairing will determine the specificity and affinity of the ribozyme for its substrate, increasing the G*C content and/or number of base-pairs*" (cf page 6) and thus, allows a variation (increase) in the length of the flanking regions. The length of both flanking regions is implicitly understood to be important for such stable interaction so as to avoid a free hanging flanking sequence and the resulting instability. These references support the functional wording (d) too, ie "*capable of hybridizing with an RNA target sequence to be cleaved*". The first priority document relates to synthetic ribozymes and clearly not to the self-cleavage RNA molecules and derivatives thereof (cf *inter alia* page 5, line 5, page 13, third line from the bottom and Figures 4 and 5). Pages 2 and 3 refer to the prior art and to the separation of enzymatic and substrate activities (cf Figures 2 and 3). The functional wording (c) and (e) - "*such RNA target sequence not being present within the compound*" and "*defined by a predetermined sequence which sequence does not naturally occur covalently bound*" - exclude these self-cleavage RNA molecules and derivatives thereof, such as the ones shown in Figure 3(c). This functional wording is formally supported by the first priority document as a whole. Figure 4 with the reference on page 6 formally support the specific

length referred to in claims 2 and 3 too (n + n'=7 + 7 and n, n' >6). Thus, **feature D**, ie the functional wording (a) to (e), has a formal basis in the first priority document.

20. The question remains whether the alternatives in respect of **feature C** (cf Section XII *supra*) are also found in the first priority document. Based on self-cleavage RNA molecules (cf Figure 2), the first priority document discloses consensus sequences of ribozymes having GUX as target RNA sequence with a stem-length and loop-size of 4 bases each (cf Figure 4). This region is identified as having "*highly conserved sequence and secondary structure*", which need to be considered for de novo design of ribozymes (cf page 5, first paragraph). It is further stated, that "*it appears that the length of the conserved base-paired stem formed in the ribozyme is not critical*" (cf page 5, second paragraph). However, this sentence, in particular the word "*critical*", cannot be understood in this context as removing all limitations on the length of the conserved stem but rather as indicating a small - not critical - variance in the stem-length within the one shown by the self-cleavage RNA molecules of Figure 2 (3 to 5 bases). Similarly, the absence of a loop in Figure 4(b) cannot be understood as indicating that such a loop is dispensable. On the contrary, as shown in Figure 4(b) itself, this absence requires a compensatory extension of the stem so as to hold the two parts of the resulting ribozyme together (cf page 5, second paragraph). There are no further references as to whether the size of the loop is critical or dispensable, and only Figure 2 discloses a loop of 6 bases in a self-cleavage RNA molecule. Thus, there is

no teaching - either explicit or implicit - of an open-ended range for the stem-length or for the loop-size, let alone for a combination thereof.

21. Thus, of the alternatives offered in claim 1 in respect of **feature C**, only that of n and n' equal to 1 and b equal to 2 is disclosed in the first priority document in combination with features A, B and D. Therefore, only this embodiment enjoys the first priority date, whilst the remaining embodiments, in particular those where the values of n and n' and b are open-ended (greater than 1 and greater than 2, respectively) are **not** entitled to the first priority. The consequence of this finding is that document D9 constitutes prior art under Article 54(2) EPC for the later embodiments.

Article 54 EPC (Claim 1)

22. There is no prior art on file anticipating the specific ribozymes which are entitled to the first priority date, namely those having GUX as target RNA sequence and with a stem-length and associated loop of 4 bases each (cf point 21 *supra*). As regards the embodiments not entitled to the first priority date, none of the prior art documents on file, including document D9, discloses a ribozyme with a stem-length or a loop-size greater than 4 bases, let alone an open-ended range for any of these two features. Thus, claim 1 is not anticipated by the prior art.

Article 56 EPC (For embodiments of claim 1 not entitled to the first priority)

23. Document D9, which is the closest prior art, discloses a model for designing ribozymes and further suggests possible modifications thereof (cf Figure 3 and page 588, left-hand column under the heading "Design of new ribozymes").

24. Starting from document D9, the objective technical problem underlying the patent in suit is the provision of alternative ribozymes. The compounds of claim 1 wherein the stem-length is greater than 4 and/or the loop-size greater than 4 are proposed as a solution to this technical problem.

25. Document D9 identifies three elements relevant for designing ribozymes, namely (i) the specificity for the target (GUC) triplet, (ii) region (B) containing highly conserved sequences and secondary structure, including the base-paired stem and the associated loop, and (iii) the base-pairing flanking-regions (cf page 588, left-hand column under the heading "Design of new ribozymes"). With regard to (ii), and commenting on the self-cleavage RNA molecules of Figure 1a, document D9 states that "*the lengths of the base-paired stem II and associated loop do not appear to be conserved and the loop may be dispensable, as for ASBV*". In fact, according to the footnote to Figure 1, the base-paired stem II can "*vary in length from 2 to 7 base pairs*" (cf page 586, line 4 of Figure 1 footnote). The ASBV mentioned in this footnote refers to the bibliographic reference "11" which corresponds to document D6 on file. Figure 1(a) of document D6 discloses the consensus

sequences for these self-cleavage RNA molecules and states that "t*he number of nucleotides in hairpin loops I, II and III vary from 2 to more than 200*", wherein loop I corresponds to the loop within region (B) of the model ribozyme in document D9. Since all this information is disclosed under the heading "Design of new ribozymes", it would be seen as an obvious suggestion to modify the length of the base-paired stem and the size of the associated loop. These modifications would only require the normal abilities of the person skilled in the art as defined in the established case law of the Boards of Appeal (cf "Case Law" *supra*, I.D.5.1.3, 111) and, being directly derived from the known naturally-occurring self-cleavage RNA molecules, the skilled person would also have a reasonable expectation of success (cf "Case Law" *supra,* I.D.6.2, 117). This expectation cannot be diminished by the mutation studies of document D9, which by their specific nature - arbitrary introduction of an unrelated linker with duplication or deletion of flanking sequences - are expected to disrupt the secondary structure of ribozymes, whereas, in the present case, the modifications are specifically located and directly derived from the ones present in the naturally-occurring self-cleavage RNA molecules.

26. Thus, claim 1 comprises an alternative group of embodiments which is obvious for a skilled person. Consequently the second auxiliary request, which comprises said claim, does not fulfil the requirements of Article 56 EPC.

Third auxiliary request
Admissibility (Rule 57a EPC)

27. This request was filed during the oral proceedings to replace previous auxiliary request 3 in order to overcome objections under Article 56 EPC. It essentially corresponds to a combination of the narrower product claims of auxiliary request 5 with the method and use claims of previous auxiliary request 3, both having been filed on 14 November 2003. Neither the board nor the absent party could be surprised by this request. Thus, the request is considered to be admissible under Rule 57a EPC.

Article 123(2),(3) and 84 EPC

28. Product claims 1 to 3 of this request are a narrower version of granted claims 3 to 5 being restricted to ribozymes having GUX as target motif in the RNA sequence and a stem-length and a loop-size of 4 bases each. These correspond to one of the embodiments of claim 1 of the second auxiliary request discussed above. The remaining claims 4 to 22 correspond in essence to granted claims 6 to 24. In the board's judgement, no additional issues of Articles 123(2),(3) and 84 EPC are raised by this request (cf points 1 to 4 and 16 *supra*).

Articles 87 to 89 EPC (Entitlement to first priority)

29. In view of document D9, a decision on the entitlement to the first priority date has to be taken for the following three aspects of the invention:

(a) The product of claims 1 to 3 which concerns **ribozymes** of the given general formula having GUX as target motif in the RNA sequence and a stem-length and associated loop-size of 4 bases each.

(b) The product of claim 4 which concerns **multimers** of a given general formula.

(c) The so-called **in vivo applications** of the said ribozymes.

30. As regards item (a), it has already been found in relation to the second auxiliary request that the compound as defined in the claims enjoys the first priority date (cf points 17 to 21 *supra*).

31. As regards item (b), Figure 5(b) of the first priority document shows the concurrent use of three ribozymes against three different target triplets of a single substrate CAT sequence. However, each ribozyme is separated from the other ones and there is no suggestion of a physical connection between them, let alone of the feasibility of such a connection (structural constraints) or of any associated advantage thereto (cf paragraph bridging pages 11 and 12). Thus, subject-matter relating to multimers is not entitled to the first priority.

32. As regards item (c), under the heading "Potential applications of the invention", the first priority document proposes in very general terms the use of ribozymes for inactivating gene transcripts in vivo (cf pages 9 to 12). However, the lack of working examples, the lack of any indication of the associated technical

problems (*inter alia* degradation by nucleases, lack of specificity), the lack of technical information about the measures to be adopted to overcome these problems (*inter alia* insertion in a carrier gene) render the disclosure of any in vivo method quite inadequate from the technical point of view to support a claim to priority. This especially in consideration of the fact that ribozymes were known to be related to self-cleavage RNA molecules associated with pathogenic organisms with a mechanism of action not understood (cf points 51 and 52 *infra*). It is established case law that priority can only be acknowledged if the claimed invention is disclosed in the priority document as a matter of substance, ie with all essential technical features (cf "Case Law" *supra*, IV.B.3, 242). In the present case, the mere reference to potential in vivo applications without any further technical information does not amount to a complete technical disclosure. Thus, claims concerned with in vivo methods do not enjoy the first priority.

Article 83 EPC

33. It has been argued that in vivo applications for short ribozymes are unfeasible as they are not active in vivo, or at least not very efficiently so, due to stability problems and degradation by nucleases, as shown in several post-published documents (to be taken as expert documents). However, the description of the patent in suit acknowledges the problem and refers to methods for stabilizing ribozymes against nuclease digestion, in particular by using large RNA molecules or carrier genes (cf page 5, lines 23 to 27 of the patent specification). This teaching is exemplified by using

long hybridisable (antisense) CAT flanking sequences in the construct pCAT19 of example 8 (cf pages 14 and 15 and Figure 9) and by using either long hybridisable CAT flanking sequences or the luciferase gene as carrier genes in the constructs of example 9 (cf pages 16 to 18). Thus, the patent in suit identifies the problems and discloses technical solutions, which are shown - both in the patent and in the post-published documents - to overcome them.

34. These examples further demonstrate the resilience in vivo of the secondary structure of ribozymes to particular modifications. Once this resilience is demonstrated, the skilled person is in a position to envisage similar modifications in line with the common general knowledge, in particular taking advantage of prior art concerned with the modification and administration of short nucleic acids into cells, such as earlier methods developed for the antisense technology (cf point 36 *infra*). The description of the patent in suit refers to both exogenous (eg parentally delivered, ribozymes produced outside the target cell) and endogenous (eg microinjection, ribozymes produced inside the target cell) methods of administration. Thus, avoiding, at least in the latter case, the degradation by nucleases present in biological fluids (blood).

35. Document D42 has been cited to support the argument that the solution disclosed in the patent in suit - insertion of short ribozymes in carrier genes - does not always achieve the intended effect. However, this document shows that, whereas for short ribozymes almost no inhibition of the target enzyme is found (cf page 723, Figure 3, pEXR with 97.5% activity), the

inhibition is relatively greater for longer ribozymes (cf page 726, Figure 6, pCGR with only 86.5% activity). Thus, even if very inefficient, the effect anticipated by the patent in suit is found in this document. Possible reasons for this inefficiency are indicated in the document itself, such as a low intrinsic catalytic activity, a specific low expression in plant cells and problems in the expression system used as shown by the low level of target mRNA (cf page 724, right-hand column, first full-paragraph). Document D42 concludes with a reference to other studies which report the successful use of ribozymes in vivo (cf page 729, left-hand column, last paragraph and page 730, left-hand column, first full paragraph). These successful results are more in line with all the post-published documents present on file, which disclose in vivo activity at least for long ribozymes.

36. The factual situation underlying decision T 994/95 (cf *supra*), concerned with therapeutic antisense oligonucleotides, was different from the present one. Contrary to antisense oligonucleotides, wherein several essential features were not taught in the patent specification (identification of the relevant portion of the mRNA encoding the target protein, synthesis of oligonucleotides of substantial complementarity to target mRNA and stabilization of such oligonucleotides), the patent in suit discloses the primary and secondary structures of ribozymes with its essential structural elements and parts thereof as well as the target cleavage site which identifies the target to be cleaved. Moreover, the patent itself identifies possible technical problems (stability and administration) and discloses methods for overcoming them. In fact, the

antisense technology referred to in decision T 994/95 was known since 1982, whereas the patent in suit was filed at the end of 1988. Thus, methods developed for overcoming the aforementioned shortcomings associated with the antisense technology were common general knowledge and available to the person skilled in the art for a relatively long time.

37. As for claims relating to methods in vivo, the ribozymes to be used therein are defined in a generic manner as having XUX as target RNA sequence and with an open-ended range for both the stem-length and the associated loop-size. On the one hand, it could be reasonable to assume that a long stem-length and/or a great loop-size could impose certain constraints on the secondary and tertiary structure of ribozymes and therefore, on their possible in vivo activity. However, on the other hand, both the stem-length and the loop-size could be irrelevant as long as the minimal structural requirements disclosed in the patent in suit are retained. Neither the patent in suit nor the prior art on file provide evidence supporting the one or the other of these contradictory assumptions. In the absence of such evidence and, relying on the established case law that requires an objection for lack of sufficient disclosure to be supported by serious doubts and substantiated by verifiable facts (cf "Case Law" *supra*, II.A.5.1.1, 150), the allegation of insufficient disclosure, in this case, is not supported. The Board considers that the skilled person working within the teachings - values and ranges - suggested and exemplified in the description of the patent in suit would achieve the desired effect with a reasonable chance of success and without undue burden.

38. It follows from the foregoing that the requirements of Article 83 EPC are fulfilled.

Article 54

39. There is no prior art on file anticipating the specific ribozymes entitled to the first priority (cf point 22 *supra*). As regards the embodiments not entitled to the first priority date, for which document D9 constitutes prior art under Article 54(2) EPC, it is observed that, consistently with the finding on the priority issue, this document cannot be considered to provide a technically meaningful disclosure of multimers and of the in vivo applications of ribozymes (cf points 31 and 32 *supra*). Thus, this request meets the conditions of Article 54 EPC.

Article 56 EPC (Claims 1 to 3 directed to specific ribozymes entitled to the first priority date)

40. Document D6, the closest prior art, discloses a trans-cleavage reaction between two oligonucleotides - 19 and 24-nucleotide synthetic fragments designated, respectively, O1 and O2 - derived from a self-cleavage RNA molecule (cf page 597, Figure 1). The cleavage site is present in oligonucleotide 02 which has a base-paired stem and an associated loop and several conserved nucleotides. Similarly, the catalytic oligonucleotide O1 has several consensus nucleotides and it is partially complementary to oligonucleotide O2. This construct has been called as a half-half ribozyme, wherein loops I and III of the self-cleavage RNA molecule have been opened and the consensus sequences

are shared by both (half/half) oligonucleotides. In contrast, the patent in suit discloses a three-quarter/one-quarter ribozyme, wherein loops II and III are opened and, apart from the target triplet to be cleaved, all other consensus sequences are in the three-quarter part of the molecule.

41. Starting from this prior art, and avoiding any hindsight knowledge (cf "Case Law" *supra*, I.D.4, 106 and 107), the technical problem underlying the patent in suit is the provision of further oligonucleotides with trans-cleavage activity. The specific ribozymes of claims 1 to 3 - three-quarter/one-quarter ribozymes - are proposed as a solution to this technical problem.

42. Although document D6 acknowledges the biological significance of trans-cleavage reactions and the interest of small RNAs as sequence specific nucleases (cf page 599, left-hand column, last two paragraphs), it characterizes the reaction of oligonucleotide O1 as being highly specific and "*not capable of cleaving a variety of other RNAs and therefore is not a general nuclease*" (cf page 600, paragraph bridging left- and right-hand columns). There is no suggestion whatsoever to modify the consensus model of Figure 1 so as to achieve such a general nuclease. However, a possible indication can be found on page 599, which states that in the model of Figure 1b "*it is likely that two base pairs could be removed from both helix I and III and one base pair from helix II*" (cf page 599, left-hand column, first paragraph) and, thus, it leaves a minimal length of 3, 2 and 4 bases for, respectively, stems I, II and III, wherein the two bases of stem II correspond to two bases of the triplet sequence identified in the

patent in suit as an essential feature of the target RNA sequence. However, there is no hint to select the specific value of 4 bases for the length of stem I, the introduction of an associated loop I of 4 bases and the elimination of loop II with an extension of the hybridisable flanking regions of both stems II and III, let alone an indication of the possible effects of all these structural changes on the activity of the resulting three-quarter/one-quarter ribozyme. In fact, document D6 states that "*it is unclear how many features of the model are essential for the cleavage reaction*" and acknowledges that "*not all of the conserved nucleotides may be needed for cleavage*", further suggesting that sequence variants be prepared in order "*to explore the sequence and structure requirements for the cleavage reaction*" (cf page 599, left-hand column, first full paragraph).

43. It has been argued that the advantages of having most of the essential consensus sequences in the three-quarter ribozyme - so as to have less constraints in the target sequence - were evident from document D3, which discloses the conversion of an intramolecular reaction (self-splicing ribosomal RNA intervening sequence) into an intermolecular one (cf page 430, Figure 1). Following this argument, document D3 would prompt the skilled person to modify accordingly the half/half ribozyme of document D6 and in doing so the claimed subject-matter would be achieved in a straightforward manner.

44. However, the board notes that document D6 refers to these teachings in a general context referring to the importance of small trans-cleaving RNAs nucleases for

biological RNA processing mechanisms (cf page 599, paragraph bridging left- and right-hand columns). There is no suggestion whatsoever that document D3 - itself not cited in document D6 - could be of any guidance for modifying the disclosed ribozyme model. In fact, the sequence shown in Figure 1 of document D3 bears little sequence and structural resemblance to the ribozyme model of document D6 and thus, no reliable extrapolation can be made in a straightforward manner. Moreover, there is no reference in document D3 to the advantageous presence of most sequence and structural constraints in the catalytic part of the trans-cleaving molecule. To consider this teaching as implicitly disclosed in document D3 and further to see it as a direct incentive to modify accordingly the ribozyme model - with different sequence and structural requirements - of document D6 entails an unacceptable degree of hindsight.

45. In conclusion, starting from document D6, the ribozymes of claims 1 to 3 were **not obvious** and thus the requirements of Article 56 EPC are fulfilled. The same conclusion applies to all remaining claims in so far as they refer to compositions containing such compounds, to their method of preparation, uses, etc. (cf claims 6 to 22).

Article 56 (Claim 4 directed to multimers)

46. Document D9, the closest prior art for this subject-matter (cf point 31 *supra*), discloses three ribozymes - $Rz_{CAT}1$, 2 and 3 - with three different arbitrarily chosen target sequences in a common chloramphenicol acetyl transferase (CAT) substrate gene (cf page 588,

Figure 4). All ribozymes and the CAT gene are cloned so as to produce the corresponding RNA transcripts in vitro. The CAT mRNA substrate is then incubated with each of the three ribozymes (cf page 589, Figure 5). There is no suggestion to have the three ribozymes in a multimeric form, let alone to the possible advantages - if any - associated with such a multimer. Moreover, the construction of a multimer requires the selection of associated intermediate sequences. This selection cannot be arbitrary but requires consideration of the structural constraints of each and every ribozyme present in the multimer and, thus, it depends on the distance between the target sites to be cleaved, the degree and extent of hybridisation - if any - of the intermediate sequences to the target substrate, as well as on their possible effects on the secondary and tertiary structures of the ribozymes. In fact, the extension of (hybridisable) nucleotide sequences flanking the catalytic domain could significantly decrease the activity of the ribozymes by favouring the formation of alternative - inactive - structures in the multimer. Since there is no information in document D9 concerning these intermediate sequences and their possible effects on the activity of ribozymes, the preparation of multimers is **not obvious**.

47. Thus, the requirements of Article 56 EPC are fulfilled for this subject-matter.

Article 56 EPC (Claims 14 to 22 directed to methods and uses of multimers and of generic and specific ribozymes)

48. Document D9, the closest prior art for this subject-matter (cf point 32 *supra*), explicitly refers to

potential in vivo applications of ribozymes, to methods of administration and to the fact that the "*anti-gene activity of the ribozymes could provide a basis for various gene and viral therapies and analysis*" (cf page 590, right-hand column and page 591).

49. Starting from this closest prior art, the objective technical problem to be solved may therefore be seen as putting the theoretical teaching of document D9 into practice. Examples 8 and 9 of the patent in suit show that this teaching has been successfully performed.

50. In accordance with the case law of the Boards of Appeal, in these cases where, in the light of the prior art, the suggested approach is obvious for the skilled person to try, then it still has to be assessed whether there is a reasonable expectation of success (cf "Case Law" *supra*, I.D.6.2, 117). In the present case, there are several factors which could not allow the skilled person to have such a reasonable expectation.

51. The model ribozyme of document D9 is derived from the consensus sequences of naturally-occurring self-cleavage RNA molecules that replicate in plants, either alone (viroid RNA) or dependent on a helper virus (satellite RNAs) (cf page 585, left-hand column, first paragraph and page 586, Figure 1). Document D2, concerned with the self-cleavage reaction of avocado sunblotch viroid (ASBV), refers to these RNA molecules as pathogenic agents with associated induction of disease symptoms upon replication (cf page 1, first paragraph and page 2, second paragraph) and summarizes several reasons that have been proposed for explaining the mechanism causing these symptoms, such as a drain

on host cell's replication machinery and metabolites or an interference with cellular RNA processing (cf page 3, second paragraph and page 49, second paragraph). Document D7, which states that some particular self-cleavage molecules do not appear to harm their host organisms and suggests possible in vivo applications (cf page 93, left-hand column, second paragraph and right-hand column), refers, however, to the mechanism of pathogenicity as being completely unknown (cf page 93, left-hand column, first paragraph). This mechanism was neither characterized nor understood and it could not be excluded that using a ribozyme in vivo resulted in the appearance of lethal effects in the host cell.

52. A low in vivo specificity and cleavage of unrelated cellular RNA transcripts had been proposed as a possible mechanism of toxicity too. The ribozymes of document D9 have flanking sequences (known to stabilize the interaction of ribozyme and its substrate) of an arbitrarily chosen length of eight nucleotides (cf page 588, left-hand column, first full paragraph and last full paragraph; right-hand column, first paragraph and Figure 4). There is, however, no information as to the effects of this length, let alone of its possible variation, on the specificity of ribozymes in vivo. Whereas short flanking sequences - associated with a low specificity - could produce more toxic effects, longer sequences - with associated higher specificity - could significantly decrease the activity of ribozymes by favouring the formation of alternative inactive structures. Furthermore, since viroids and satellite RNAs can be specific for certain host cells and dependent on helper virus, it could not be excluded

that the in vivo activity of ribozymes would require - or would be influenced by - the presence of specific cellular RNA components or of other viruses (cf page 599, left-hand column, last paragraph in document D6).

53. Contrary to the patent in suit, there is no reference in document D9 to the possible lack of in vivo stability of ribozymes due to protease degradation, let alone a disclosure of methods for overcoming this problem. Even if the problem was known in the prior art and the experience gained from oligonucleotide-related techniques was, in principle, available to the skilled person, the particular sequence and structural constraints of ribozymes did not allow that person to expect a straightforward extrapolation. It is only once the resilience of ribozymes - in particular to certain modifications such as the introduction of long flanking sequences - has been demonstrated in vivo that such an extrapolation can reasonably be made (cf points 34 and 36 *supra*). In fact, the claimed subject-matter is not limited to short ribozymes since the length and composition of the flanking sequences as well as the content and extent of the hybridization is functionally defined in the wording of the claims.

54. The present situation is not a case of "try-and-see" whether the ribozymes of document D9 are active in vivo but a case which requires certain assumptions to be made and, accordingly, the selection of appropriate parameters - such as ribozyme flanking sequences (specificity) with possible associated toxic effects, stability to protease degradation, etc. - none of them referred to in document D9. It follows from the

foregoing that the skilled person had no reasonable expectation of success before performing the actual experiments in vivo.

55. It has also been argued that the subject-matter does not solve the technical problem over the whole claimed range as required by the established case law (cf "Case Law" *supra*, I.D.6.9.2, 125). Document D12 fails to detect any cleavage for certain target triplets, in particular those not having uracil at the second position or having guanine at the third position as well as for the specific target triplet AUC (cf abstract, page 162 Table II). For the latter target triplet, however, the results are contradictory with the prior art and possible reasons for this difference are given in the document (cf page 160, right-hand column, second paragraph), whereas for the former cases, the claimed subject-matter always requires the presence of uracil at the second position of the target triplet and page 5, lines 45 to 48 of the patent in suit clearly excludes the presence of guanine in the third position of the target triplet. Accordingly, and in the light of the conclusions reached with regard to Article 83 EPC, the board considers that the claimed subject-matter provides an effective and inventive solution to the objective technical problem over the whole range claimed (cf points 33 to 38 *supra*).

56. Thus, the requirements of Article 56 EPC are fulfilled.

Adaptation of the description

57. Appellant I requests that the description on file be replaced by an amended description, consisting of pages 3, 3a, 4, 4a, 4b, 5, 5a, 6, 6a, 6b and 7 to 18.

58. The requested amendments are an appropriate adaptation of the description to the claims of the third auxiliary request and they are in compliance with the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent in amended form on the basis of the auxiliary request 3 filed during the oral proceedings, the description as amended during the oral proceedings and the drawings of the patent as granted.

The Registrar: The Chairman:

A. Wolinski L. Galligani