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Datasheet for the decision of 5 November 2013

Case Number: T 1720/10 - 3.3.08

Application Number: 99967788.3

Publication Number: 1228237

IPC: C12N 15/90, C12N 5/10, C07K 14/47

Language of the proceedings: EN

Title of invention:
Expression vectors, transfection systems, and method of use thereof

Applicant:
Novartis Vaccines and Diagnostics, Inc.

Headword:
Impaired markers, transcriptionally active hot spots/NOVARTIS

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (no)"

Decisions cited:
-

Catchword:
-

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Case Number: T 1720/10 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 5 November 2013

Appellant: (Applicant)
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Decision under appeal:
Decision of the Examining Division of the European Patent Office posted 8 March 2010 refusing European patent application No. 99967788.3 pursuant to Article 97(2) EPC.

Composition of the Board:

Chairman: M. Wieser
Members: P. Julià
J. Geschwind

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division to refuse the European patent application no. 99 967 788.3, published as International patent application WO 01/32901 (hereinafter "*the application*").

II. The examining division considered that, in the light of documents D1 and D3 (*infra*), the Main and sole Request, filed on 15 January 2010 and containing 9 claims, did not fulfil the requirements of Article 56 EPC.

III. The applicant (appellant) filed a notice of appeal and a statement of Grounds of Appeal together with a Main Request and Auxiliary Requests 1 to 3. The Main Request was essentially identical to the request underlying the decision under appeal.

IV. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons to oral proceedings, the board informed the appellant of its preliminary, non-binding opinion on the issues of the case, in particular those concerning Article 56 EPC and the admissibility of the requests.

V. In reply to the board's communication, the appellant withdrew its previous Auxiliary Request 2 and made the previous Auxiliary Request 1 its Main Request. Previous Auxiliary Request 3 and Main Request were made Auxiliary Requests 1 and 2, respectively.

VI. Oral proceedings were held on 5 November 2013. At these proceedings, the appellant withdrew all its requests

except for its Auxiliary Request 2 which was made its (sole) Main Request and was identical to the request underlying the decision under appeal.

VII. Claim 1 of appellant's **Main Request** read as follows:

"1. An expression vector comprising:

(a) a first polynucleotide encoding a first, crippled selectable marker;

(b) a second polynucleotide encoding a heterologous polypeptide of interest; and

(c) a third polynucleotide encoding a second, amplifiable selectable marker,

wherein the crippled selectable marker includes one or more mutations that diminish, but do not destroy, the function marker, and wherein the crippled selectable marker is selected from the group consisting of: a sequence coding for a neomycin resistance gene having a mutation at amino acid residue 182, a sequence coding for a neomycin resistance gene having a mutation at amino acid residue 261."

Claims 2 and 4 were directed to preferred embodiments of claim 1. Claims 5 to 8 were directed to a method for producing a polypeptide of interest in a host cell in which an expression vector according to any one of claims 1 to 4 had been introduced. Claim 9 was directed to a host cell line that produced a polypeptide of interest according to the method of claims 5 to 8.

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

D1: WO-A1-98/41645 (publication date: 24 September 1998);

D3: R.L. Yenofsky et al., Proc. Natl. Acad. Sci. USA, May 1990, Vol. 87, pages 3435 to 3439;

D5: WO-A2-94/11523 (publication date: 26 May 1994).

IX. Appellant's arguments, insofar as relevant to the present decision, may be summarized as follows:

Article 56 EPC
Closest prior art

Document D1, with reference to document D5, represented the closest prior art since it had the same purpose as the claimed invention, namely expression vectors for identifying high-expression loci. In order to identify these loci, both the production and translation of mRNA of a selectable marker were impaired by translational impairment and insertion of artificial introns. The structure of the vector disclosed on pages 4 to 6 of document D1 (shown in Figure 6 of document D5) was complex, containing a translationally impaired neomycin phosphotransferase (neo) gene artificially engineered to contain an intron in which a DHFR gene and the gene of interest were inserted. Both features, translational impairment and insertion of the gene of interest in an artificial intron, were disclosed as being of relevance for the function of the vector. The remainder of document D1 was directed to a more complex system based

on several vectors (neo exons split over several targeting plasmids), which was also in line with the teachings of document D5.

Document D5 disclosed three sequential developments (impaired Kozak sequence, out-of-frame start codon and engineered artificial intron). Each of them impaired the production and translation of mRNA and the expression of the neo gene. In Figure 7A, a vector with no modification (TCAE 5.2) was compared with a vector (NEOSPLA) having all three modifications. In Figures 7B and 7C, the vector NEOSPLA was compared with vectors ANEX 2 (Kozak impaired, out-of-frame) and GKNEOSPLA (out-of-frame, artificial intron) of lower complexity. The highest expression loci were always identified using the expression vectors of highest complexity (see also Figure 5, in which vectors TCAE 5.2, ANEX 1 (Kozak impaired) and ANEX 2 were compared).

Technical problem

Starting from this prior art, the technical problem to be solved was the provision of an alternative vector for identification of high-expression loci and its use for the production of a polypeptide of interest.

Non-obviousness

The teaching of documents D1 and D5 was narrow, specific and limited to translationally impaired markers. Starting therefrom, a skilled person would have looked only for alternative markers with impaired expression and for other ways of impairing the expression of the neo gene. There was no suggestion or

indication that any kind of modification other than impairment of expression could be useful. The teaching of documents D1 and D5 could not be generalized beyond markers with impaired expression. Any generalization to other types of modification required hindsight of the application.

In view of this specific teaching, a skilled person would not have consulted document D3 when looking for alternative vectors. Document D3 was not concerned with expression vectors for identifying high-expression loci. There was no suggestion in document D3 that could have led a skilled person to consider neo mutants of reduced intrinsic activity to be useful for solving the relevant technical problem. Although certain plant transformants containing the neo mutant gene had higher levels of neo mRNA and protein, the only conclusion to be drawn therefrom was that the neo mutant had reduced activity rather than reduced stability but not that neo overexpression could overcome the effect of the neo mutation. A skilled person would not have considered the simple neo mutation disclosed in document D3 (changing the amino acid sequence and affecting an unrelated process) to be compatible with insertion in an artificial intron (as disclosed in document D1) and/or to be comparable with the marker mutations and changes present in the complex vectors disclosed in documents D1 and/or D5 for which the best results were obtained, let alone to consider that such a simple mutation would likely provide any improvement.

Further evidence of non-obviousness and of inventive step

The chronology of the relevant disclosures did not support a combination of documents D1 and/or D5 with document D3, since these documents were published long after document D3. Despite the fact that neo mutants with reduced intrinsic activity were available to the authors of documents D1 and D5, they did not make the generalisation from translational impaired markers to markers with reduced intrinsic activity, let alone to modifications of the marker gene that could be overcome by an overproduction of the marker. This generalisation required hindsight of the application.

The expression impairments used in documents D1 and D5 provided only a small improvement in the expression of the gene of interest. Although not directly comparable, yields in document D5 were only 1-2 mg/L (Figures 7A-C), whereas yields in the application were around 100-200 mg/L (Table 3 and page 38, lines 14-17). The vectors of the application were also simpler than those disclosed in documents D1 and D5. The use of crippled markers with reduced intrinsic activity did not require to impair the expression of the marker gene for identifying high-expression loci. The selectable marker and the gene of interest were untangled and there was no need to clone the gene of interest within an artificial intron.

X. The appellant (applicant) requested that the decision under appeal be set aside and that a patent be granted on the basis of the Main Request filed at the oral proceedings on 5 November 2013.

Reasons for the Decision

Main Request

1. The Main Request is identical to the request considered by the examining division. In points 1 and 2.1 of the decision under appeal, the examining division acknowledged the request to fulfil the requirements of Articles 123(2) and 54 EPC. The remaining parts of the decision under appeal, namely points 2.2 to 2.4, dealt with the issue of Article 56 EPC, which was held by the examining division not to be fulfilled (cf. point 3 of the decision under appeal). Thus, the sole issue before the board concerns inventive step.

Article 56 EPC

Closest prior art

2. Document D1, identified as the closest prior art document, describes a "*method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site*" as well as "*vectors suitable for accomplishing the above*" (cf. page 1, lines 10 to 22). The method obtains a high level of gene expression of the desired DNA and avoids the deficiencies of other methods based on the random nature of the integration event generally referred to as "*position effects*", in particular wide variations in the expression level of integrated genes (cf. page 3, line 3 to page 4, line 6).

3. In this context, reference is made to previous studies of the authors of document D1 describing "*the use of*

DNA vectors containing transcriptionally impaired dominant selectable markers in mammalian gene expression", in particular, "*vectors containing a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted*" (cf. page 4, lines 7 to 16 of document D1). These studies are described in detail in document D5, an earlier patent application of the authors of document D1.

4. The gist or basic, general technical teaching on which the methods and vectors of documents D1 and/or D5 are based, is explicitly disclosed in both documents. In essence, it is the recognition that, using a transcriptionally impaired selectable marker, the overall number of drug resistant transfected cells (viable colonies) decreases and that a higher percentage of the viable, "*surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high, resulting in* ***overproduction*** *of neo* [transcriptionally impaired marker gene], *thereby allowing the cells to* ***overcome the impairment*** *of the neo gene*" (highlighted in bold by the board) (cf. page 4, line 24 to page 5, line 6 of document D1; page 5, line 28 to page 6, line 2 and page 12, lines 6 to 24 of document D5).

5. Based on this general teaching, document D5 discloses expression vectors comprising transcriptionally impaired selectable markers, starting with a simple impairment, such as fully impaired consensus Kozak sequences (cf. *inter alia*, page 6, lines 14 to 25,

page 12, line 26 to page 17, line 13), and increasing their complexity by combining additional impairments, such as out-of-frame start codons (cf. *inter alia*, page 7, line 5 to page 8, line 2, page 22, line 8 to page 23, line 13) and/or secondary (hairpin or stem-loop) structure (cf. *inter alia*, page 8, lines 4 to 8, page 23, line 15 to page 24, line 3). As a preferred (optional) embodiment, document D5 discloses transcriptionally impaired markers comprising "e*ither a natural intronic insertion region or artificial intronic insertion region, and at lest one gene product of interest is encoded by DNA located within such insertion region*" (cf. *inter alia*, page 5, lines 12 to 15, page 25, lines 25 to 27, page 24, lines 5 to 8 and claims 29 and 30).

6. These expression vectors are referred to in document D1 as the technical background for the construction of even more complex vectors, such as a triply spliced neo construct (neo gene artificially split into three exons) which relies on a "*target plasmid*" (containing exons 1 and 2 of the neo gene separated by an intervening intron into which at least one gene of interest is cloned) and a "*marker plasmid*" (containing exon 3 of the neo gene) (cf. *inter alia*, page 16, line 8 to page 17, line 13 of document D1). This system, based on homologous recombination, enables the identification of sites in the mammalian genome at which gene expression is high, i.e. transcriptionally active hot spots (cf. *inter alia*, page 10, line 12 to page 11, line 2 of document D1).

Objective technical problem

7. Starting from this closest prior art, the objective technical problem to be solved is seen as the provision of alternative expression vectors for identification of transcriptionally active hot spots and their use in the expression of a polynucleotide encoding a (heterologous) polypeptide of interest (cf. page 3, lines 1 and 2 of the decision under appeal).

8. There is no doubt that the expression vectors of claims 1 to 4 of the Main Request and the methods of claims 5 to 8 solve this technical problem (cf. point VII *supra*).

Obviousness

9. Although there was agreement in the formulation of the objective technical problem between the appellant and the examining division, the appellant interpreted this problem in a very narrow sense. According to the appellant, when looking for alternative expression vectors, a skilled person would have limited himself/herself, only and exclusively, to expression vectors comprising the impaired markers disclosed in the closest prior art, i.e. transcriptionally impaired, since there was no reason to look for other types of impairment. In the appellant's view, hindsight would have been required in order to do the latter (cf. point IX *supra*).

10. The board, however, does not agree to appellant's argumentation. Although documents D1 and D5 disclose only transcriptionally impaired markers, the mechanisms and systems underlying these various impairments are

very different. Whereas, for simple impaired markers, the impairment is overcome by the ribosomal system, other systems or mechanisms are required for more complex impaired markers, such as the splicing mechanism (with artificially engineered introns) and/or the homologous recombination system (with artificially split exons over different vectors) (cf. page 25, lines 21 and 22 of document D5; page 10, line 12 to page 11, line 2 of document D1). The common feature of all these markers is not only that they are all transcriptionally impaired but, more importantly, that all transfected cells **overcoming** these impairments **overproduce** the marker (cf. point 4 *supra*). Indeed, it is this second common feature which enables a skilled person to achieve the actual purpose underlying these prior art documents, namely the identification of transcriptionally active hot spots and their use for expressing a gene encoding a polypeptide of interest. The first common feature may be considered as being merely a tool for achieving the second. In the board's view, no hindsight is required for identifying this second common feature. Thereby, the above formulated objective technical problem can be interpreted more broadly than the appellant has done it, namely as being the provision of alternative expression vectors comprising impaired markers (not limited to transcriptionally impaired) for which the impairment is overcome by overproduction of the marker.

11. In view of this formulation of the technical problem, the board does not share appellant's argument according to which a skilled person, when looking for these alternative expression vectors, had no reason to consult the disclosure of document D3. On the contrary,

the board is convinced that there were good reasons for a skilled person to consult this disclosure. Document D3 discloses an impaired selectable (neo gene) marker of reduced intrinsic activity resulting from a mutation that involves a glutamic to aspartic acid conversion at residue 182. According to document D3, "*the presence of the mutant enzyme results in a decreased ability of tobacco leaf discs to grow on elevated concentrations of the antibiotic G418, as compared with the normal enzyme*" and, "*at the same time, the transformants containing the mutant enzyme had substantially higher levels of the mutant protein*" (cf. page 3438, left-hand column, last paragraph of document D3). A situation absolutely comparable to that described in documents D1 and D5 for the expression vectors containing transcriptionally impaired markers (cf. point 4 *supra*). More importantly, document D3 further suggests that "*the higher NPTII mRNA and protein levels seen in tobacco leaf tissue containing the mutation as opposed to the normal gene probably reflect differences in* ***transcriptional activity*** *and not altered stability of the mutant mRNA or protein product*" (highlighted in bold by the board) (cf. page 3438, right-hand column, first paragraph of document D3). This corresponds in fact to the second common feature identified in point 10 above.

12. In the light thereof, the board agrees with the examining division that a skilled person would not have hesitated to combine the teachings of documents D1 and/or D5 with these of document D3. Document D3 offers an obvious alternative way to cripple the selectable (neo gene) marker in the expression vectors disclosed

in documents D1 and D5 (cf. page 4 of the decision under appeal).

13. The appellant has also given several reasons which, in its opinion, speak against this combination and support the presence of an inventive step:

13.1 As a first reason, it argues that although document D3 was published much earlier than documents D1 and D5, the authors of these two documents disregarded the use of the impaired selectable marker with a reduced intrinsic activity disclosed in document D3 (cf. point IX *supra*). Whereas it is true that there is no reference in documents D1 and/or D5 to the impaired marker of document D3, it is also true that the reasons for the absence of such a reference are absolutely unknown. They might well be of practical nature related to the commercial and/or scientific interests of the applicant of documents D1 and D5. The absence in documents D1 and D5 of any reference to document D3 cannot be interpreted as supporting the assertion that the authors of these two documents deliberately disregarded the disclosure of document D3, let alone that they considered the impaired marker of document D3 to be unsuitable or not appropriate for the expression vectors disclosed in documents D1 and D5.

13.2 As a second reason, the appellant argues on the basis of the simplicity of the claimed expression vectors when compared to those of document D1 (cf. point IX *supra*). However, in the board's view, documents D1 and D5 disclose a general teaching on which basis several vectors are provided (cf. points 4 to 6 *supra*). The disclosure of these documents is not limited to the

more complex vectors but includes also the simplest expression vectors, such as those comprising only an impaired Kozak sequence or an out-of-frame start codon. The complexity of these vectors and impairments is comparable to those of document D3 which comprise an impaired marker obtained by a mutation involving only a glutamic to an aspartic acid conversion at residue 182.

13.3 As a third reason, the appellant refers to the higher yield of the polypeptide of interest obtained when using the claimed expression vectors compared to the yield obtained with the vectors of documents D1 and D5 (cf. point IX *supra*). However, the board is not convinced by this argument. According to the case law of the Boards of Appeal, if comparative tests are chosen to demonstrate an inventive step on the basis of an improved effect, the nature of the comparison with the closest prior art must be such that the alleged advantage is convincingly shown to have its origin in the distinguishing feature of the invention compared with the closest state of the art (cf. "Case Law of the Boards of Appeal of the EPO", 7th edition 2013, I.D.10.9, page 231). In the present case, no comparative tests have been provided. Reference is made only to the results obtained in the Examples of documents D1 and D5 and those of the present application. There are however both, intrinsic (gene of interest, elements of the vector, such as promoter, enhancer, codon use, etc.) and extrinsic factors (culture conditions, such as medium composition, temperature, host cells, etc.), that render such a comparison meaningless. In this context, it is noted that it is shown in Table 2 of the application that the levels of CAB protein expression are dependent on the

expression plasmid used (from ≤ 9.0 to 733 ng/mL), and that the range of CAB protein expression shown in Table 3 is also very broad. A similar degree of expression variability is indicated for other (uPAR or VEGF-D) proteins of interest, namely "*expression levels between about 250 mg to about 1 mg/liter have been achieved*" (cf. page 38, lines 14 to 17 of the application). Under these circumstances, the board considers that the conditions required by the established case law for acknowledging an improvement are not fulfilled.

Conclusion on Article 56 EPC

14. In the light of the above considerations, the board does not see any reason to deviate from the decision of the first instance as regards Article 56 EPC. Thus, the Main Request is considered not to fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski M. Wieser