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D E C I S I O N
of 14 July 2005

Case Number: T 1189/02 - 3.3.8

Application Number: 95119039.6

Publication Number: 0747485

IPC: C12N 15/85

Language of the proceedings: EN

Title of invention:
Production of proteins using homologous recombination

Patentee:
Cell Genesys, Inc.

Opponents:
F. Hoffmann-La Roche & Co. Aktiengesellschaft
Institut Pasteur
Transkaryotic Therapies Inc.
Applied Research Systems ARS Holding NV

Headword:
Homologous recombination/CELL GENESYS

Relevant legal provisions:
EPC Art. 76(1)

Keyword:
"Main request and auxiliary request - subject matter extending beyond the content of the earlier application (yes)"

Decisions cited:
G 0001/93

Catchword:
-

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Case Number: T 1189/02 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 14 July 2005

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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 6 September 2002 revoking European patent No. 0747485 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: M. R. Vega Laso
C. Rennie-Smith

Summary of Facts and Submissions

I. The appeal lies from the decision of the opposition division posted on 6 September 2002, whereby the European patent No. 0 747 485 (European application No. 95 119 039.6) with the title "Production of proteins using homologous recombination" was revoked pursuant to Article 102(1) EPC. The patent had been granted on a divisional application of the earlier European application No. 91 900 640.3, published as WO 91/06667 (in the following referred to as "the earlier application"), and contained 14 claims for the Contracting States AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, NL, SE, and 11 claims for the Contracting State ES.

II. The patent was opposed by four parties on the grounds of Article 100(a), namely lack of novelty and lack of inventive step, 100(b) and 100(c) EPC. The opposition division decided that the patent was to be revoked because, contrary to the requirements of Article 76(1) EPC, the subject-matter of the claims of both the main request (claims as granted) and the auxiliary request (claims 1 to 13 as filed on 22 March 2002) extended beyond the content of the earlier application. In the view of the opposition division, a one-step method as claimed in claim 1 of both requests was not disclosed in the earlier application. Moreover, because the term "and/**or**" implied that the amplifiable gene and the heterologous nucleotide regulatory sequences were alternatives, claim 1 encompassed methods not disclosed in the earlier application.

III. Claim 1 of the **main request** (claims as granted for all designated Contracting States) read as follows:

"1. A method for producing mammalian proteins comprising:

transforming mammalian host cells comprising an endogenous target gene with a construct comprising an amplifiable gene and/or a heterologous nucleotide regulatory sequence and at least one flanking region homologous to a region of the host cell genome within or proximal to said endogenous target gene, so that the amplifiable gene and/or heterologous nucleotide regulatory sequence are integrated via homologous recombination into the genome of the mammalian cells and the amplifiable gene and/or heterologous regulatory sequence become operatively associated with said endogenous target gene so that said endogenous target gene is capable of being amplified when said amplifiable gene is amplified and so that expression of said endogenous target gene is controlled by said heterologous regulatory sequence;

selecting for cells comprising said construct by means of said amplifiable gene or other marker present in said construct; and

culturing said cells comprising said construct under conditions wherein the targeted gene is expressed and the protein encoded by the targeted gene is produced."

Independent claim 2 was directed to a method for integrating an amplifiable gene and/or a heterologous nucleotide regulatory sequence into the genome of a

mammalian cell. Independent claim 3 related to a method for amplifying gene expression in a mammalian host cell, and dependent claim 4 concerned a particular embodiment of this method. In the dependent claims 5 to 11, different embodiments of the methods according to the previous claims were claimed. Independent claim 12 was directed to a mammalian host cell, and claims 13 and 14 to particular embodiments of the host cell of claim 12.

Claims 2 to 11 for the Contracting State ES were identical to the corresponding claims for the other Contracting States.

IV. The amended claim 1 of the **auxiliary request** differed from the corresponding claim of the main request in that the first step of the method read:

"1. ...

transforming mammalian host cells comprising an endogenous target gene with a construct comprising an amplifiable gene and/or a **transcriptional regulation region changed or different from that of the endogenous target gene** and at least one flanking region **of a total of at least about 150bp** homologous to a region of the host cell genome within **100kb of the transcribed region of** said endogenous target gene, so that the amplifiable gene and/or **the changed transcriptional regulation region** are integrated via homologous recombination into the genome of the mammalian cells and the amplifiable gene and/or heterologous regulatory sequence become operatively associated with said endogenous target gene so that said endogenous target gene is amplified when said amplifiable gene is amplified and so that

expression of said endogenous target gene is controlled by said **changed transcriptional regulation region**;

..." (amendments emphasised by the board)

Claims 2 to 4 were amended in a similar manner. Previous claim 5 was omitted, and claims 6 to 14 were renumbered and the back-references amended. Additionally, in amended claim 11 the terms "*heterologous nucleotide regulatory sequence*" were replaced by "*changed transcriptional regulation region*".

V. On appeal, the appellant (proprietor of the patent) pursued further the requests on which the decision of the opposition division was based. In support of its case, the appellant submitted with the statement of grounds of appeal a declaration of Dr. Skoultchi, the sole inventor of the patent in suit (document D26), which had not been admitted into the proceedings by the opposition division as it was considered to be late-filed. Oral proceedings pursuant to Article 116 EPC were requested in the event that the board did not intend to set aside the contested decision.

VI. Respondents I, II and IV (opponents 01, 02 and 04) each filed a response to the statement of grounds of appeal and requested dismissal of the appeal. Respondent I further requested that the declaration filed by the appellant be not admitted into the proceedings. As a subsidiary request, all respondents requested oral proceedings.

VII. The parties were summoned to oral proceedings. In a communication pursuant to Article 11(1) of the Rules of

Procedure of the Boards of Appeal sent with the summons, the board indicated the essential issue to be discussed at the oral proceedings, namely whether or not the claims of the main request and the auxiliary request contained subject-matter that extended beyond the content of the earlier application as filed.

VIII. Respondent I submitted additional arguments in writing and notified the board that it would not attend the oral proceedings, withdrawing its corresponding request.

IX. At oral proceedings, which took place on 14 July 2005, the appellant and respondents II, III and IV were represented.

X. The submissions made by the appellant may be summarised as follows:

Secondary host cells used for production of the protein

The earlier application disclosed both a two-step method, in which the gene of interest was transferred into secondary host cells where the desired protein was produced, and a one-step method wherein the optional step of transformation of the secondary host cells was omitted, the desired protein being produced in the primary host cells. These two methods were clearly optional alternatives to solve the technical problem set out in the specification of the earlier patent, namely to produce a desired protein avoiding the prior art problem of having to identify and isolate the sequence coding for the target protein.

There was formal literal support in the description of the earlier application for expression of a desired protein in the primary host cells. For instance, on page 4, lines 25 to 29 of the earlier application it was disclosed that the resulting cell lines were screened for production of the target protein and secondary cell lines selected for desired levels of production, which cells could be expanded and used for production of the desired protein in culture. Since the term "*resulting cell lines*" in this passage referred to either primary or secondary host cells, the passage clearly foreshadowed primary cell lines being screened for the production of the desired protein.

There was no explicit statement in the description that secondary host cells were essential. On the contrary, the skilled person would undoubtedly understand from the earlier application that expression in the secondary host cell was not necessary to achieve the technical effect of the invention, namely to obtain expression of protein products which are only produced in small quantities in native primary cells. Thus, secondary host cells were only a matter of convenience.

Furthermore, the specification made it plain in a number of places (for instance on page 4, lines 13-17 and on page 10, line 34 to page 11, line 2) that amplification could take place in the primary cells and could be associated with a screen for production of the protein of interest. Amplification in primary cells necessarily disclosed protein expression in these cells. The skilled person was clearly in a position to recognise that some expression must be occurring in the

primary cells, rendering the transformation into secondary cells an optional step.

The contested decision was predicated on the suggestion that the only way of solving the problems stated on page 2, lines 4 to 17 was the use of a secondary host cell. This was incorrect. Increasing the expression level of a protein produced in a primary cell by upregulating expression using an altered regulatory sequence solved the problem of low expression, making a secondary cell line irrelevant. It was technically nonsensical to suggest that by merely transferring the DNA into a suitable host, a non-expressing construct could be made to express.

Optional amplifiable gene

The contribution of the earlier application was the idea of using homologous recombination to engineer greater expression of a gene of interest *in situ* by modifying the regulatory sequences and/or amplifying the gene. Thus, the earlier application disclosed two alternative independent ways to increase protein expression, namely amplification of the gene (which gave rise to a copy number improvement - more copies of the gene encoding the target protein), and heterologous regulatory sequences which directly altered the level of expression of the gene encoding the target protein.

The specification contained no explicit statement of the essentiality of amplification. Reading the description of the earlier application, the skilled person would realise that, if the endogenous gene could

be expressed at a reasonable level by alteration of its regulation, amplification would be unnecessary.

XI. The submissions of the respondents may be summarized as follows:

There was no literal support, formal or otherwise, for omitting the secondary host cells. The skilled person, on reading the earlier application, would not appreciate that the secondary host cells were optional. Indeed, the whole tenor of the teaching was that expression in the secondary host cells was essential to solve the problem addressed by the earlier application. The appellant's reasoning was based on a misreading of the passage on page 4, lines 13 to 29 of the earlier application.

Taking account of the statements in the Summary of the Invention on page 3 of the earlier application that "other regulatory sequences" could possibly be integrated *in addition* to the amplifiable gene, it was clear that the integration of the amplifiable gene was obligatory. There was no support in the earlier application for a process which did not involve an amplifiable gene.

XII. The appellant requested that the decision under appeal be set aside and that the case be remitted to the opposition division for further prosecution on the basis of the main request (claims as granted) or of the auxiliary request filed on 22 March 2002.

The respondents requested that the appeal be dismissed.

Reasons for the Decision

Admissibility of document D26

1. As regards document D26 which the opposition division did not admit in the first instance proceedings (cf. V above), the appellant submitted this had been "caught up" with a large number of other documents all of which had been excluded for late filing. The respondents objected to the introduction of this document in the appeal proceedings on the grounds that it was late-filed and of insufficient relevance.

2. The Board agrees with the respondents for several reasons. First, there must always be exceptional reasons to admit into opposition appeal proceedings evidence which - for good reasons - has already been excluded for late filing at first instance - if that were not so, parties would be encouraged to file evidence at a late stage in opposition proceedings knowing they could always circumvent a lateness objection on appeal. Second, in the case of the declaration in question, there can be no adequate reason for its late filing at first instance - one person whose evidence can be obtained and produced in time is the inventor. Third, it is clear beyond doubt that the inventor's evidence would be of very limited value, indeed almost certainly of no value, in the proceedings - any inventor is of course likely to give evidence favouring the patentee's case, *a fortiori* in the present case when the issue is the extent of the invention and when the inventor (as his declaration states) was at the time of the invention acting in an

advisory capacity to the patentee. However accurate his evidence might be, its pertinence or relevance would by reason of his association with the appellant be at best minimal. Therefore, exercising its discretionary power under Article 114(2) EPC, the board decided to not admit the declaration in question in the proceedings.

The Article 76(1) EPC issue

3. The question at issue in the present appeal is whether claim 1 of the main request and the auxiliary request contains subject-matter that extends beyond the content of the earlier application as filed (cf. Article 76(1) EPC, second sentence).

4. The purpose of Article 76(1) EPC, second sentence being the same as the purpose of Article 123(2) EPC, ie to guarantee legal certainty to third parties and to create a fair balance between the interests of applicants and other parties (cf. G 1/93, OJ EPO 1994, 541), the boards of appeal of the European Patent Office have extended the principles set out in rulings on Article 123(2) EPC to the relationship between the subject-matter claimed in a divisional application and the disclosure in the earlier application from which the divisional derives.

5. Thus, the question to be decided by the board is whether the subject-matter of claim 1 of the main request and the auxiliary request is directly and unambiguously derivable from the disclosure in the earlier application.

6. Claim 1 of the main request is directed to a method of producing a desired mammalian protein, which method consists essentially of integrating by homologous recombination a construct comprising an amplifiable gene and/or a heterologous regulatory sequence, into the genome of mammalian host cells, the integration locus being near to the target gene encoding the protein of interest. The host cells containing the construct are then cultured under conditions allowing the target gene to be expressed and the desired protein produced.

7. In the board's judgement, the claimed method is not directly and unambiguously derivable from the earlier application as filed. In particular, nowhere in the earlier application is there a disclosure of the production of a desired protein in a **primary** host cell, ie in the host cell where the amplifiable gene and regulatory sequences have been integrated by homologous recombination. Rather, in the earlier application only secondary host cells are contemplated for expression of the target gene and production of the protein (cf. paragraph under the heading "Summary of the Invention" on page 3, as well as lines 6 to 29 on page 4 of the earlier application), the secondary host cells obtained by transformation with DNA from the primary host cells being referred to as "expression host" throughout the earlier application (cf. *inter alia* page 4, lines 22-23; page 5, lines 19-20; page 10, line 29; page 11, line 14 and page 19, line 28).

8. The alleged literal disclosure on page 4, lines 25 to 28 of the earlier application is not such as to teach the person skilled in the art directly and

unambiguously that the desired protein may be produced in primary cells, secondary host cells being optional and only a matter of convenience. Read in the proper context, the phrase "t*he resulting cell lines*" used in the passage cited by the appellant refers to the "*secondary expression host cells*" mentioned in the previous sentence, and there is no indication which would induce the skilled person to interpret that phrase as referring to either primary or secondary host cells.

9. The skilled person could also not infer from the technical problem formulated in the earlier application that the desired protein may be produced in primary host cells. It is clear from the statements on page 2, lines 3 to 17 that the problem to be solved in the earlier application was to provide an alternative method for producing a desired protein in a cell **different** from the source of the target gene encoding the protein, the fact that the coding sequence does not need to be identified precisely being solely an advantage of the disclosed method.

10. Although there is no explicit statement in the earlier application that secondary host cells are essential, it follows from the description that the transfer of the target gene and the amplifiable gene to secondary host cells is an integral part of the disclosed method. Moreover, it might be true that secondary host cells are not necessary to achieve the technical effect of the invention, ie to obtain expression of a desired protein which is either not produced, or produced in small quantities in native primary cells, and that secondary host cells can be used only for a more

efficient and economical production. Yet, only a method for producing a mammalian protein in secondary host cells is disclosed in the earlier application, nothing else.

11. It is true that the possibility of carrying out amplification of the target gene in the primary host cells is mentioned in the earlier application. However, amplification in primary cells is disclosed in the earlier application only as a possible preliminary step to the transfer of the amplifiable region to secondary host cells, where the target gene is expressed and the desired protein produced. Contrary to the appellant's view, carrying out amplification of the gene in primary host cells does not necessarily mean that the protein of interest is produced in these cells, but only that the number of copies of the construct in the genome of the primary host cells can be increased before it is transferred to the secondary expression host cells in order for the protein to be produced.

12. It follows from the above that, a method for producing a mammalian protein in **primary** host cells not being disclosed in the earlier application, the subject-matter of claim 1 of the main request extends beyond the content of the earlier application as filed.

13. Moreover, in its decision to revoke the patent the opposition division also held that, because of the language "*and/**or***" in claim 1, the claimed method does not necessarily involve the use of an amplifiable gene. This has been admitted by the appellant.

14. However, in the method disclosed in the earlier application, production of mammalian proteins is achieved by employing homologous recombination for integration of an amplifiable gene **and** regulatory sequences in proximity to a gene of interest (cf. page 3, lines 12-15), and in embodiments where an enhancer is integrated, the enhancer sequence is associated with the amplifiable gene (cf. page 7, lines 31-32). Both methods exemplified in the earlier application for producing human t-PA and EPO protein in CHO cells use an amplifiable gene (DHFR), either with or without an associated regulatory sequence, ie an enhancer.

15. It follows that, even if not expressly stated in the earlier application, the presence of an amplifiable gene is essential for producing a mammalian protein according to the method disclosed in the earlier application. Contrary to the appellant's allegation, the earlier application does not disclose amplification and regulatory control as **alternative** independent ways of increasing the expression of a target gene, regulatory sequences being always used in addition to the amplifiable gene.

16. Thus, a method for producing a mammalian protein by expression of the corresponding gene ***in situ***, ie in primary host cells, under the control of a regulatory sequence, but **in the absence** of an amplifiable gene is not disclosed in the earlier application. The contrary interpretation of the disclosure of the earlier application made by the appellant could only be possible in full knowledge of later developments in the field of gene activation.

17. The findings above also apply to the identical claim 1 of the set of claims for the Contracting State Spain, and, *mutatis mutandis*, to claim 1 of the auxiliary request, the latter being directed to a method analogous to that of claim 1 of the main request in so far as neither secondary host cells nor an amplifiable gene are required for the production of a mammalian protein.

18. Consequently, none of the requests on file satisfies the requirements of Article 76(1) EPC, second sentence, as they comprise subject-matter which extends beyond the content of the earlier application as filed. The decision taken by the opposition division being correct, there is no reason to set it aside.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani