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**Datasheet for the decision
of 23 September 2009**

Case Number: T 1440/07 - 3.3.08

Application Number: 96203412.0

Publication Number: 0779362

IPC: C12N 15/67

Language of the proceedings: EN

Title of invention:

DNA constructs for endogenous gene activation and expression modification

Patentee:

Laboratoires Serono SA

Opponent:

CELLECTIS

Headword:

Gene expression/SERONO

Relevant legal provisions:

EPC Art. 123(2)

Relevant legal provisions (EPC 1973):

EPC Art. 76(1), 83, 111

Keyword:

"Main request - added subject-matter (no)"

"Sufficiency of disclosure (no)"

"Auxiliary request - sufficiency of disclosure (yes)"

Decisions cited:

T 0019/90, T 0984/00, T 0397/02

Catchword:

-



Case Number: T 1440/07 - 3.3.08

DECISION
of the Technical Board of Appeal 3.3.08
of 23 September 2009

Appellant: Laboratoires Serono SA
(Patent Proprietor) Centre Industriel
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CH-Vaud (CH)

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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 20 June 2007
revoking European patent No. 0779362 pursuant
to Article 102(1) EPC 1973.

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
T. Karamanli

Summary of Facts and Submissions

I. European patent No. 0 779 362 with the title "DNA constructs for endogenous gene activation and expression modification", filed as a divisional application of the European patent application No. 91 903 051 (European patent No. 0 505 500), was granted with 24 claims, based on European patent application No. 96 203 412.0.

Granted claims 1, 13, 17 and 22 read as follows:

"1. A DNA construct suitable for use in modifying the expression characteristics of a preselected gene in a predetermined eukaryotic host cell line and suitable for targeting said preselected gene by homologous recombination, the construct comprising:

a DNA regulatory segment (F) which activates and/or enhances expression of said preselected gene when operatively linked thereto,

two DNA targeting segments (A,B) homologous to a region of the genome within or proximal to the preselected gene within the host cell line, one of which (A) is homologous to a region of the genome located downstream of the specific region at which the regulatory segment (F) is inserted, and the other of which (B) is homologous to a region of the genome located upstream of the specific region at which the regulatory segment (F) is inserted,

a positive selectable marker,

a negative selectable marker, and an amplifiable gene.

13. Use of a DNA construct as claimed in any of the preceding claims, in modifying the expression characteristics of a preselected gene in a predetermined eukaryotic host cell line.

17. A eukaryotic host cell transfected with a DNA construct as claimed in any of claims 1 to 12.

22. Process for preparation of a gene product comprising the step of culturing the cell according to claim 17."

Claims 2 to 12, 14 to 16 respectively related to further features of the DNA construct of claim 1 and the use of claim 13. Claim 18 related to a genome comprising a DNA construct as claimed in any of claims 1 to 12. Claim 19 to 21 were directed to uses of the eukaryotic host cell of claim 17. Dependent claims 23 to 24 related to further features of the process of claim 22.

II. Three oppositions were initially filed under Article 100(a) to (c) EPC 1973. Opponents 1 and 2 withdrew their oppositions on 13 September 2005 and 21 November 2007, respectively. The opposition division revoked the patent for lack of sufficient disclosure in relation to the subject-matter of the sole claim request then on file which differed from the granted claims in that in claim 1, the positive and negative selectable markers were specified to be genes and the position of these genes and of the amplifiable gene in the construct was indicated. Reference was made inter

- alia to the findings in decision T 397/02 of 10 October 2003 which was issued in relation to the parent patent EP-0 505 500.
- III. The appellant (patentee) filed an appeal and submitted a statement of grounds of appeal together with the request refused by the opposition division as sole request.
- IV. The respondent (opponent 03) replied to the statement of grounds of appeal.
- V. The board sent a summons to oral proceedings together with a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal, indicating its preliminary, non-binding opinion, in particular on the issue of sufficiency of disclosure.
- VI. By fax letter dated 11 May 2009, the respondent informed the board of its intention not to take part in the oral proceedings.
- VII. On 11 August 2009, the appellant filed further submissions.
- VIII. At the oral proceedings which took place on 23 September 2009, the appellant replaced the request then on file by a new main request and an auxiliary request. The new main request consisted of 16 claims corresponding to granted claims 1 to 12, 17, 22 to 24 (see section I supra). The auxiliary request consisted of granted claims 1 to 12 and 17.

IX. The documents which are cited in the present decision are the following:

- (23) : Declaration of Professor F. Grosveld made on 17 April 2003;
- (26) : Declaration of Professor E.J. Louis made on 9 March 2001;
- (27) : Declaration of Professor J. Haber made on 9 March 2001;
- (43) : Declaration of Professor T. Maniatis made on 13 February 2003;
- (45) : Declaration of Professor D. Martin made on 24 September 2003;
- (48) : Declaration of Dr. S. Chappel made on 24 September 2003;
- (49) : Declaration of Dr. P. Dupraz made on 25 August 2005;
- (54) : Second declaration of Dr. P. Dupraz made on 4 August 2006;
- (77) : Second declaration of Dr. C.A.Kelton made on 23 September 2003.

X. The appellant's submissions in writing and during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Articles 76(1) EPC 1973 and 123(2) EPC

The respondent cited page 9, lines 19 to 22 of the application as filed as allegedly disclosing (only) an expression due to a previous genetic manipulation, and then argued that such genetic manipulation did not imply a relationship to a gene which was endogenous. First, it should be noted that claim 1 did not recite "endogenous" at all. Further, the passage on page 9, lines 19 to 22 allowed two alternative options for expression, the first being "natural expression" and the second being "expression which has been caused by previous genetic manipulation of the cell line or microorganism". The respondent focused only on the second of these two alternatives and completely overlooked the first. This was incorrect. Even if claim 1 were explicitly restricted to endogenous expression as the respondent implied, "natural expression" as disclosed on page 9, line 20 supported the notion of expression of an endogenous gene.

All elements of the now claimed DNA construct were disclosed as such on page 16, lines 4 to 16 of the application as filed.

The contents of the parent application were the same as the content of the application as filed.

The requirements of Article 76(1) EPC 1973 and Article 123(2) EPC were fulfilled.

Article 83 EPC 1973; sufficiency of disclosure

- Numerous decisions of the Boards of Appeal made it clear that an example was not necessary for the requirement of sufficiency of disclosure to be complied with. Thus, T 984/00 of 18 June 2002 established that in some cases, it was enough that precise instructions were given on what to do to put the invention into practice. These findings directly applied to the present case. Claims 1 to 13 were directed to DNA constructs and to a eukaryotic host cell transfected therewith. The skilled person would have had no difficulty in reproducing these constructs on the basis of the instructions given in the patent specification. On page 7, lines 5 to 8, 35 to 41, it was disclosed how to choose the DNA targeting segments A and B starting from the known sequence of the gene to be expressed. Suitable positive and negative selection markers, amplifiable genes were identified from page 7, line 43 to page 8, line 9. On page 8, lines 40 and 41, it was taught that any promiscuous promoter could be used as a regulatory element ie that the invention was not limited to using the RSV promoter as in the example. Finally, Figure 1 gave a clear picture of what the DNA construct according to the invention should "look like". Claims 14 to 16 were directed to processes for the preparation of a gene product, the expression of said gene being activated by the transformation of the DNA construct into the eukaryotic cells. It would be fully expected that the transformed cells would express such gene product. Indeed, this only required homologous recombination to take place, a mechanism most likely to occur since there was homology between the locus of the silent gene and the DNA construct.

In view of this extensive information, the skilled person would have had no difficulty in reproducing the claimed subject-matter, irrespective of whether or not an example had been given.

- In fact, lack of sufficient disclosure had been argued by taking into account, in particular, results which were not described in the patent but were obtained in experiments carried out at a later date - which, in any case, did not prove lack of enablement but rather the contrary. This approach was not the correct one as sufficiency of disclosure must be assessed on behalf of the skilled person at the filing/priority date. If for the sake of argument, one would regard an example as being necessary, then it remained that the skilled person reading the example on pages 14 and 15 of the patent in suit would have realized that the host transformed with a DNA construct according to claim 1 produced a molecular species that was not produced prior to transformation, namely, a new transcript containing TSH β RNA. He/she would have deduced therefrom that there had been expression of the preselected gene and, therefore, that the invention could be put into practice. Conversely, one may ask oneself what in the patent could instil doubts as to whether or not the invention could be reproduced. There was nothing at all to this effect. In summary, the inventors were the first to develop this totally new approach to gene expression which only required well known methods to be put into practice without undue burden.

- The opposition division appeared to have misconceived the aim of the example contained in the patent. This example was simply designed for showing that the

constructs according to the invention could successfully be used to activate the expression of the preselected gene. It was not intended to illustrate the feasibility of obtaining a gene product as the TSH β system would not, in any case, have been the best system for doing so. It delivered results which were both coherent and reproducible as confirmed by the later experiments filed by the appellant.

As for the assertion that the skilled person would not be able to redesign the construct without undue burden so as to obtain the direct transcript of the TSH β gene. ie the gene product, it was irrelevant. He/she would not even attempt to do so as the TSH β gene was not such a useful target. At the relevant date, the skilled person was aware that the nature of the inventive construct - for example, the sequences incorporated for targeted homologous recombination - may have to be modified to meet the needs of each case at hand. It was without a doubt that he/she could and would have made the modifications necessary without any burden, eg. given prior knowledge of the genomic sequence surrounding the endogenous gene to be activated.

- The respondent's objection to sufficiency of disclosure were entirely theoretical in nature. They were based on speculative arguments for which no proof had been provided nor any support given other than unsubstantiated references to seemingly random documents.

For these reasons, the requirements of Article 83 EPC 1973 were fulfilled in relation to the subject-matter of all claims.

XI. The respondent's submissions in writing insofar as relevant to the present decision may be summarized as follows:

Articles 76(1) EPC 1973 and 123(2) EPC

The passage on page 9, lines 19 to 22 of the application as filed taught that the gene expression which was modified may be natural expression or expression that had been caused by previous genetic manipulation of the cell line or microorganism. The fact that an earlier genetic manipulation of the cell may have taken place did not imply that the gene could be any other than an endogenous gene. Thus, it was clear and unambiguous that the invention concerned the modification of the expression of an endogenous gene by means of homologous recombination and did not concern the insertion of transgenic sequences. The same passage was found in the parent application as filed.

The requirements of Article 76(1) EPC 1973 and Article 123(2) EPC were not fulfilled.

Article 83 EPC 1973; sufficiency of disclosure

What was aimed at in the patent in suit was to express a silent endogenous gene in order to produce the corresponding protein.

- There was no proof given that the addition of a regulatory sequence such as sequence F was enough to achieve this aim. The relevance of the given example was at best doubtful. The transformation rate of the DNA construct was too high compared to that of the negative control plasmid. The recombination rate was also much

higher than that expected. No marker genes were present in the DNA construct which would eliminate the possibility of heterologous recombination. One could not be sure that the expression observed was due to homologous recombination as the test carried out in this respect (Northern blot on total RNA) was not meaningful.

- Post-published documents showed that the instructions given in the patent specification were not sufficient to reproduce the invention. The regulatory segment F would not be expected to function in any and all settings as it may negatively interact with other neighbouring promoters. The selectable marker could act as a repressor. The specific insertion site of the DNA construct was crucial. Chromatin regulation was equally important for transcription. Furthermore, modifications of the genome structure due to the introduction of segment F could inhibit transcription. Alternative cryptic splice sites could be created.

- The fact that a novel transcript was observed did not necessarily imply that a protein would be expressed nor, if expressed, that it would be the correct one. This was evident from the provided example itself wherein the mRNA produced was a chimeric one. And, besides, the example did not show protein production although this clearly was the intended goal.

- The appellant had produced a series of declarations and supplementary data. However, none of the declarations were suited to demonstrate that homologous recombination occurred. Moreover, none of the supplementary data were of sufficient quality to demonstrate unambiguously that it did.

- If additional steps or modifications were needed to put the claimed invention into practice, then the patent in suit failed to provide any instructions in this respect.

For these reasons, the requirements of Article 83 EPC 1973 were not fulfilled.

XII. The appellant requested that the decision under appeal be set aside and the patent be maintained in amended form on the basis of the claims according to the main request or first auxiliary request, both filed during the oral proceedings.

The respondent requested in writing that the appeal be dismissed.

Reasons for the decision

Article 76(1) EPC 1973 and Article 123(2) EPC; added subject-matter

1. The European patent application No. 96 203 412.0 corresponding to the patent in suit is a divisional application of the earlier European patent application No. 91 903 051 (EP-0 505 500). For the requirements of Article 76(1) EPC 1973 and Article 123(2) EPC to be fulfilled, it is necessary that the content of the patent in suit does not go beyond that of either the parent application as filed or the divisional application as filed.

2. Present claim 1 relates to the same subject-matter as claim 2 of the application as filed, namely a DNA construct suitable for use in modifying the expression characteristics of a preselected **gene**. The equivalent subject-matter is found in claim 22 as filed of the parent application which is directed to "A method for modifying the expression characteristics of a **gene** within the genome of a cell line ...". In the parent application (A1 version, page 16, lines 4 to 16) and in the divisional application as filed (A1 version, page 6, lines 54 to page 7, line 1), the DNA construct is described as comprising segments A, B and F as well as a positive selectable marker gene, a negative selectable marker gene and an amplifiable gene. Thus, there is a formal basis in both of these applications for the subject-matter of claim 1.

3. The respondent argued on the basis of the passage on page 9, lines 19 to 22 of the divisional application as filed and of the parent application as filed that the originally disclosed invention was only directed to endogenous genes. The following disclosure is given on page 9:

"The gene expression which is modified in this manner may be natural expression or expression which has been caused by previous genetic manipulation of the cell line or microorganism. The **previous** genetic manipulation may have been by conventional techniques or **by means of homologous recombination in accordance with the present invention.**" (emphasis added)

This disclosure is found in a section dealing with the modification of the expression characteristics of a

specific gene which already expresses a product in the cell line or microorganism of interest. The board understands it as meaning that the previous expression of the specific gene - which is to be modified - may already have been obtained by any techniques and that it is contemplated that it would be further modified, in accordance with the present invention. This disclosure is not informative on whether the gene was an endogenous gene or not.

4. For the reasons given in point 2 supra, the requirements of Article 76(1) EPC 1973 and Article 123(2) EPC are fulfilled.

Article 83 EPC 1973; sufficiency of disclosure

Main request; claims 1 to 13 (DNA construct and eukaryotic host cell transfected therewith)

5. In its decision, the opposition division denied sufficiency of disclosure with regard to DNA constructs corresponding to those now claimed on the basis that they could not be used for the given purpose. In the board's judgment, whether or not the contemplated use is achievable may have been relevant to the earlier use claims which have now been deleted. Whether or not the claimed DNA constructs are reproducible is an all together different issue. Otherwise stated, the question to be answered as regards the DNA constructs is: would the skilled person be able to assemble together DNA segments A, B, F, a positive, a negative and an amplifiable genes as prescribed in claim 1 on the basis of the information given in the patent specification ? In this respect, one may turn for guidance to pages 7, 8 and 11. There, advice is given on how to choose segments

A and B, numerous examples are provided of the other necessary elements. And besides, there is no evidence on file that they could not be assembled. Once isolated, the DNA constructs could be used at the relevant date to transform a host cell in a routine manner.

6. For these reasons, the board judges that reproducing the DNA constructs and the transfected eukaryotic host cell was possible without undue burden at the filing date.

Main request; claims 14 to 16 (processes for the preparation of a gene product)

The teachings of the patent in suit

7. At the beginning of the patent specification, the field of the invention is identified as being that of gene expression. On pages 2 and 3, it is taught that the previous approach to obtaining a gene expression product - identified as the encoded protein - involves transfecting the host cell with a DNA construct comprising, in particular, the gene to be expressed in combination with suitable regulatory sequences. This DNA may integrate at random into the genome where it will thereafter be transcribed and translated. This approach is said to have numerous shortcomings.
8. It is readily apparent on page 4, [0022] of the patent that the invention, which is intended to eliminate these shortcomings, does not entail modifying the already existing method. To the contrary, a totally different path is taken. The idea is, thus, proposed that preselected genes in the host cells may be activated. This should be achievable by inserting DNA regulatory

segments upstream of or within or otherwise proximal to said genes, taking advantage of the known natural mechanism of homologous recombination. This method significantly departs from the previous method of gene expression and has oft been argued by the appellant to amount to a new concept, which the board agrees to.

9. From page 4 to page 9 of the patent, the molecular entities to be combined in the DNA construct so that its fate within the host cell may be followed (selectable markers), homologous recombination may take place (targeting segments) and transcription of the preselected gene may occur (DNA regulatory segment) are described in detail.

10. The soundness of the concept is then tested in the example on pages 9 to 16. A DNA construct is described which contains such regulatory element and targeting segments as should enable homologous recombination within the silent thyrotropin beta subunit (TSH β gene) present in the genome of the GH3 cell line. The DNA is transfected into GH3 cells with the aim of triggering the activation of said gene. The outcome of the experiment is evaluated as follows: total RNA is extracted from the transformed cells and converted into cDNA. DNA primers derived from the known sequences of the TSH β exons 2 and 3 are used to amplify by PCR any TSH β cDNA sequence that may be present in the cDNA population, ie. in the total RNA. The primers are chosen so that a 247 bp fragment of TSH β cDNA is observed in case the transcription of the TSH β locus is activated. Fig. 15 is intended to show that this was indeed the case.

The relevance of this teaching to sufficiency of disclosure

11. Here, it becomes important to keep in mind the claimed subject-matter, namely a process for **the preparation of a gene product** comprising the step of culturing cells transformed with the DNA construct. Much was said during the proceedings on the significance to be given to the term "gene product". Initially, as found in the patent specification, the term implied the synthesis of the protein encoded by that gene. Later on, it was argued also to cover the mRNA resulting from the transcription of that gene. For the sake of argument, the board is prepared to accept the two interpretations. Irrespective thereof, the relevant point is that showing that a piece of TSH β mRNA is present in the total RNA population produced by the transformed cells is in no way equivalent to showing that a TSH β gene product in the form of TSH β mRNA has been synthesized. The difference between the two is that mRNA species other than TSH β mRNA but containing some piece of TSH β mRNA may have been transcribed from the TSH β locus, which would explain the result in Figure 15, yet not allow the production of the gene product. This point has not been denied by the appellant which does not argue that the example shows the synthesis of TSH β mRNA but rather that transcription has been activated at the TSH β locus. Consequently, the example does not illustrate the claimed subject-matter which is, as already mentioned, the preparation of the bona fide TSH β gene product in the form of at least TSH β mRNA. In fact there is no example to illustrate the claimed invention.

12. The appellant put forward the argument that no example was necessary as the skilled person was given all

information needed "to reach the state" where activation of transcription would take place and if he/she was to find out that the mRNA resulting from this activation was not a bona fide transcript of the gene of interest, he/she would know on the basis of common general knowledge how to modify the DNA construct so that its insertion into the genome by homologous recombination would lead to the proper transcription.

13. The board cannot agree with either of the two parts of this argument. As regards the necessity to have an example for the requirements of sufficiency of disclosure to be fulfilled, the case law establishes that it has to be decided on a case by case basis (see eg T 397/02 of 10 October 2003; points 8 to 11 of the Reasons). The present invention, as already above mentioned, is conceptually different from the approach taught in the prior art, even if known methods are used when attempting to put it into practice. In fact, by developing a new concept, the appellant has entered uncharted territory. Combining known independent methods and mechanisms does not necessarily guarantee that the end result expected from the combination will be achieved. Under such circumstances, it is the board's judgment that an example is necessary to establish sufficiency of disclosure and also that such an example as the present one which shows some effect of the invention (activation of transcription) but not the expected claimed invention (synthesis of the desired gene product) is simply not sufficient to establish workability. At best, it shows that the approach could be promising, leaving to the skilled reader the burden to find out the proper ways to operate.

14. As for the suggestion that the skilled person would be able to modify an "unsatisfactory" DNA construct - such as the one used in the example - without undue burden, it is also not convincing. It should be kept in mind that the DNA construct was "custom built" to allow for many steps to be taken before transcription such as selection, counter-selection, amplification and homologous recombination as well as to allow for transcription activation. In the board's judgment, adapting the DNA construct specifically to the last function which it is intended to perform without altering its other characteristics may amount to developing a new research program. And, in any case, in the absence of any guidance in the patent specification, this certainly involves an undue burden.

15. In conclusion, the patent in suit discloses a new approach for gene expression. As this approach significantly departs from the approaches known in the art, it is conceptually different. For the requirement of sufficiency of disclosure to be fulfilled under such circumstances, there is a need for an example to show that the concept has some practicality. The patent in suit provides the tools necessary to test the concept. The use of these tools leads to some effect on gene expression (transcription activation at the relevant locus). Yet, activation of bona fide transcription is not demonstrated. In the event that it does not occur, and in the absence of any guidance, it is an undue burden for the skilled person to reproduce the claimed subject-matter.

16. For sake of completion, the following observations are made:

- A great part of the written proceedings was devoted to evaluating the significance of the presence of a TSH β DNA fragment in the cDNA population obtained from the transformed cells (see point 10, supra), to finding out whether or not homologous recombination has taken place, or which transcripts were generated after insertion of the construct in the TSH β locus. All these points prompted the filing by the appellant of numerous additional experiments (eg documents (49), (54) or (77)). And much was, thus, learnt on the intrinsic molecular mechanisms which had taken place. Yet, as shown above, the issue of sufficiency of disclosure can be decided by applying the simple general principles established in the case law (necessity for an example, undue burden...). Accordingly, while these additional data were undoubtedly informative, they need not be discussed.

- A number of declarations were also produced by the appellant to the avail that the essence of the method was that the transcription of a silent locus can be activated by targeted insertion of a regulatory element (see, for example, documents (43), (45) or (48)). This is a scientific point which the board does not challenge. This is not, however, the subject-matter of claims 14 to 16. And it is in relation to these claims that sufficiency of disclosure within the meaning of Article 83 EPC fails.

- The respondent contested the scientific validity of essentially all data in the patent in suit or of the additional experiments, filing a number of declarations

in this respect. Many documents were also cited describing various biological mechanisms which could in principle have a negative impact on putting the concept into practice (eg documents (23), (26) or (27)). As no data were presented as evidence that they would indeed have an impact on the present invention, they cannot be regarded as relevant (see T 19/90, OJ EPO 1990, 476).

17. For the reasons given in points 11 to 15, supra, the main request is refused for failing to fulfil the requirements of Article 83 EPC 1973.

First auxiliary request

18. This request is limited to DNA constructs and a host transfected therewith. Sufficiency of disclosure has already been established in this respect (see points 5 and 6 supra). The requirements of Article 83 EPC 1973 are fulfilled.

Article 111 EPC 1973; remittal to the first instance

19. Since in its decision, the opposition division did not deal with the other requirements for patentability such as novelty, inventive step or industrial applicability which were challenged by the respondent, the board remits the case to the first instance for further prosecution in accordance with Article 111 EPC 1973.

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 for further prosecution.

The Registrar

The Chairman

A. Wolinski

L. Galligani