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D E C I S I O N
of 19 April 2005

Case Number: T 0968/00 - 3.3.8

Application Number: 92904720.7

Publication Number: 0556345

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Language of the proceedings: EN

Title of invention:

Retroviral vectors useful for gene therapy

Patentee:

CELL GENESYS, INC., et al

Opponent:

Genetic Therapy, Inc.

Headword:

Retroviral vectors/CELL GENESYS

Relevant legal provisions:

EPC Art. 56, 123(2)

Keyword:

"Main request - inventive step - no"

"First to third auxiliary request - admissibility of disclaimer - no"

Decisions cited:

G 0009/92, G 0001/03

Catchword:

-



Case Number: T 0968/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 19 April 2005

Appellant: CELL GENESYS, INC.
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Decision under appeal: Interlocutory decision of the Opposition
Division of the European Patent Office posted
24 July 2000 concerning maintenance of European
patent No. 0556345 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: F. L. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

- I. European patent No. 0 556 345 with the title "Retroviral vectors useful for gene therapy" was granted with eight claims on the basis of the European patent application No. 92 904 720.7.

Granted claim 1 read as follows:

"1. A retroviral vector comprising in operable combination: a 5' LTR and a 3' LTR derived from a retrovirus of interest; a portion of gag sequence encoding a splice donor site; an insertion site for a gene of interest; a splice acceptor site located upstream of said insertion site; wherein said vector does not contain a complete gag, env, or pol gene and said vector does not contain a selectable marker."

- II. An opposition was filed under Article 100(a)-(c) EPC for lack of novelty and inventive step, lack of sufficient disclosure and added subject-matter. The opposition division rejected the main, first and second auxiliary claim requests then on file for lack of inventive step and maintained the patent in amended form on the basis of the fourth auxiliary claim request, the third auxiliary request having been withdrawn.
- III. The appellant (patentee) filed an appeal and submitted a statement of grounds of appeal together with a main request and three auxiliary requests, the main, the first and second auxiliary requests being those refused by the opposition division.

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

"1. A retroviral vector comprising in operable combination: a 5' LTR and a 3' LTR derived from a retrovirus of interest; a psi site; a portion of gag sequence encoding a splice donor site; an insertion site for a gene of interest; a splice acceptor site located upstream of said insertion site; wherein said vector does not contain a complete gag, env, or pol gene and said vector does not contain a gene encoding a selectable marker."

Claims 2 to 5 related to further features of the retroviral vector of claim 1. Claim 6 related to a packaging cell line transfected with a retroviral vector according to any of the preceding claims and claim 7 was directed to a cell transduced with retroviral particles obtained from the packaging cell line of claim 6.

Claim 1 of the **first auxiliary request** read as follows:

"1. A retroviral vector comprising in operable combination: a 5' LTR and a 3' LTR derived from a retrovirus of interest; a portion of gag sequence encoding a splice donor site; an insertion site for a gene of interest; a psi site; a splice acceptor site located upstream of said insertion site **which is not a cryptic splice acceptor site**; wherein said vector does not contain a complete gag, env, or pol gene and said vector does not contain a gene encoding a selectable marker." (*emphasis added by the board*)

Claim 1 of each of the second and third auxiliary requests also contained the disclaimer "a splice

- acceptor site... which is not a cryptic splice acceptor site".
- IV. The respondent (opponent) filed a reply to the appellant's statement of grounds of appeal.
- V. On 17 September 2004, the board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal stating its preliminary non-binding opinion, in particular with regard to the inventive step of claim 1 of the main request and also putting in question the allowability of the disclaimer present in claim 1 of the auxiliary requests.
- VI. The respondent withdrew its opposition in a letter dated 14 January 2005.
- VII. In a letter dated 9 February 2005, the appellant informed the board that it would not attend oral proceedings and withdrew its request for oral proceedings.
- VIII. On 11 February 2005, the board advised the parties by fax that the oral proceedings were cancelled.
- IX. The documents mentioned in the present decision are the following:
- (1): Bender, M.A. et al., J. Virol., Vol. 61, No. 5, pages 1639 to 1646, May 1987;
 - (2): WO-A-89/02468;
 - (3): WO-A-89/07136;

(9): Armentano, D. et al., J. Virol., Vol. 61,
No. 5, pages 1647 to 1650, May 1987;

(18): Belmont, J.W. et al., Mol. Cell. Biol.,
Vol. 8, No. 12, pages 5116 to 5125,
December 1988.

X. The appellant's arguments, so far as relevant to the present decision may be summarised as follows:

Main request; claim 1, inventive step

The closest prior art was document (9) which disclosed the retroviral vector N2 comprising all the structural elements of the retroviral vector of claim 1 and, in addition, a selective marker gene (*neo*).

The technical problem underlying the invention was to provide a reliable retroviral vector for somatic gene therapy which efficiently integrated into the genome, expressed high levels of the gene product of interest and was produced in high titres without the negative side effect of co-production or expression of marker products.

The solution given in claim 1 was a retroviral vector such as described in document (9) but wherein the selective marker *neo* gene had been replaced by a cloning site for the insertion of the gene of interest. Thus, once integrated in this site, the gene of interest was expressed in the same manner as the *neo* gene.

Document (9) did not suggest constructing the vectors of claim 1. The skilled person would have doubted that the transcript of the gene of interest could be spliced in the same manner as that of the *neo* gene ie by interaction between a natural splice donor site and a **cryptic** splice acceptor site and thus, that the gene of interest could be expressed. Indeed, he/she was aware that a cryptic splice acceptor site was only functional when activated and its activation depended on its environment. It would not have been expected that the 3' cryptic splice acceptor site which ensured the splicing of the *neo* mRNA would remain functional after this gene had been replaced by another gene. Document (9) did not give any instructions as to how to carry out this replacement while keeping active the 3' cryptic acceptor splice site. The person skilled in the art would, thus, be reluctant to rely on vectors with cryptic acceptor sites for gene therapy.

For these reasons, the claimed subject-matter was inventive.

The same applied to claim 1 of all auxiliary requests.

XI. The former respondent's arguments, so far as relevant to the present decision may be summarised as follows:

Main request; claim 1, inventive step

The closest prior art document was document (9) which disclosed the retroviral vector N2 comprising all the structural elements of the retroviral vector of claim 1 and, in addition, a selective marker gene (*neo*).

The problem as formulated by the appellant - including efficient integration of the viral DNA into the genome, high levels of expression of the gene product of interest... - did not exist, as all these properties were well-known inherent features of retroviruses whether or not they carried a selective marker gene.

In fact, starting from the closest prior art, the problem to be solved should be defined as "the provision of a retroviral vector for use in gene therapy without the presence of a selectable marker".

The provided solution -the N2 vector without the selectable marker gene but with an insertion site for incorporating the gene of interest- was obvious. The skilled person interested in human gene therapy would, of course, want to eliminate the selective marker gene as expressing it in the human host would lead to an immune response. Furthermore, contrary to the appellant's allegation, the skilled person would have had no doubts that the gene introduced in place of the selective marker gene would be expressed (ie that the cryptic splice acceptor site would be active) because document (9) provided two examples that it was. Document (18) provided a further such example since the human ADA protein was expressed from the vector pANN2ADA (derived from N2 by replacement of the *neo* gene with the *ADA* gene), which expression would not have been observed in the absence of a functional cryptic splice acceptor site.

For these reasons, inventive step was to be denied to the subject-matter of claim 1.

Auxiliary requests

All auxiliary requests comprised the disclaimer "a splice acceptor site ... which is not a cryptic splice acceptor site" which had no basis in the application as filed. This disclaimer clearly served only to establish inventive step over at least the prior art document (9). In accordance with the case law, it was not allowable.

- XII. The appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request or on the basis of one of the three auxiliary requests filed with the grounds of appeal.

Reasons for the Decision

1. The appellant's appeal lies from the decision of the opposition division to maintain the patent in amended form. In the course of appeal proceedings, the opponent withdrew its opposition. Thus, the only issues which remain to be considered by the board are those which were decided against the patentee as well as the formal issues which may arise from the filing of amended claims, the claims as maintained by the opposition division not being subject to review (cf. G 0009/92, OJ EPO 1994, 879).

Main request; claim 1, inventive step

2. Document (9) is considered to be the closest prior art. It is concerned with finding the effect of internal

viral sequences on the utility of retroviral vectors. For this purpose, a viral vector is constructed : N2 which is derived from moloney murine leukemia virus (M-MuLV) and presents the following structural features in the 5' to 3' direction (Fig. 1B, pages 1647 and 1648, right-hand columns):

- a 5' long terminal repeat (LTR) which comprises a promoter,
- a donor splice site 68 bp downstream of the 5' LTR,
- a sequence encoding the packaging signal (*psi*),
- 418 bp of the *gag* region of the virus comprising a 3' cryptic acceptor splice site,
- a selectable marker gene encoding neomycin resistance (*neo*),
- a 3' LTR.

Cells infected by N2 express two RNA transcripts which are both initiated from the 5' LTR promoter: a long transcript corresponding in size to the predicted 5' LTR promoter-driven transcript and a transcript which, albeit being shorter, comprises *neo* mRNA. This latter transcript is generated by the splicing of 500 nucleotides involving the interaction of the naturally occurring donor splice site with a cryptic acceptor splice site present in the *gag* region, 100 nucleotides upstream from the *neo* gene.

On page 1648 (right-hand column), the section entitled " N2-derived ADA vectors" is devoted to testing "the usefulness of the N2 vector system for transfer and expression of **non-selectable** genes." (emphasis added). The human *ADA* cDNA (preceded by the mouse metallothionein I (MT) promoter or by the simian virus

- 40 (SV40) early promoter) is inserted downstream from the *neo* gene. Transcription leads to the synthesis of *ADA* mRNA starting from the MT or SV40 promoter; the spliced *neo* mRNA is also synthesised starting from the 5' LTR promoter but to a lesser extent than in the N2 vector per se.
3. Starting from the closest prior art, the problem to be solved may be defined as providing an alternative retroviral vector system for the transfer and expression of non-selectable genes.
 4. The solution given in claim 1 is a vector system comprising the same structural features as the N2 vector except that the selective marker gene has been eliminated and that an insertion site is present in its place where the gene of interest may be introduced. This feature has definite consequences for the expression of the gene of interest: like for the *neo* gene, one may expect that it will be transcribed from the 5' LTR promoter and, thus, that the correct translation will only occur after splicing. This is, of course, in contrast with what happens with the N2-derived *ADA* vectors (see point 2, supra) as in these vectors, the *ADA* cDNA is transcribed from its own promoter.
 5. Formulating the problem of providing an alternative retroviral system is not per se inventive: it is readily apparent from document (9) (eg. page 1647, first paragraph) that although, at the priority date, retrovirus were appreciated for their potential as vectors, their draw-backs were also known and, consequently, there was a need for vector improvement.

6. In the same manner, the skilled person would have been aware of the possible use of retroviral vectors in human gene therapy (document (9), page 1649, reference to in vivo transfer and expression) and, thus, would undoubtedly avoid the expression of genes of non-human origin - such as selective marker genes - because this expression would be likely to trigger an immune response. Accordingly, taking away the selective marker gene was equally obvious.

7. Yet, the question remains whether or not the skilled person aware of the teachings of document (9) would have contemplated constructing a vector wherein the gene of interest would replace the selective marker gene, and if he/she had considered the possibility of doing so, whether or not there would have been a reasonable expectation of success that the vector would be effective at producing the protein of interest.

8. The appellant argued there would not because of the peculiar conditions for gene expression created by the mRNA having to be spliced through the interaction of a natural splice donor site and a cryptic splice acceptor site. The skilled person would have been aware that cryptic acceptor sites were not always functional, that their functionality strongly depended on the environment, ie could very easily change when only slight modifications were carried out in the surrounding DNA. In the appellant's view, such a vector would have been thought unreliable and, thus, neither its isolation nor a reasonable expectation of success in its use would have been envisaged.

9. The board does not find this argument convincing. Firstly, the N2 vector itself was made in spite of this alleged reluctance of the skilled person to have the "foreign" gene - in that case the *neo* gene - transcribed from the 5' LTR promoter and its RNA spliced thereafter. Two other constructs N1 and N3 are also mentioned in document (9) which carry various amounts of *gag* DNA upstream of the *neo* gene and, yet, are capable of splicing 5' LTR-initiated *neo* mRNA. Secondly, the *neo* mRNA is transcribed and spliced in the N2-ADA recombinant vector (point 2, supra) although the introduction into the vector of the further ADA transcriptional unit may be considered a significant modification at the transcriptional level.

10. Document (18) is also of interest in this context. There, the recombinant vector p Δ NN2ADA is described which was obtained by replacing the *neo* gene in the N2 vector by the ADA cDNA. Like the *neo* gene in the N2 vector, the ADA cDNA is expressed in p Δ NN2ADA. The authors do not specify the size of the corresponding mRNA but state that "the possible utilisation of an alternative cryptic splice acceptor in p Δ NN2ADA is being further evaluated by RNase protection analysis" which implies that the ADA mRNA must have been spliced.

11. On the basis of this evidence, the board concludes that whatever misgivings the skilled person might have had about cryptic splice sites in general, he/she would have contemplated constructing the now claimed specific vector system involving the spliced transcription of the mRNA encoded by the gene of interest by interaction between a natural *gag* splice donor site and a cryptic *gag* splice acceptor site and would have done so with a

reasonable expectation of success. Claim 1, thus, lacks inventive step.

12. The main request is rejected for failing to fulfil the requirements of Article 56 EPC.

Auxiliary requests 1 to 3; allowability of the disclaimer

13. In each of auxiliary requests 1 to 3, granted claim 1 was amended, in particular by the feature:

"... a splice acceptor site located upstream of said insertion site **which is not a cryptic splice acceptor site**;..." (*emphasis added*).

ie cryptic acceptor sites have been excluded from the claim by way of disclaimer. Said disclaimer is not found in the application as filed.

14. The criteria for the allowability of a disclaimer which is not disclosed in the application as filed are given in the Enlarged Board of Appeal's decision G 1/03 (OJ EPO 2004, 413). Points 2.1 and 2.3 of the Order are most relevant to the present case:

"2.1 A disclaimer may be allowable in order to:

- restore novelty by delimiting a claim against state of the art under Article 54(3) and (4) EPC;

- restore novelty by delimiting a claim against an accidental anticipation under Article 54(2) EPC;

- disclaim subject-matter which, under Articles 52 to 57 EPC, is excluded from patentability for non-technical reasons."

and

"2.3 A disclaimer which is or becomes relevant for the assessment of inventive step or sufficiency of disclosure adds subject-matter contrary to Article 123(2) EPC."

15. The opposition division acknowledged the novelty of claim 1 over each of documents (1) and (9) because the vectors therein described contained a selectable marker gene. None of documents (2), (3) and (18) were considered to be a clear and unambiguous disclosure of vectors such as claimed and, thus, to affect novelty. Consequently, the fact that the splice acceptor site is not a cryptic splice acceptor site is not decisive for delimiting the claimed subject-matter against the prior art. In the same manner, the disclaimed subject-matter is not excluded from patentability for non-technical reasons. On the contrary, in view of the conclusion reached in point 12 supra that the claimed retroviral vector carrying a cryptic splice site lacks inventive step, the disclaimer of cryptic splice sites could be relevant for establishing inventive step. In accordance with the quoted decision G 1/03 (supra), this disclaimer adds subject-matter contrary to Article 123(2) EPC.

16. The auxiliary requests 1 to 3 are refused for failing to comply with the requirements of Article 123(2) EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:

A. Wolinski

L. Galligani