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**Datasheet for the decision
of 30 October 2019**

Case Number: T 0738/15 - 3.3.04

Application Number: 99910592.7

Publication Number: 1068311

IPC: C12N15/82, C12N15/11

Language of the proceedings: EN

Title of invention:

Methods and Means for Obtaining Modified Phenotypes

Patent Proprietor:

Commonwealth Scientific and Industrial Research
Organisation

Opponents:

BASF SE
Strawman Limited
Carnegie Institution of Washington / University of
Massachusetts
Syngenta International Ag

Headword:

Gene silencing/CSIRO

Relevant legal provisions:

EPC Art. 54, 56, 83, 57, 123(2), 123(3)

Keyword:

Novelty - (yes)

Inventive step - (yes)

Industrial application - (yes)

Sufficiency of disclosure - (yes)

Amendments - added subject-matter (no) - broadening of claim
(no)

Decisions cited:

G 0007/93

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 0738/15 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 30 October 2019

Respondent: Commonwealth Scientific and Industrial Research
(Patent Proprietor) Organisation
Black Mountain Science and Innovation Park
Clunies Ross Street
Acton ACT 2601 (AU)

Representative: Almond-Martin, Carol
Ernest Gutmann - Yves Plasseraud S.A.S.
88, Boulevard des Belges
69452 Lyon Cedex 06 (FR)

Appellant: Strawman Limited
(Opponent 2) Orchard Lea
Horns Lane
Combe, Witney
Oxfordshire OX29 8NH (GB)

Representative: Wainwright, Jane
Potter Clarkson
The Belgrave Centre
Talbot Street
Nottingham NG1 5GG (GB)

Decision under appeal: **Interlocutory decision of the Opposition
Division of the European Patent Office posted on
19 February 2015 concerning maintenance of the
European Patent No. 1068311 in amended form.**

Composition of the Board:

Chairman P. de Heij
Members: A. Chakravarty
D. Luis Alves

Summary of Facts and Submissions

- I. European patent EP 1 068 311, entitled "*Methods and Means for Obtaining Modified Phenotypes*" derives from European application 99 910 592.7 and was published as international application WO 1999/053050. The patent was opposed by four parties, opponents 1 to 4.
- II. In an interlocutory decision dated 19 February 2015, the opposition division decided that, account being taken of the amendments in the form of auxiliary request 3, the patent and the invention to which it related met the requirements of the EPC. In that decision, that opposition division also held that claim 11 of the main request had been amended contrary to Rule 139 EPC and that the subject-matter of auxiliary requests 1 and 2 did not meet the requirements of Article 54 EPC.
- III. Opponent 3 withdrew their opposition on 23 March 2015.
- IV. Appeals were filed by the patent proprietor (appellant I) and by opponents 1, 2 and 4 (appellants II, III and IV, respectively) against said interlocutory decision. Appellants II and IV subsequently withdrew their respective oppositions and appeals. Appellant I withdrew their appeal at oral proceedings before the board.
- V. The parties to the appeal are the patent proprietor and opponent 2. Opponent 2 is the appellant, while the patent proprietor is respondent. For ease of reference, the parties will be referred to as the patent proprietor and opponent 2 in this decision.

VI. The board set out its preliminary opinion on the appeals in a communication pursuant to Article 15(1) RPBA.

VII. Claim 1 of the set of claims considered allowable by the opposition division (main request) reads:

"1. A method for reducing the phenotypic expression of a nucleic acid of interest, which can be expressed in a eucaryotic [sic] cell, comprising the step of introducing into said cell a chimeric DNA comprising the following operably linked parts:

a) a promoter, operative in said eukaryotic cell;
b) a DNA region, which when transcribed, yields an RNA molecule with a nucleotide sequence comprising

i. a sense nucleotide sequence including at least 15 consecutive nucleotides having 100% sequence identity with at least part of a coding region of said nucleic acid of interest; and

ii. an antisense nucleotide sequence including at least 15 consecutive nucleotides, having 100% sequence identity with the complement of said at least 15 consecutive nucleotides of said sense nucleotide sequence;

wherein the RNA is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence such that at least said 15 consecutive nucleotides of the sense sequence base-pair with said 15 consecutive nucleotides of the antisense sequence; wherein said nucleic acid of interest is a gene integrated in the genome of said eukaryotic cell,

and wherein said method is not a method of treatment of the human or animal body by surgery or therapy, or a diagnostic method practised on the human or animal body".

Claim 17 reads:

"17. A eucaryotic [sic] cell comprising a nucleic acid of interest, which can be phenotypically expressed, further comprising a chimeric DNA molecule comprising the following operably linked parts:

- a) a promoter, operative in said eucaryotic cell;
- b) a DNA region, which when transcribed, yields an RNA molecule with at least one RNA region with a nucleotide sequence comprising
 - i) a sense nucleotide sequence including at least 15 consecutive nucleotides having 100% sequence identity with at least part of a coding region of the nucleic acid of interest; and
 - ii) an antisense nucleotide sequence including at least 15 consecutive nucleotides, having 100% sequence identity with the complement of said at least 15 consecutive nucleotides of said sense nucleotide sequence;

wherein the RNA is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence, such that at least said at least 15 consecutive nucleotides of the sense sequence base pair with said at least 15 consecutive nucleotides of the antisense sequence and wherein said nucleic acid of interest is a gene integrated in the genome of said eucaryotic cell".

VIII. The following documents are mentioned in this decision.

E1: Hunter, Tony et al., "The Characteristics of Inhibition of Protein Synthesis by Double-Stranded Ribonucleic Acid in Reticulocyte Lysates", *The Journal of Biological Chemistry*, 1975, 250(2), 409-417.

E7: Hobbs, Shaun L.A. et al., "Transgene copy number can be positively or negatively associated with transgene expression", *Plant Molecular Biology*, 21, 1993, 17-26.

E11: WO 94/01550.

E12: Bureau, Thomas E., et al., "Stowaway: A new Family of Inverted Repeat Elements Associated with Genes of both Monocotyledonous and Dicotyledonous Plants", *The Plant Cell*, 6, June 1994, 907-916.

E21: Sijen, Titia et al., "RNA-Mediated Virus Resistance: Role of Repeated Transgenes and Delineation of Targeted Regions", *The Plant Cell*, December 1996, 8, 2277-2294.

E24: WO 97/01952.

E30: Stam, Maïke et al., "Post-transcriptional silencing of chalcone synthase in *Petunia* by inverted transgene repeats", *The Plant Journal*, July 1997, 12(1), 63-82.

E40: Fire, Andrew et al., "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*", *Nature*, Vol. 391, 19 February 1998, 806-811.

E45: WO 99/32619.

E45a: Provisional US Application No. 60/068562
(Priority of WO 99/32619).

E46: WO 99/49029.

E46a: Australian patent application PP2492
(priority doc of WO99/49029).

E46b: Australian patent application PP2499
(priority doc of WO99/49029).

E51: Bridge, Alan J. *et al.*, "Induction of an interferon response by E51 RNAi vectors in mammalian cells", *Nature Genetics*, 2003, 34(3), 263-264.

E60: Experimental data re-filed by Appellant III with the statement of grounds of appeal.

E61: Rang A. *et al.*, "Detection of RNA variants transcribed from the transgene in Roundup Ready soybean", *Eur. Food Res. Technol.*, 2005, 220, 438-443.

E67: Yi, C. E. *et al.*, "Specific and Potent RNA Interference in Terminally Differentiated Myotubes", *Journal of Biological Chemistry*, 2003, 278(2), 934-939.

E68: McIntyre G.J. *et al.*, "The effects of stem length and core placement on shRNA activity", *BMC Molecular Biology* 2011, 12-34.

E69: Ge Q. *et al.*, "Minimal-length short hairpin RNAs: The relationship of structure and RNAi activity", *RNA* (2010), 16, 106-117.

E70: Mellow C. and Fire A., "DNA Transformation", *Methods in Cell Biology*, 1995, 48, 451-482.

- IX. Oral proceedings before the board took place on 30 October 2019. At the end of the proceedings, the Chair announced the board's decision.
- X. The arguments of opponent 2 in relation to the main request as far as relevant to the present decision are summarised as follows.

Admission of documents E67 to E69

The opposition division had been wrong to admit these documents into the proceedings because they had been filed late. Specifically, they were filed on the 24 December 2014, only shortly before the oral proceedings before the opposition division on 7 January. In addition, the patent proprietor had been aware of at least document E67 and its relevance to the present case since May 2014 and could and should therefore have filed them earlier. Also weighing against their admission into the opposition proceedings was the fact that the new documents required extensive preparation, which was not possible given the very short notice.

Amendments - Article 123(2) EPC

Claim 1

The amendment to introduce the feature of "at least 15 consecutive nucleotides" into claim 1 was not allowable under Article 123(2) EPC. The skilled person would not, directly and unambiguously, have understood from the application as filed that any sequence in the specification was intended to be at least 15 nucleotides long, as 15 nucleotides was only disclosed

as filed as a single preferred length (i.e. exactly 15).

The amendment to claim 1: "sense nucleotide sequence including ... " had no basis in the application as filed. Said application disclosed a sense nucleotide sequence with a total length of 15 consecutive nucleotides but did not disclose a sense nucleotide sequence being longer and only including 15 consecutive nucleotides with defined sequence identity to the target. The application as filed did not provide basis for a sense nucleotide sequence including a sequence of 15 consecutive nucleotides having 100% sequence identity with at least part of a coding region of said nucleic acid of interest.

Claim 17

There was no disclosure in the application as filed for the subject matter of independent claim 17. It did not disclose a eukaryotic cell as claimed in combination with a length of 15 consecutive nucleotides. Moreover, the feature "wherein said nucleic acid of interest is a gene integrated into the genome of said eucaryotic cell" was not disclosed in the application as filed.

Amendments - Article 123(3) EPC

Claim 1 as amended extended the protection conferred by the patent beyond that of the granted claims.

Claim 1 (see section (b)(i)) as granted read:

"... a sense nucleotide sequence **of** at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of a coding region of said nucleic acid of interest: and ... ",

whereas claim 1 as amended in the main request read:

"... a sense nucleotide sequence **including** at least 15 consecutive nucleotides having 100% sequence identity with at least part of a coding region of said nucleic acid of interest; and ... " (emphasis added by the board).

In claim 1 as granted, the entire sense sequence had to possess at least 10 consecutive nucleotides and the entire sense sequence across its whole length had to have 75 to 100% sequence identity to the target sequence. In claim 1 as amended the sense sequence included a portion that had 15 nucleotides being 100% identical to the target sequence, but there was no further limitation on what the remainder of the sense sequence contains.

The proprietor had argued that the claim did not impose any restriction on the identity of the nucleotides which were to be regarded as 100% identical to the target. However, such a definition was in contradiction to claim 24 as granted, according to which the sense sequence had to correspond in length to the target. The degree of identity was then calculated based on this entire length.

Amended case - Article 13(1) RPBA

The line of argument brought by the patent proprietor in relation to Article 83 EPC that no evidence had been provided that the claimed method could not be carried out in relation to the invention as claimed, i.e. in relation to methods where there was expression of hairpin RNAs from a chimeric DNA introduced into a eukaryotic cell, had not been made in writing and was

brought forward for the first time at oral proceedings before the board. However, opponent 2 stated that they were willing and able to deal with this question.

*Disclosure of the invention - Article 100(b) EPC/
Article 83 EPC*

Claim 1

The subject-matter of claim 1 was a method of gene silencing using double-stranded hairpin RNAs (dshRNA) expressed from a DNA which had been introduced into a cell. The claim included RNA molecules having a sense strand with only 15 consecutive nucleotides having identity to a target. Moreover, the claim specified no upper limit of the length of the RNA.

By way of background, document E40, dating from 1998, was a ground breaking disclosure as it showed that double stranded RNA (dsRNA) could be used to silence genes in non-vertebrate animals (i.e. in nematodes). By contrast, dsRNA in plants had already been known to silence genes since at least 1992, see for example documents E7, E24 and E30. Hence, the patent in suit was not ground-breaking in the same way as document E40.

The skilled person reading the patent would understand it to be about plants. There was no disclosure in the patent which would allow for expression of constructs in non-plant systems, for example only plant expression promoters were mentioned as were the preferred plasmids etc and the preferred phenotypic characteristics to be inhibited. Finally, the experimental examples were all plants.

The application provided no specific teaching in respect of animal cells. To find out which animal cells might be suitable, the skilled person had to experimentally investigate each and every RNA construct, cell type, culture conditions, dose etc, to see if they worked in the claimed method. As acknowledged by the proprietor and opposition division, there were a number of cell types that would not work depending on the specific circumstances (size of hairpin, cell type, dose of dsRNA etc). No guidance was provided in the application as filed as to the "circumstances" which would work and those which would not.

'Short' and 'long' dshRNAs

Document E60 provided experimental evidence that short hairpin RNAs did not work in a method as claimed, while document E1 showed global protein suppression (PKR response) occurring when using long RNAs, proving that the claimed method would not work when using long RNAs.

Long sequences

There was a significant body of evidence (verifiable facts) dating back to 1975 (see document E1) demonstrating that dsRNA constructs over 50 nucleotides in length (later shown to be the case even for constructs over 30 nucleotides in length, see E51) induce a global suppression of protein synthesis in most vertebrate animal cells. The PKR response of vertebrate cells represented a significant technical hurdle to be overcome. The application as filed provided no guidance on this.

It was therefore impossible to achieve target-specific inhibition in most vertebrate animal cells according to the claimed method.

Short RNA sequences

Short RNA sequences were those having a stem of ca. 15-18 nucleotides (nt) in length. There were serious doubts substantiated by verifiable facts that the claimed method would work using such short double-stranded hairpin RNA sequences because they were too short to anneal specifically to a target to induce gene-specific silencing. This was confirmed by the data in document E60, which demonstrated that none of the constructs of 10, 11, 15 or 16 nucleotides (nt) had any effect on gene expression, while the control constructs of 19nt based on the same gene sequence had a clear inhibitory effect on gene expression, as reflected in the resulting phenotype.

Document E68 was cited by the patent proprietor in support of sufficiency of the constructs having stem lengths shorter than 19 nucleotides. However, a skilled person would be aware that introducing any kind of molecule into the target cells would lead to variations in gene expression due to variable nature of biological systems. What was reported in document E68 was not a phenotypic reduction in gene expression as claimed, but an observation of variability of gene expression due to intrinsic characteristics of the biological system. It should be noted that figure 2A of document E68 clearly indicated that the constructs below 19nt were inactive.

The quality of the results presented in document E60 was clear from page 18, paragraphs 1 and 2, which reported that quality control was done.

Moreover, document E68 was not relevant to the claimed invention because it concerned siRNA and not hairpin RNA.

In summary, document E60 was evidence that shRNA sequences having stem lengths of under 19nt did not work in the claimed method.

Novelty - Article 54 EPC

With respect to document E45 - Claims 1 and 17

In the decision under appeal the opposition division held that document E45 did not disclose a DNA coding region, e.g. a viral vector that has a DNA stage and therefore did not anticipate the claimed subject matter.

This decision was incorrect because page 7, lines 7 to 8 referred explicitly to transcription by RNA polymerase of the RNA strand. It was known to the person skilled in the art that transcription is the process by which RNA is produced from an initial DNA sequence, and additionally that RNA polymerase is the enzyme which is recognised as producing the primary transcript RNA from a DNA template. Therefore, by referring to *in vivo* production of RNA by transcription by RNA polymerase in the cell, only production from a DNA vector could have been intended. This was reinforced by the disclosure on page 13, lines 8 to 12 and also in claim 21 (claim 20 in document E45a). Here explicit reference was made to the synthesis of RNA *in vivo* using endogenous or cloned RNA polymerase to mediate transcription. Therefore, this must have been from a DNA template (i.e. a vector) as discussed above.

With respect to document E30 and E7, E21, E24 - claim 1

Document E30 disclosed transgenic plants containing T-DNA loci having a *ChsA* gene in an inverted repeat structure which when expressed led to the formation of an RNA as defined in claim 1 (see Figure 7, PSE19-1). Moreover, document E30 disclosed that some plants containing insert PSE19-1 exhibited a phenotype corresponding to silencing of the *ChsA* gene. The formation of a hairpin RNA as defined in the claim occurred either because the *nos*-terminator was leaky, leading to the production of read-through transcripts or because the *ChsA* gene inserted into the plants had no terminator. The same conclusion applied to documents E7, E21 and E24. Thus, the subject-matter of claim 1 lacked novelty with respect to the disclosure in document E30 and documents E7, E21 and E24.

With respect to document E46 - claim 1 and claim 17

All the features of claim 1 and claim 17 were present together in the embodiment of figure 25 and were also disclosed in the priority documents of document E46 (documents E46a and E46b). These clearly disclosed inverted repeats/hairpins, see lines 16 to 20 on page 18 and lines 15 to 24 on page 21 of document E46a. Furthermore, Figure 15 of E46a clearly disclosed a plasmid containing an inverted repeat of the BEV.2 gene separated by a stuffer fragment which would thus form a hairpin structure.

Inventive step - Article 56 EPC - Claim 1

Document E30 as closest prior art

Even if the board held that document E30 did not disclose a method according to claim 1 because it did not explicitly disclose read-through transcript generation, it would have been obvious to a skilled person to modify the teaching of E30 to arrive at the claimed subject matter. Document E30 clearly demonstrated that a recombination of different inserts produced inverted repeats that were effective in gene expression inhibition. From this, the skilled person would have modified the original DNA constructs used in document E30 to remove the *nos* terminator, solving the problem of providing an alternative means of inhibiting phenotypic expression of a nucleic acid of interest. The subject-matter of claims for the methods using such constructs was obvious because the constructs were already produced in the transformants disclosed in E30 and their efficiency was clearly demonstrated.

Another reason why the claimed subject-matter was obvious in view of document E30 was because the *nos* terminator was 'leaky', making the constructs disclosed in document E30 produce at least some RNA molecules containing an inverted repeat. Document E61 provided conclusive evidence that the *nos* terminator was leaky and that read-through must therefore have occurred.

Document E40 as closest prior art

Additionally, document E40 was closest prior art for subject-matter relating to animal cells. It disclosed the breakthrough discovery that dsRNA can be used to efficiently reduce expression of a target gene in a nematode.

When starting from document E40, the technical problem to be solved was 'the provision of an alternative

method of gene silencing'. Faced with this problem, the skilled person would have immediately considered exploring the concept disclosed in document E40 further and developed the idea with a view to adapting the effects disclosed therein. The skilled person would have directly modified the method of document E40 to use DNA expressing RNA instead of direct introduction of RNA and would have used a dsRNA made single stranded (i.e. sense and antisense on the same strand capable of forming a hairpin structure). These two modifications were linked. Once it was decided to use a DNA to introduce the RNA into the cell, it was clearly easier to have both the sense and antisense sequences under the control of a single promoter.

The change from direct injection of dsRNA, as done in document E40, was obvious in the light of the disclosure of document E70 (referred to in document E40 as reference 27). Document E70 was from the same authors and described different methods of working with *C. elegans*, specifically including the use of DNA instead of RNA. The content of document E70 was common general knowledge of a person working with gene expression.

The claimed subject-matter was also obvious in view of the disclosure in document E40 in combination with that in document E11. Document E11 clearly described the use of self-complementary RNA which formed hairpin structures so as to be resistant to nucleolytic degradation. This would have been a beneficial property of any dsRNA.

Failure of the claimed subject matter to solve the technical problem over the whole scope of the claims

There could only be an "invention" if the application as filed made it plausible that its teaching credibly solved the technical problem (see decision T 1329/04). In the present case, the information in the application as filed, solely related to plants (as discussed above) and so the skilled person had to apply their common general knowledge in relation solving the problem in animal cells. That common general knowledge was that vertebrate animal cells exhibited a global non-specific cellular response after exposure to longer length dsRNA and as such, the skilled person would have doubts as to the plausibility of the effectiveness of longer RNA hairpins in animal cells. As the problem was not solved over the whole scope of the claims, the requirements of Article 56 EPC were not satisfied.

Industrial application - Article 57 EPC

The claimed subject-matter did not meet the requirements of Article 57 EPC for the same reasons that it did not meet the requirements of Articles 56 and 83 EPC.

- XI. The arguments of the patent proprietor, as far as relevant to the present decision, are summarised as follows.

Admission of documents E67 to E69

The opposition division was correct in admitting documents E67 to E69 into the proceedings. They were filed in response to new submissions of the opponent on 7 November 2014 and as soon as possible thereafter.

Amendments - Article 123(2) EPC

The opponent argued that there was no basis in the application as filed for the feature in claims 1 and 17 of the main request that the sense and antisense sequences include "at least 15 consecutive nucleotides". However, the length feature of "15 nucleotides for the sense and antisense sequences was supported at page 18, line 30 to page 19, line 3 in association with page 21, line 25, and page 21, lines 12 to 14.

The disclosure of "15 consecutive nucleotides" at page 18, line 30, at page 19, line 3 and at page 21, line 25 did not explicitly comprise the words "at least" before the number "15". However, the passage at page 21, line 25 explicitly stated that the antisense sequence preferably "always includes a sequence of about 10, preferably 15 consecutive nucleotides ... with 100% sequence identity to the corresponding part of the sense nucleotide". By virtue of the presence of the word "includes", said passage disclosed a series of nested values, in which all the values in the series were encompassed by the preceding value. This was an implicit disclosure of "at least". The word "includes" clearly indicated that the length of the sequence in question was not limited to the number recited thereafter and conveyed the meaning "at least".

Opponent 2 considered there was no support in the application as filed for the feature "sense nucleotide including". However, the disclosure of the application at page 21, lines 12 to 14 stated that the length of the antisense nucleotide sequence preferably corresponds to that of the sense nucleotide sequence. Consequently the teaching at page 21, lines 24 to 28 relating to the antisense sequence also related to the sense sequence.

The subject-matter of claim 17 was directly and unambiguously disclosed in the application as filed. In particular, a disclosure of a eukaryotic cell as claimed in combination with a length of 15 consecutive nucleotides could be found in the application as filed at page 21, lines 12 to 14, where it was stated that the length of the antisense nucleotide sequence preferably corresponds to that of the sense nucleotide sequence. Consequently the teaching of page 21, lines 24 to 28 relating to the antisense sequence also related to the sequence. The basis for the inclusion of such a sequence in a eukaryotic cell was to be found in the application as filed page 28, lines 14 to 16 and also at page 18, line 21 to page 19, line 3, at page 20, lines 20 to 21, at page 21 lines 12 to 14 and lines 23 to 27. The feature that the nucleic acid of interest is integrated into the genome of the eukaryotic cell was disclosed in the application as filed at page 19, lines 28 to 30 as well as at page 8, lines 9 and 10, as well as in claim 5.

Amendments - Article 123(3) EPC

Opponent 2 considered that the change in claim 1 from "of at least 10 consecutive nucleotides" to "including at least 15 consecutive nucleotides" extended the protection conferred by the patent.

However, the decision under appeal was correct. The claims as granted specified the presence of a DNA region comprising a sense nucleotide sequence of at least 10 consecutive nucleotides having an identity of 75 to 100% with the target nucleic acid of interest. The "comprising" language was 'open' and permitted the presence of additional nucleotides in the DNA region.

These additional nucleotides could be considered to be part of the 'DNA region' or part of the 'sense sequence'. Irrespective of the terminology used to designate these additional nucleotides, their presence was encompassed by the claims as granted. Consequently, the only requirement of the claims as granted with respect to the sense strand was that it contained a stretch of at least 10 nucleotides having an identity of 75 to 100% with the target sequence.

*Disclosure of the invention - Article 100(b) EPC/
Article 83 EPC*

Short RNA sequences

Opponent 2 considered that the evidence on file demonstrated that hairpins having a stem length of 15 base pairs did not have an effect on gene expression. The evidence referred to was essentially document E60, an experimental report provided by opponent 2 and document E68. However, neither of these documents showed that RNA hairpins having a stem of 15 base pairs as described in the claims cannot down-regulate target gene expression.

Two assay systems were reported in document E60. One was a phenotypic assay measuring epithelial to mesenchymal transition (EMT) via MMP10 secretion, as explained on page 4 of the report. The other was based on the same rationale, but SMAD3 and SMAD4 mRNA levels were measured directly.

The design of each of these assays was flawed and the results did not provide reliable information as to the capacity of the tested shRNAs to down-regulate target gene expression.

Even if the results reported in document E60 were accepted, Figure 8 (top graph) showed the expression of SMAD3 was affected by RNAs having a 15 nt stem. The experiment could be interpreted as meaning that silencing of up to 40% had been achieved due to the large error bars. Thus, contrary to the arguments of the opponent, Figure 8 did show inhibition using RNA molecules having a stem length of 15 nucleotides.

Document E68

Also contrary to the opponent's arguments, document E68 did not show that hairpins having a stem length of 15nt did not have an effect on gene expression. In fact, the authors of this document concluded that "*there is no fixed correlation between stem length and suppressive activity, though in some cases the activity of hairpins of at least 23 bp may be improved by stem extensions. From a purely activity point of view, neither short nor long hairpins should be discounted as potentially potent suppressors*" (see page 9, Conclusion).

RNA hairpins having long (>50nt) stem length in vertebrate cells

No evidence had been provided the skilled person would not be able to carry out the invention claimed over the whole range claimed. In relation to whether or not the claimed method worked in vertebrate cells, it should be noted that the claimed method comprised introducing into the cell a chimeric DNA from which the RNA capable of forming an artificial hairpin RNA structure was expressed. Although a PKR response existed in vertebrate cells, as documented e.g. in document E58, no evidence had been presented that the claimed method

would not work when expressing the RNA from a chimeric DNA as claimed.

In document E1, the PKR response was demonstrated in cell lysates. As such, this document could not serve as evidence that the PKR would be problematic when expressing the double-stranded RNA from a chimeric DNA introduced into the cell.

Novelty - Article 54 EPC

With respect to document E45 - Claims 1 and 17

The disclosure in document E45 did not anticipate the subject-matter of either claim 1 or 17.

None of the passages cited by the opponent disclosed that the RNA was produced by transcription in a cell in which the nucleic acid of interest was also present. The passages at pages 7, 13 and 14 (and the corresponding passages of priority document E45a) referred generally to transcription from a transgene *in vivo* or to an expression construct, but were silent as to whether the transcription took place in a cell in which the target gene to be silenced also occurs. Claim 36 of document E45 had no counterpart in the priority application, document E45a. This disclosure was thus not part of the prior art. In any event, claim 36 clearly related to a situation in which the target gene is NOT in the same cell as the cell containing the expression construct, because it explicitly stated that the RNA produced by transcription is "*contacted with an organism containing the target gene*".

With respect to document E30 - claim 1

There was no evidence in document E30 that RNAs capable of forming an artificial hairpin structure were produced in the transgenic plants disclosed. The authors of document E30 themselves concluded that the silencing mechanism might not involve RNA transcripts from the transgenes and that the transgenes may not be transcribed at all (see page 78, column 2, lines 2 to 25). It was emphasised that the palindromic arrangement of the transgene sequences in the genome appeared to play a crucial role (see page 78, column 1, lines 41-44) and that a DNA-DNA pairing mechanism between the inverted repeat transgene sequences and the endogenous target genes (see page 80, second full paragraph) might play a role, giving rise to aberrant RNAs from the endogenous target gene (see page 79, column 2 - page 80 and column 1; abstract).

On the question of whether or not the insert contained in plant PSE19-1 contained *nos* promoters at all, this could be answered by reference to Figure 1a which showed the presence of a *nos* promoter between the restriction sites B and E of the T-DNA construct (see also Figure 7 - white box). Regarding the question of whether or not the *nos* promoter was leaky and notwithstanding the disclosure in document E61, no evidence had been provided showing the production of read-through transcripts in the plants in question.

With respect to document E46 - claims 1 and 17

The disclosure of document E46 did not affect the novelty of any of the claims. The passage referred to by Opponent 2 on page 50, lines 10-22 disclosed the vector pCMV.BEV.GFP.VEB. This construct provides an RNA molecule targeting a gene of bovine enterovirus (BEV). BEV was a member of the picornavirus family,

characterised by a single-stranded positive RNA genome. Such viruses had no DNA intermediate in their replication cycle and did not integrate into the host genome. The nucleic acid of interest disclosed in this specific embodiment of document E46 was thus not a gene integrated in the genome of the eukaryotic cell and consequently did not meet the requirements of the present claims.

The remaining passages of document E46 also did not disclose all the features of the claims. For example, there was no disclosure of the targeting of a coding sequence. Moreover, even if the passages of document E46 cited by opponent 2 did disclose all of the features of the present claims, multiple selections and combinations were needed to arrive at subject matter having claimed features.

Inventive step - Article 56 EPC - Claim 1

Document E30 as closest prior art

The skilled person would have found no motivation in document E30 to modify the DNA constructs disclosed therein, particularly PSE19, such that they comprised a chimeric DNA according to the terms of the present claims.

The difference between the cell comprising the PSE19-1 locus in document E30 and a cell according the present invention was that the transcription of the transformed locus of E30 did not give rise to a hairpin RNA. In particular, the locus in the plants disclosed in document E30 comprised a full *Chs* gene comprising the *Chs* coding sequence under the control of a 35S promoter and a *nos* terminator, as well as an inverted fragment

of a Chs gene which no longer had the 35S promoter but still had the nos terminator (see Figures 7, 4(d) and 1(a)). Transcription of this locus resulted in a sense transcript.

The technical effect of this difference was that, according to the invention, efficient and reliable silencing of the target gene is achieved. The technical problem was thus the provision of a means for efficient and reliable silencing of a target gene.

The claimed solution i.e. the provision of a chimeric DNA which on transcription, gives rise to a hairpin RNA having a specific structure as recited in the claim, was not obvious from document E30. In order to arrive at a structure capable of reliably giving rise to a hairpin RNA, the PSE19-1 locus would have to be modified by removing at least one nos terminator at the 3' extremity of the sense gene. However, the skilled person seeking to solve the technical problem would not have done this. The authors of document E30 had noted that the inverted repeat (IR) loci were not detectably transcribed and had concluded that transcription of the transgenes was not required for silencing. Instead, an inverted repeat structure in the DNA genome of the plant was required, because of interaction between the endogenous target gene DNA and the transgene DNA, which generated aberrant RNA from the endogenous target gene, not from the transgene (see page 77, column 1, lines 19-23).

Document E40 as closest prior art

Document E40 disclosed that double-stranded RNA causes potent and specific interference in *C. elegans*. The authors however explicitly stated that the mechanisms

underlying the RNA mediated interference were not yet known (see page 809, column 2, lines 27 to 28), and that the target of the dsRNA was unknown (see page 810, column 1, final paragraph).

The skilled person, seeking to provide an alternative, more efficient means of gene silencing, would have been very cautious about making any type of modification to the system disclosed in document E40 and not have modified either the structure of the effector molecule from a duplex RNA molecule to a single stranded RNA molecule capable of forming a hairpin duplex RNA, or the means of delivery of the effector molecule from injection into the body cavities, gonads or cytoplasm of intestinal cells of *C.elegans*, to the expression of RNA by transcription from a chimeric DNA.

The combination of the disclosure in document E40 and that in document E11 also did not render the claimed invention obvious. The main teaching of document E11 related to stabilised oligonucleotides which activate RNase H. Document E11 however stipulated that these different embodiments provided for the activation of RNase H "*as long as four or more contiguous deoxyribonucleotide phosphodiester, phosphorothioates or phosphorodithioates are present*" (see page 14, line 8). Since the presence of at least 4 deoxyribonucleotides is required in the target hybridizing region, it was evident that the stabilised oligonucleotides could not be produced by transcription. Consequently, a skilled man would not have concluded that hairpin RNA molecules could be produced by transcription from a chimeric DNA in gene-silencing of the type disclosed in document E40.

Concerning the objection of failure of the claimed subject matter to solve the technical problem over the whole scope of the claims

The claimed subject matter solved the problem over the whole of the claimed scope. It was noted that the arguments put forward by Opponent 2 had been made under Article 83 EPC before the opposition division, both in writing and at the oral proceedings. They indeed related to sufficiency of disclosure and were dealt with under that heading.

Industrial application - Article 57 EPC

Opponent 2's submissions on this topic were contingent on the objections relating to Articles 56 and 83 EPC. However as set out above the requirements of these articles were met.

- XII. The patent proprietor (respondent) requested that the appeal of opponent 2 (appellant) be dismissed, i.e. that the patent be maintained in amended form on the basis of the set of claims of auxiliary request 2, filed during oral proceedings before the opposition division as auxiliary request III and upheld by the opposition division. Alternatively, the patent should be maintained on the basis of the set of claims of auxiliary requests 3, 4 or 5 on file (filed on 24 December 2014, filed during oral proceedings before the opposition division, and filed with letter dated 16 December 2016, respectively).

Furthermore, the proprietor requested that document E76 be admitted into the proceedings and that the opponent's line of argument on inventive step, based on document E30, not be admitted into the proceedings.

Finally, it was requested that Mr de Feyter be given permission to address the board. The conditional request for a different apportionment of costs was withdrawn.

- XIII. Opponent 2 (appellant) requested that the decision under appeal be set aside and the patent be revoked. Furthermore, documents E67, E68 and E69 should be removed from further consideration in the proceedings and auxiliary requests 3 and 4 should not be admitted into the proceedings.

Reasons for the Decision

1. In the following, reference to the rules of procedure of the boards of appeal (RPBA) is to the version in force as of 2007.
2. The appeals comply with Articles 106 to 108 and Rule 99 EPC and are therefore admissible.
3. The patent in suit has expired. However, both the patent proprietor and the opponent requested a decision in the present case.

Admission of documents E67 to E69

4. The board has the discretion to hold inadmissible requests which were not admitted in the first instance proceedings (Article 12(4) RPBA). This discretion does not apply to documents E67 to E69 because they were admitted at the discretion of the opposition division.
5. In line with the established case (see Case Law of the Boards of Appeal of the European Patent Office, 9th edition, V.A.3.5.1 and the case law cited there) the

board, unless justified by a new legal or factual situation, limits its review to the opposition division's exercise of its discretion. Such a review takes into consideration whether or not the opposition division exercised its discretion in accordance with the right principles and whether or not it exercised its discretion in an unreasonable way (cf. decision G 7/93, reasons 2.6).

6. In the present case, the opposition division considered the timing of the submissions, the justification for their late filing and their complexity. Thus the board is satisfied that the opposition division exercised its discretion according to the right criteria. Moreover, this was not contested by the opponent.
7. The opponent argues that the patent proprietor was already aware of document E67 and its relevance in May 2014. However, the opposition division reasoned that the documents were filed in response to new issues which had been raised by the opponent in their submission of November 2014. Clearly the opposition division held that the documents only became relevant to the case because of these new issues. The opponent has not provided any arguments to counter this assessment, other than the general statement that the patent proprietor was aware of its relevance in May 2014. This argument is therefore not persuasive.
8. In addition, the opponent argues that dealing with the documents required lengthy preparations in an unreasonably short period before the oral proceedings (held 7 January 2015). The board notes that documents E67 to E69 contain ca. 30 pages in total, including references. Also taking the content of these documents into account, the board considers that the opposition

division could reasonably have arrived at the conclusion that, in the circumstances of the present case, lengthy preparations were not needed.

9. In view of the above, the board concludes that the opposition division exercised its discretion in accordance with the right principles and that it exercised its discretion in a reasonable way. The board therefore decided not to overturn the decision of the opposition division to admit documents E67 to E69 into the proceedings.

Amendments - Article 123(2) EPC

10. Opponent 2 considered that claims 1 and 17 as amended contained subject-matter that extended beyond the content of the application as filed (see Section X.).
11. Concerning the objection to claim 17, the patent proprietor argued at the oral proceedings that the objection had been newly raised. However, the board decided that the objection had already been part of the proceedings. As the objection was not successful (see below), this decision needs no further reasoning.
12. The board construes the relevant part of claim 1 of the main request as defining an RNA molecule capable of forming an artificial hairpin RNA structure with a double-stranded RNA stem formed by base pairing between the regions with sense and antisense nucleotide sequences. The sense strand is defined as including (i.e. containing) at least 15 consecutive nucleotides identical to those in (part of) the coding region of the target. In the board's view, the wording of the claim confers no further structural limitations on the sense strand, in particular, it may or may not contain

further nucleotide sequences with identity to the target.

13. There is a *verbatim* disclosure in the application as filed for "a sense nucleotide sequence of at least 10 nt, preferably 15 nt consecutive nucleotides having [...] 100% sequence identity with at least part of the nucleotide sequence of the nucleic acid of interest" (see paragraph bridging pages 18 and 19). The question to be answered is therefore whether or not the application as filed, directly and unambiguously discloses that above mentioned sense nucleotide sequence may include at least 15 consecutive nucleotides having 100% sequence identity with at least part of the nucleotide sequence of the nucleic acid of interest.

14. The skilled person reading the passage on page 20, lines 18 to 21, that "[t]he length of the sense nucleotide sequence may vary from about 10 nucleotides (nt) up to a length equaling the length [...] of the target nucleic acid. Preferably the total length of the sense nucleotide sequence is at least 10 nt, preferably 15 nt ..." would understand that the length of the sense nucleotide strand may be any length starting from 10 nucleotides, up to the length of the target sequence. Given that 15 nucleotides is indicated as the preferred length and the maximum length is the length of the target sequence, the board considers that the application as filed discloses "a sense nucleotide sequence including at least 15 consecutive nucleotides". It is also apparent that the application as filed discloses that the sense nucleotide sequence may be 100% identical to the target sequence, see page 21, lines 5 and 9: "However, it is preferred that the sense nucleotide sequence always includes a sequence of

about 10 consecutive nucleotides, particularly about 20 nt, more particularly about 50 nt, especially about 100 nt, quite especially about 150 nt with 100% sequence identity to the corresponding part of the target nucleic acid.". Thus, the board is satisfied that the subject matter of claim 1, part b) (i) is directly and unambiguously disclosed in the application as filed.

15. On the question of whether or not the application as filed directly and unambiguously discloses the subject-matter of claim 17, the board considers that the disclosure on page 28 lines 14 to 17: "*It is a further object of the invention to provide eucaryotic cells, preferably plant cells and organisms (preferably plants) comprising the chimeric genes for the reduction of the phenotypic expression of a target nucleic acid as described in the invention*" provides a disclosure of the subject-matter as claimed. That the nucleic acid of interest is integrated in the genome of the cell is disclosed on page 8, lines 9 and 10 and also in claim 5 of the application as filed.
16. In view of the above, the board considers that claims 1 and 17 meet the requirements of Article 123(2) EPC.

Amendments - Article 123(3) EPC

17. Opponent 2 argued that the amendment of claim 1 in part b) (i) from "a sense nucleotide sequence **of** at least **10 consecutive nucleotides having between 75 and 100% sequence identity** with at least part of a coding region of said nucleic acid of interest" to
- "... a sense nucleotide sequence **including** at least **15 consecutive nucleotides having 100% sequence identity** with at least part of a coding region of said nucleic

acid of interest" (emphasis added by the board) extended the scope of protection to include methods using hairpin structures not covered by the original claim.

18. The board is of the view that the RNA molecule defined in claim 1 as amended is a sequence comprising a sense strand that includes (i.e. contains) a sequence of at least 15 consecutive nucleotides having 100% identity to a target sequence. The claim as granted covered a sequence comprising a sense strand of at least 10 consecutive nucleotides having 100% identity with the target sequence. Any sequence comprising at least 15 consecutive nucleotides having 100% sequence identity with the target region also fulfils the requirement of comprising at least 10 consecutive nucleotides having between 75 and 100% sequence identity and was therefore included within the ambit of the claim as granted.

19. That the sense sequence could include nucleotides which were not so termed in the claim as granted, is irrelevant. For instance, the construct depicted on page 6 of the grounds of appeal of opponent 2 fell within the scope of granted claim 1 because it contains two sequences of 10 nucleotides which are 100% identical to at least a part of the coding region of the nucleic acid of interest (the target sequence). The opponent's suggestion that this would be in contradiction with claim 24 as granted is not persuasive because this claim does not prescribe a fixed length of the nucleic acid of interest (target). In fact the target may be defined as being any sequence and could be a sequence of 10 nt that corresponds to the sense strand of the hairpin construct.

20. The amended claim therefore complies with Article 123(3) EPC.

Disclosure of the invention - Article 100(b) EPC/Article 83 EPC

Claim 1

21. At the oral proceedings, the patent proprietor argued that there was no evidence on file that the claimed method did not work in mammalian cells. Opponent 2 stated at the oral proceedings that this was a new line of argument, not raised in the written procedure but that they could deal with it without an adjournment of the oral proceedings.
22. In as far as the above mentioned line of argument represents an amendment of the patent proprietor's case, the board saw no reason to hold it inadmissible *ex officio*.
23. Article 83 EPC provides that a European patent must disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. It is established case law that an objection of lack of sufficient disclosure presupposes that there are serious doubts, substantiated by verifiable facts, and that in order to establish insufficiency, the burden of proof is upon an opponent to establish on the balance of probabilities that a skilled reader of the patent, using their common general knowledge, would be unable to carry out the invention (see Case Law of the Boards of Appeal of the European Patent Office, 9th edition, II.C.5.3 7.1.4).
24. One line of argument of opponent 2 was that the skilled person reading the patent would realise that it

concerned only methods carried out in plants and that no other methods were disclosed sufficiently to be carried out by the skilled person.

25. The board recognises that the patent does indeed only contain examples where plants and plant cells are used. However, the fact that the patent lacks examples in which the claimed method is carried out in animal cells is in itself not evidence that the person skilled in the art cannot carry out the method in animal cells as claimed. As set out in the paragraph above, this would require demonstrating that there were serious doubts substantiated by verifiable facts that the invention cannot be carried out in animal cells.
26. Opponent 2 also argued that the claimed method could not be carried out when using longer (over 50 nucleotides) dsRNA in vertebrate cells, due to the induction of a non-specific inflammatory response (PKR response) caused by the presence of foreign dsRNA in these cells.
27. Opponent 2 stated in writing "*We have demonstrated by reference to E1, E6, E17, E40, E43, E44 and E47 that the prevailing technical opinion at the time of filing (and even after the filing date, see E51 and E53) was that dsRNA could not induce gene-specific silencing in vertebrates due to longer length sequences inducing a global non-specific response*" (see statement of grounds of appeal, point 7.9.3). However, no substantiated arguments were presented in appeal except with respect to documents E1 and E51.
28. Document E1 discloses that "*all types of double-stranded RNA (dsRNA) tested inhibit protein synthesis in rabbit reticulocyte lysates*" (see page 409,

abstract). The inhibition of protein synthesis by dsRNA is reported only in reticulocyte lysates. None of the results relate to inhibition of protein synthesis in a system where the dsRNA is expressed from chimeric DNA that has been introduced into a eukaryotic cell. Thus, the disclosure in document E1 does not demonstrate that the claimed method cannot be carried out by the skilled person.

29. Document E51, published in July 2003, concerns the "*Induction of an interferon response by RNAi vectors in mammalian cells*" (see title) and concludes "*we show that a commonly used shRNA construct can induce an interferon response. Although this may not be a concern in initial screens with shRNA banks, we recommend testing for interferon induction before attributing a particular response to the gene targeted. One simple precaution to limit the risk of inducing an interferon response is to use the lowest effective dose of shRNA vector. Finally, we note that many commonly used tumor cells have a defective interferon response, which may explain why these effects have not previously been reported*" (see paragraph bridging pages 263 and 264).
30. In the board's view, although document E51 does report the induction of an interferon response by dsRNAs, it does not provide persuasive evidence that "*in most vertebrate cells it is impossible to achieve target-specific inhibition*" (cf. statement of grounds of appeal of opponent 2, point 7.4.4).
31. Opponent 2 had also argued that the skilled person at the relevant date of the patent did not know techniques to avoid the PKR response, However, document E51 suggests that low doses of the dsRNA vector may be used

to avoid such a response (see page 264, final paragraph).

32. In view of the above, the board must conclude that it has not been established that there are serious doubts substantiated by verifiable facts that the invention cannot be carried out with respect to methods using 'long' dsRNAs in vertebrate cells.
33. Opponent 2 further argued that the claimed method could not be carried out using RNA hairpin constructs having a stem length of 10 to 16 nucleotides ('short' dsRNAs). Document E60 was submitted in evidence.
34. Document E60 contains the results of experiments which purport to show by phenotypic cellular assays, as well as by measurements of the mRNA levels of the target genes, that where the shRNA includes a double-stranded (stem) region of between 10 and 16 nt there is no effect on the expression of the target gene. The experiments reported in document E60 relate to the expression of the transcription factors SMAD3 and SMAD4 and their expression in human bronchial epithelial cells (HBECS) (see page 18, "*Cell culture*").

- in Fig. 7, the graphs relating to the effect of 10nt shRNAs against SMAD3 and SMAD4 show that these do have some inhibitory effect on expression, achieving at best ~40% inhibition. While the 'longer' 19nt shRNAs were indeed more effective, it cannot be concluded from this figure that the 'shorter' shRNA were entirely ineffective.

- in Fig. 8, the graph relating to the effect of 15nt shRNA on SMAD4 (see lower graph) shows that these RNAs inhibit expression at a level of just less than 40%,

while the 19nt shRNAs achieved inhibition of expression of ca. 50%. While the 'longer' shRNAs were indeed more effective, it cannot be concluded from the evidence provided that the 'shorter' shRNA were entirely ineffective.

35. Opponent 2, referring to Fig 2A of document E68 argued that this was evidence that hairpins having a stem length of 15nt did not have an effect on gene expression. On the other hand, the patent proprietor citing the abstract argued that document E68 taught that there was no correlation between stem length and activity. The patent proprietor further cited document E69, which was said to show that a construct having a 15nt stem with a GUUC loop was effective in a method as claimed (see page 109, final paragraph).
36. The above cited documents taken as a whole show that shRNA constructs having 'shorter' stem regions (e.g. ca. 15nt) are generally less effective in gene silencing than longer ones, although no fixed correlation between stem length and activity has been established beyond this. Moreover, the available evidence does not demonstrate that the 'shorter' constructs are entirely ineffective.
37. In view of the above, the board concludes that the evidence presented in document E60 cannot be regarded as showing that the claimed method cannot be carried out when using shRNA having a stem region of less than 19nt.
38. In summary, on the basis of the evidence provided, the board considers that it has not been shown that there are serious doubts, substantiated by verifiable facts that the invention as claimed cannot be carried out

over the whole scope claimed. The claimed subject-matter therefore meets the requirements of Article 83 EPC.

Novelty - Article 54 EPC

Claims 1 and 17

With respect to document D45

39. Opponent 2 was of the view that the subject matter of claim 1 was anticipated by the disclosure in document E45. This document is an international patent application with a filing date of 21 December 1998. It was common ground that the document was comprised in the state-of-the-art pursuant to Article 54(3) EPC only for subject-matter disclosed therein that was entitled to an effective date corresponding to the earliest claimed priority, i.e. 23 December 1997.
40. It was disputed between patent proprietor and opponent 2, whether or not the document disclosed the feature of claim 1 that the RNA is produced by transcription in a cell in which the nucleic acid of interest is also present. Opponent 2 considered that this feature was disclosed on page 7, lines 3 to 16, page 13, lines 8 to 12 and claims 21 and 36 of document E45.
41. The passage on page 7 reads "*RNA may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region may be used to transcribe the RNA strand (or strands)*".

The passage on page 13, lines 8 to 10 is essentially identical.

42. The board considers that the reference to RNA synthesis *in vivo* could theoretically refer to the production of RNA in a separate cell or in the same cell as the nucleic acid of interest. However, in the context of document E45, the board is persuaded that the RNA synthesis referred to is only that in a separate cell. This view is supported by the disclosure on page 7 of document E45 that "*The RNA may be directly introduced into the cell (i. e., intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing RNA*" (see lines 9 to 12). None of these alternatives correspond to the expression of the RNA construct from the chimeric DNA which has been introduced into the eukaryotic cell containing the target nucleic acid of interest.
43. Claim 21 of document E45 reads "*The method of claim 1 in which an expression construct in the cell produces the RNA*". While not explicitly stated, this includes the possibility of a DNA expression construct. However, as correctly noted by the patent proprietor, document E45a, the application from which a priority of 23 December 1997 is claimed, does not contain the identical claim. Instead, claim 20 of document E45a reads "*the method of any one of claims 1-12 in which an expression vector in a cell produces the RNA*". This differs from claim 21 of document E45 in that it refers to "*a cell*" not to "*the cell*". Thus, claim 20 of document E45a does not unambiguously relate to the production of the RNA from a vector in the cell containing the target nucleic acid sequence. Moreover,

the description of document E45a does not contain any disclosure of production of RNA from an expression vector in the cell containing the target gene, as evidenced by the passages on pages 7 and 13 referred to above. Thus, the subject-matter of claim 21 of document E45 has as its effective date, the filing date of document E45 and is not comprised in the state of the art for the presently claimed subject-matter.

44. Claim 36 of document E45 reads *"A cell containing an expression construct, wherein the expression construct transcribes at least one ribonucleic acid (RNA) and the RNA forms a double-stranded structure with duplexed strands of ribonucleic acid, whereby said cell contains the double-stranded RNA structure and is able to inhibit expression of a target gene when the RNA is contacted with an organism containing the target gene."*

45. The board is of the view that the subject-matter of this claim relates to a situation in which the target gene is not in the same cell as the cell containing the expression construct, because it is explicitly stated that the RNA produced by transcription is *"contacted with an organism containing the target gene"*.

46. In view of the above considerations, the subject-matter of claims 1 and 17 is novel with respect to the disclosure of document E45. This conclusion applies equally to the subject-matter of the remaining claims. *With respect to document Document E30 (and E7, E21 and E24) - claim 1*

47. At the oral proceedings the proprietor put forward that the line of argument made by opponent 2, based on the disclosure in document E30 of plants (PSE19-1) containing the T-DNA insert termed PSE19, was newly

raised. The patent proprietor did however not object to this and the board saw no reason to hold the line of argument inadmissible *ex officio*.

48. Document E30 discloses *Petunia* plants transformed with a number of promoterless chalcone synthase (*Chs*) constructs having a phenotype corresponding to the silencing of their *Chs* gene. Some of these plants contained T-DNA loci having the construct PSE19 in an inverted repeat (see Figure 7; PSE19-1 - IR_{ct}).
49. Document E30 does not explicitly disclose the formation of RNA which is capable of forming an artificial hairpin structure. Opponent 2 argued that the formation of such RNAs was the inevitable consequence of the expression of the genes on the T-DNA in the transgenic plants and was proven by the presence of the corresponding silencing phenotype. The production of an RNA capable of forming a hairpin structure was said to be an inherent feature of these plants due to the read-through transcription of the IR_{ct} insert. This read-through transcription was said to be inevitable because the *Chs* gene did not contain a terminator.
50. In the board's view, document E30 does not directly and unambiguously disclose subject-matter falling within the ambit of claim 1. Firstly, the PSE-19 construct in fact contains a *nos* terminator as can be seen in figure 1 (a). Thus, it cannot be assumed that a read-through transcript was inherently produced. Secondly, the document discloses that "*silencing by these loci cannot be explained by the RNA threshold model. However, it is possible that in most plant tissues these IR_c loci produce low quantities of aberrant transcripts sufficient to activate the RNA degradation machinery*" (see page 76, right-hand column,

paragraph 1). Furthermore, it is disclosed that "*it is unlikely that the IR_C loci carrying the promoterless Chs transgenes produce aberrant transcripts as these sequences are not detectably transcribed. Moreover, there was no detectable read-through transcription from one repeat into the other*" (see page 79, left-hand column, paragraph 2). From these passages and in the context of the content of the document as a whole, it can be concluded that the authors were unsure of the mechanism by which gene silencing occurred, although they had a number of different theories about this. One was the production of aberrant transcripts, another was that a mechanism of DNA-DNA pairing could interfere with normal processing (see page 79, right-hand column, 1st paragraph). Nowhere do the authors even speculate that dsRNA species are involved. In summary, there is no direct and unambiguous disclosure in document E30 of the production of RNAs capable of forming a hair-pin structure.

51. A further line of argument of opponent 2 concerns the allegation that the *nos* terminator was "leaky", leading to the inevitable production of read-through transcripts. This line of argument was supported by the disclosure in document E61.

52. Document E61 concerns "*Roundup Ready*" soybean varieties into which a glyphosate-resistant EPSPS gene was introduced. In these plants, transcription of CP4 EPSPS is controlled by a CaMV-promoter and the *nos* terminator derived from Cauliflower Mosaic Virus and the nopaline synthase gene from *A. tumefaciens*, respectively. It is reported that "*The presented data demonstrate read-through of the nos terminator and at least partial transcription of the epsps-gene fragment located downstream of the terminator region*" (see page 438,

right-hand column, second paragraph). The board considers the above disclosure, while showing that read-through transcription occurs in the particular situation reported on, cannot be relied upon to show that the same also occurs in the system disclosed in document E30. The differences in genetic background and construct are such that it cannot simply be assumed that read-through was an inherent feature of the plants disclosed in document E30. Thus, it has not been proven that RNAs capable of forming hairpin structures were inherently produced in the plants disclosed in document E30.

53. In view of the above, the board concludes that skilled person reading document E30, even in the light of the disclosure in document E61, would find no direct and unambiguous disclosure of subject-matter according to present claim 1.
54. For the same reasons, documents E7, E21 and E24 do not anticipate the claimed subject-matter.

With respect to document E46 - claim 1 and claim 17

55. This document has been cited as prior art pursuant to Article 54(3) EPC, as anticipating the subject matter of claims 1 and 17. It was filed on 19 March 1999 and claims priority from two Australian applications, both filed on 20 March 1998 (documents E46a and E46b). In view of the fact that the earliest priority date of the patent in suit is 8 April 1998, only subject matter which has a valid claim to priority based on either of the two priority documents could be relevant to novelty.

56. The most relevant passages cited by the appellant are the passage on page 10, lines 10 to 14 together with the passages on page 18, lines 11 to 20 of document E46a as disclosing a DNA region which encodes an RNA capable of forming an artificial hairpin structure. The first passage reads *"accordingly, the structural gene component of the synthetic gene may comprise a nucleotide sequence which is at least about 80% identical to the least about 30 continuous nucleotides of an endogenous target gene [...] or a complimentary sequence led to"*, while the second passage reads *"The individual structural gene units for the multiple structural gene according to the embodiments described herein may be spatially connected in any orientation relative to each other, for example head-to-head, head-to-tail or tail-to-tail and all such configurations are within the scope of the invention. Preferably, the multiple structural gene unit comprises two structural genes in a head-to-tail or head-to-head configuration. More preferably, the multiple structural gene unit comprises two identical or substantially identical structural genes or homologue, analogue or derivative thereof in a head-to-tail configuration as a direct repeat of alternatively, in a head-to-head configuration as an inverted repeat of palindrome "*.
57. Document E46b, page 17, lines 15 to 20 was also cited as a basis for the above subject matter. This passage reads as follows: *"Wherein the structural gene region comprises more than one dispersed nucleic acid molecule or foreign nucleic acid molecule it shall be referred to herein as "multiple structural gene region" or similar term. The present invention clearly extends to the use of multiple structural gene regions which preferably comprise a direct repeat sequence, inverted repeat sequence or interrupted palindrome sequence of a*

particular structural gene or fragment thereof". A similar disclosure is found in document E46a, page 18, lines 16 to 20.

58. There is no disclosure in any of the above cited passages that the so-called "*multiple structural gene*" in any arrangement was recognised as encoding or in fact encoded an RNA capable of forming an artificial hairpin structure. Accordingly, neither of the priority documents of document E46 provides a basis for a valid claim to priority for subject-matter corresponding to that of present claim 1. Thus, document E46 does not anticipate the subject-matter of either claim 1 or claim 17.

Inventive step - Article 56 EPC

59. Opponent 2 presented a line of argument starting from document E30 representing the closest prior art. The patent proprietor requested that this line of argument not be admitted. The board decided to admit the line of argument but since it was unsuccessful, the reasons for its admittance are moot. Opponent 2 also argued that the claimed invention lacked inventive step when starting from document E40 representing the closest prior art. The board considered both lines of argument.

Inventive step when considering document E40 as closest prior art - all claims

The objective technical problem

60. The difference between the disclosure in document E40 and the subject-matter of claim 1 is primarily the fact that the RNA species used to achieve gene silencing in document E40 consist of separate sense and antisense

strands (see page 810, "Methods"). These are not capable of forming an artificial hairpin structure. A second difference is that they are not expressed from a chimeric DNA which has been introduced into the cell which contains the nucleic acid of interest but rather the sense and antisense RNAs are introduced into the cell by injection (*ibid.*).

61. The technical effect of the above-mentioned differences is that silencing of a target gene is achieved in a more straightforward fashion and is better targeted to each cell. In view of the above mentioned differences and the technical effect thereof, the problem to be solved can be formulated as the provision of an alternative method for reducing the phenotypic expression (i.e. silencing) of a nucleic acid of interest in a eukaryotic cell.

Obviousness

62. The question to be answered is therefore whether or not the skilled person starting from the disclosure in document E40 of methods for the specific genetic interference by double-stranded RNA in *C.elegans* would have modified those methods to arrive at the claimed subject-matter.
63. The answer to this question could be yes if there were a disclosure in document E40 that would have led the skilled person to the claimed subject-matter. However, the document contains no such disclosure. In particular, it does not contain anything from which it could be inferred that a suitable alternative method for reducing the phenotypic expression of a nucleic acid of interest in a eukaryotic cell would be to combine the sense and antisense RNA strands into a

single strand expressed from a DNA which has been introduced into the cell. Nor is there any suggestion to modify the RNA species such that they can form a hairpin structure by base pairing between the sense and antisense nucleotide sequences.

64. It remains to be assessed whether or not there was other teaching in the state-of-the-art that would have led the person skilled in the art starting from document E40, to arrive at the claimed solution to the technical problem. Opponent 2 argued that documents E11 and E70 were such a disclosure.
65. Document E11 is concerned with self-stabilised oligonucleotides as therapeutic agents (see title). These oligonucleotides comprise a target hybridising region and a self complementary region (see claim 1). This oligonucleotide is said to form a "*totally or partially double-stranded structure that is resistant to nucleolytic degradation*" (see page 5, paragraph 2). Examples of such structures are shown in Figure 1, from which it is apparent that these molecules can form a hairpin structure with a double-stranded stem. That the oligonucleotides may be RNAs can be derived from page 8, paragraph 2.
66. However, there is nothing in the disclosure of document E40 to suggest that nucleolytic degradation may be a problem with the silencing methods disclosed therein. There is therefore nothing in document E40 that provides a link to the self-stabilised oligonucleotides disclosed in document E11. It is also noted that the purpose of the oligonucleotides disclosed in document E11 is their use as therapeutic agents in the treatment of virus infections, infections by pathogenic organisms, and diseases arising from abnormal gene

expression or gene products (see page 8, paragraph 1). There is nothing in document E11 that would provide a link to methods of gene silencing. The board therefore concludes that although the skilled person could have combined the teaching in document E40 with the disclosure in document E11, they would not have done so.

67. Document E70 is a review article that is concerned with transformation of *C. elegans* by microinjection (see parts II to VII) cited as representing common general knowledge by opponent 2. Although it does disclose a transformation method using the microinjection of DNA constructs, it does not contain any teaching that would have led to skilled person to modify the sense and antisense RNA strands mentioned in document E40 such that a single RNA strand is expressed from a DNA.
68. Opponent 2 also argued under the heading of inventive step that the patent failed to disclose a solution to the technical problem. In particular, opponent 2 considered that the problem had not been solved over the whole scope claimed because short sequences under 19 nucleotides and long sequences over 30 nucleotides would not be effective in vertebrate animal cells.
69. The board notes that the claimed method contains an indication of the desired result, namely "reducing the phenotypic expression of a nucleic acid of interest", which is a feature of the claimed subject-matter. All claimed subject-matter therefore (by definition) represents a solution to a technical problem aimed at achieving this result. Objections concerning whether or not the skilled person can carry out the invention as claimed must be dealt with under the heading of

disclosure of the invention, i.e. Article 83 EPC, and have been assessed by the board in that context.

70. In view of the above considerations, the board concludes that the subject-matter of claim 1 involves an inventive step starting from document E40 as the closest prior art.
71. This conclusion applies *mutatis mutandis* to the subject matter of dependent claims 2 to 16 as well as to claims 17 to 25.

Inventive step when considering document E30 as closest prior art - all claims

72. The board also assessed the inventive step of the claimed subject-matter when taking document E30 to represent the closest prior art.
73. As set out above, document E30 discloses the generation of genetically transformed *Petunia* plants by the introduction of T-DNA containing a promoterless chalcone synthase gene. Some of the resulting transformants had phenotypes corresponding to a silenced chalcone synthase gene and contained T-DNA loci having the construct PSE19 in an inverted repeat.
74. The difference between the method of claim 1 and that disclosed in document E30 is that the claimed method introduces a DNA construct which can express an RNA capable of forming a double stranded hairpin structure into cells, whereas the method disclosed in document E30 involves the introduction of promoterless (sense) chalcone synthase constructs into cells.

75. In view of this difference, the problem to be solved can again be formulated as the provision of an alternative method for reducing the phenotypic expression (i.e. silencing) of a nucleic acid of interest in a eukaryotic cell.
76. The question to be answered is therefore whether or not the skilled person starting from the disclosure in document E30 would have modified those methods to arrive at the claimed subject matter.
77. As set out in paragraph 50. above, document E30 does not contain any disclosure that would indicate to the skilled person that dshRNA would be useful in methods of gene silencing. It follows that the skilled person seeking a solution to the technical problem, would not find any suggestion in that document to modify the promoterless sense constructs disclosed therein in such a way as to allow the production of dshRNAs. As already discussed under the heading of novelty, the authors of document E30 had various theories as to why T-DNA inserts in inverted repeat formations could cause gene silencing and none of these involved the formation of dshRNAs.
78. In view of the considerations above, the subject-matter of claim 1 is considered not to have been obvious to the skilled person, also when starting from document E30 alone. This conclusion applies equally to all claims which therefore meet the requirements of Article 56 EPC.

Industrial application - Article 57 EPC

79. Since opponent 2 provided no arguments on this topic beyond those already given under the headings of

Articles 56 and 83 EPC, and the board has already held the claims of the main request to meet the requirements of those articles, no further reasons need to be given here. The subject-matter of the main request is held to meet the requirements of Article 57 EPC for industrial application.

Admission of document E76

80. Admission of document E76 need not be considered as the document is not relevant for the board's decision.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:



I. Aperribay

P. de Heij

Decision electronically authenticated