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# D E C I S I O N
## of 16 November 2004

**Case Number:** T 0960/02 - 3.3.8

**Application Number:** 92924367.3

**Publication Number:** 0649464

**IPC:** C12N 5/10

**Language of the proceedings:** EN

**Title of invention:**
Transfection of vertebrate cells e.g. by homologous recombination

**Patentee:**
TRANSKARYOTIC THERAPIES, INC.

**Opponents:**
Genetic Therapy, Inc.
Institut Pasteur
Applied Research Systems ARS Holding NV
Cell Genesys, Inc.
Roche Diagnostics GmbH

**Headword:**
Transfection/TRANSKARYOTIC THERAPIES

**Relevant legal provisions:**
EPC Art. 123(2)(3), 84, 54, 56

**Keyword:**
"Main request; auxiliary requests 1 to 3 - added subject-matter - yes" "Clarity - no"
"Auxiliary requests 4 to 7 - added subject-matter - yes"
"Auxiliary requests 8 and 9 - inventive step - no"
"Auxiliary requests 10 and 11 - extension of protection conferred - yes"

**Decisions cited:**
G 0004/92, T 0279/93

**Catchword:**-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

**Case Number:** T 0960/02 - 3.3.8

# D E C I S I O N
## of the Technical Board of Appeal 3.3.8
## of 16 November 2004

**Appellant:** (Proprietor of the patent)
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**Decision under appeal:**
**Decision of the Opposition Division of the European Patent Office posted 3 June 2002 revoking European patent No. 0649464 pursuant to Article 102(1) EPC.**

**Composition of the Board:**

**Chairman:** L. Galligani
**Members:** F. L. Davison-Brunel
M. B. Günzel

## Summary of Facts and Submissions

I. European patent No. 0 649 464 with the title "Transfection of vertebrate cells e.g. by homologous recombination" was granted with 22 claims for all designated Contracting States on the basis of the European patent application No. 92 924 367.3 published as WO 93/09222. The latter contained ninety-nine claims of which claims 65 and 78 (relevant to the present decision) read as follows:

"65. A method of introducing exogenous DNA into a preselected site of the genome of a primary or a secondary cell of vertebrate (e.g., mammalian) origin, comprising the steps of:

a) transfecting the primary or the secondary cell with a
DNA construct comprising exogenous DNA which includes DNA sequences homologous to genomic DNA sequences of the primary or secondary cell, thereby producing transfected primary or secondary cells and

b) maintaining transfected primary or secondary cells under
conditions appropriate for homologous recombination between DNA sequences in the DNA construct and genomic DNA to occur;

thereby producing homologously recombinant primary or secondary cells."

"78. A method of targeting exogenous DNA into a preselected site in genomic DNA of a primary or secondary cell of vertebrate origin, comprising the steps of:

a) providing a DNA construct comprising:

1) exogenous DNA selected from the group consisting of:

a) DNA sequences which repair, alter, delete or replace a resident gene in the primary or secondary cell;

b) DNA sequences encoding a product not normally expressed in the primary or secondary cells or not expressed in significant levels in the primary or secondary cells as obtained;

c) DNA sequences which repair, alter, delete or replace a regulatory sequence present in the primary or secondary cells;

d) DNA sequences which encode a regulatory sequence not normally functionally linked to a gene to be expressed in the primary or secondary cells as obtained; and

e) DNA sequences which inactivate or remove a gene or gene portion in the primary or secondary cells;

2) DNA sequences homologous with genomic DNA sequences in the primary or secondary cells; and

3) DNA sequences encoding at least one selectable marker;

b) transfecting primary or secondary cells with the DNA construct provided in (a), thereby producing transfected primary or secondary cells containing the DNA construct provided in (a); and

c) maintaining primary or secondary cells produced in (b) under conditions appropriate for homologous recombination to occur between DNA sequences homologous with genomic DNA sequences and genomic DNA sequences,

thereby producing primary or secondary cells of vertebrate origin having the DNA construct of (a) integrated into genomic DNA of the primary or secondary cells."

Granted claim 14 which is relevant for the present decision read as follows:

"14. A barrier device containing a cell strain obtainable by the process of any one of the preceding claims, wherein the barrier device is made of a material which permits passage of the therapeutic agent (e.g. erythropoietin, insulinotropin or human growth hormone) produced by the cell strain contained therein into the circulation or tissues of a mammal and prevents contact between the immune system of the mammal and the cell strain contained within the barrier device to a sufficient extent to prevent a deleterious immune response by the mammal."

II. Five oppositions were filed under Article 100(a) to (c) EPC. The opposition division revoked the patent for lack of novelty and lack of inventive step of respectively, the main and the auxiliary requests then on file.

III. The appellant (patentee) filed an appeal and submitted a statement of grounds of appeal together with a main request (granted claims) and three auxiliary requests.

IV. Respondents II, III and V (opponents 2, 3 and 5) answered to the statement of grounds of appeal.

V. On 29 June 2004, the board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal, indicating its preliminary, non-binding opinion.

VI. With letters dated 14 and 15 October respectively, respondents II and III sent further submissions in answer to this communication. With letters dated 19 October 2004 and 8 September 2004 respectively, respondents I and IV informed the board that they would not take part in the oral proceedings.

VII. With letter dated 15 October 2004, the appellant submitted a new main request and 11 auxiliary requests in replacement of all requests then on file and informed the board that the appellant would not take part nor be represented at oral proceedings, which took place on 16 November 2004.

**Claim 15 of the main request and auxiliary requests 1 to 3** read as follows:

"15. A method of introducing exogenous DNA into a preselected site of the genome of a primary or secondary cell of vertebrate (e.g., mammalian) origin, comprising the steps of:

(a) transfecting the cell with a DNA construct comprising exogenous DNA which e.g. encodes a therapeutic product and which includes DNA sequences homologous to genomic DNA sequences of the cell (and which e.g. also encodes a selectable marker), thereby producing transfected primary or secondary cells;

(b) maintaining the transfected cells of step (a) under conditions appropriate for homologous recombination between DNA sequences in the DNA construct and genomic DNA to occur; thereby producing homologously recombinant primary or secondary cells which express an endogenous gene at a higher level than occurs in the corresponding non-transfected cells; and

(c) propagating in vitro the homologously recombinant cells of step (b) to produce a clonal strain of homologously recombinant secondary cells; or

(d) exposing the homologously recombinant cells of step (b) to a selective agent which selects for a selectable marker present in the DNA construct whereby cells that have

not properly integrated the selectable marker are killed and cells that have stably integrated the marker can survive and form colonies, followed by screening the colonies to identify homologously recombinant primary or secondary cells, wherein for example the selective marker and selective agent is neo and G418 respectively."

**Claim 15 of auxiliary requests 4 to 7** read as follows:

"15. A method of introducing exogenous DNA into a preselected site of the genome of a primary or secondary cell of vertebrate (e.g., mammalian) origin, comprising the steps of:

(a) transfecting the cell with a DNA construct comprising exogenous DNA which is selected from:

(i) sequences which repair, alter, delete or replace a resident gene or regulatory sequence in the primary or secondary cell;

(ii) sequences which encode a regulatory sequence not normally functionally linked to a gene to be expressed in the cell as obtained; and

(iii) sequences which inactivate or remove a gene or gene portion in the cells;

and which also includes DNA sequences homologous to genomic DNA sequences of the cell (and which e.g. also encodes a selectable marker), thereby producing transfected primary or secondary cells;

(b) to (d): [*as in claim 15 of the main request*]".

**Claim 1 of auxiliary request 8** read as follows:

"1. A process for producing a cell strain comprising human transfected primary or secondary somatic cells for delivering a therapeutic product in vivo comprising the steps of:

(a) providing primary or secondary human somatic cells;

(b) transfecting the primary or secondary cells with DNA, which DNA encodes a therapeutic product;

(c) isolating a non-immortalized cell strain from the transfected cells of step (b); and

(d) expanding the cell strain of step (c) in vitro."

Claims 2 to 13, 15 and 16 of this request related to further features of the process of claim 1. Claim 14 related to a barrier device containing a cell strain obtainable by the process of any one of the preceding claims and claim 17 related to a process for producing a therapeutic product in vitro.

**Claim 1 of auxiliary request 9** was identical to claim 1 of the eighth auxiliary request but for step (d) which read:

"1. (d) expanding the cell strain of step (c) in vitro, comprising maintaining the cell strain under appropriate conditions for a sufficient time for at least 20 cell doublings to occur."

**Claim 1 of auxiliary request 10** read as follows:

"1. Use, in a manufacture of a vehicle or delivery system for delivering a therapeutic product in vivo of a cell strain comprising human transfected primary or secondary somatic cells, said use comprising the steps of:

(a) to (d): [*as in claim 1 of the eighth auxiliary request]*".

**Claim 1 of auxiliary request 11** read as follows:

"1. Use, in a manufacture of a vehicle or delivery system for delivering a therapeutic product in vivo of a cell strain comprising human transfected primary or secondary somatic cells, said use comprising the steps of:

(a) to (d): [*as in claim 1 of the ninth auxiliary request]*".

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

(2): Fountain, J.W. et al., Gene, Vol. 68, pages 167 to 172, 1988;

(12): WO-A-91/06667;

(76): First declaration of Dr D. Barnes received on 29 January 2001;

(77): Dusty-Miller, A., Blood, Vol. 76, No. 2, pages 271 to 278, 1990 attached to the declaration of Prof. K. Kurachi dated 11 January 2001;

(98): Declaration of Prof. D. Kipling dated 1 October 2002;

DB2: Cristofalo, V.J. and Stanulis-Praeger, B.M., Advances in Cell Culture, Vol. 2, pages 1 to 83, 1982, Academic Press Inc;

DB4: Goldstein, S., The Journal of Investigative Dermatology, Vol. 73, pages 19 to 23, 1979;

DK3: Holliday, R. et al., Science, Vol. 213, pages 1503 to 1505, 1981.

IX. The appellant's arguments in writing insofar as they are relevant to the present decision may be summarized as follows:

***Article 84 EPC***

*Claim 15; main request, auxiliary requests 1 to 3*
From the wording of the claim it would be immediately apparent to the skilled person that the sequence encoding a therapeutic product (part (a) of the claim) was not related to those parts of the constructs that

brought about the technical effect now introduced in part (b) of the claim. Therefore, the claim was clear.

*Claim 15; auxiliary requests 4 to 7*
The claim was limited to aspects of the DNA constructs that might bring about the technical effect (part (b)). The claimed sequences (part (a) of the claim) could clearly result in up regulation of an endogenous gene. Therefore, the claim was clear.

***Article 123(2) EPC; Article 84 EPC***

*Claim 1; auxiliary request 8*
It was abundantly clear from page 16, line 33 and also from the many examples given in the application as filed that the claimed process was to be carried out in particular with human cells. For this reason, the requirements of Article 123(2) EPC were fulfilled.

*Claim 1; auxiliary request 9*
This claim corresponded to claim 1 limited to incorporate the features of claim 13. This limitation rendered clear and unambiguous the requirement of an extended expansion period (step (c)) when producing cells suitable for the delivery of a therapeutic product *in vivo*.

*Claim 1; auxiliary requests 10 and 11*
These claims had been reformulated as use claims to explicitly emphasize the need to develop a cell strain beyond an initial production point to a point where there is sufficient cells for delivery of a therapeutic product *in vivo*. Basis for the amendment could be found in the application as filed for example at page 8,

line 16 to page 9, line 5 as well as at page 36, line 17 to page 38 line 19. Production of a delivery system was described in further detail from page 33 onwards.

***Article 54 EPC, novelty***

*Claim 1; auxiliary requests 8 and 9*
The limitation of claim 1 of auxiliary requests 8 or 9 to human cell lines imparted novelty to said claims.

***Article 56 EPC, inventive step***

*Claim 1; auxiliary requests 8 and 9*
Despite being part of the preamble and, of course, not limiting the claims to the use of those cell strains for delivering a therapeutic product *in vivo*, the feature "for delivering a therapeutic product *in vivo*" provided the absolute requirement that cell strains produced according to the processes in claim 1 of auxiliary requests 8 or 9 are suitable for delivering a therapeutic product *in vivo*. Accordingly, the nature of the product of the process must be relevant to the assessment of inventive step.

Because of the nature of the starting material (primary or secondary human cells), the skilled person would have been of the opinion that the experimental step of expanding or maintaining the cell strain to a point at which it can be ascertained reliably that the appropriate strain has been generated, and in sufficient quantity to deliver a therapeutic product *in vivo* would be jeopardized in view of the then unpredictable nature of cells having undergone the

onset of senescence. Otherwise stated, the onset of senescence would have been seen as a serious barrier to the provision of a cell strain suitable for the delivery of a therapeutic product *in vivo*. Therefore, the skilled person would not have had a reasonable expectation of success while trying to implement a process as claimed.

In claim 1 of auxiliary request 9, the amount of time for cell expansion was further specified to be that necessary for at least 20 doublings. When added to the time necessary to accomplish steps (a) to (c), this explicit time requirement took the cell strain well beyond the point at which the onset of senescence will have occurred.

X. The respondents' arguments in writing and during oral proceedings insofar as they are relevant to the present decision may be summarized as follows:

***Articles 123(2)(3) and 84 EPC***

*Claim 1; auxiliary request 8*

- The application as filed provided isolated examples of transfecting human fibroblasts with a gene encoding a therapeutic product and expressing said product in said cells but a generic disclosure relating to human cells in general was missing.
Furthermore, step (c) of the claimed method was not described in the application as filed and could not be implicitly derived from the fact that the cells were human cells i.e. from the fact that they would not immortalize while cultured *in vitro*. Indeed, the

eventuality that the biopsy providing the primary cells also contained tumorigenic cells i.e. that some cells would be immortalized from the start could not be discarded. Such cells would be transfected just as non-immortalized cells were. Step (c) was thus compulsory to discard immortalized (tumorigenic) transfected human cells, which step was not disclosed in the application as filed.

For both these reasons, the requirements of Article 123(2) EPC were not fulfilled.

- The claimed process found no support in the description as required under Article 84 EPC since the examples concerned with the delivery of the therapeutic product were carried out in mice and not in humans and, thus, there was no evidence that the cell strain would deliver a therapeutic product *in vivo.*

*Claim 1; auxiliary request 9*

The appellant had failed to point out a basis in the application as filed for the feature that the cells should be maintained in culture for at least twenty cell doublings. The closest process to that claimed was found in originally filed claim 43 referring to claim 41. Yet, even then, the originally claimed process was one of producing a clonal cell strain (rather than a mixture of transfected cells) starting from secondary cells (rather than from primary or secondary cells). The other alleged basis on page 104 of the application as filed concerned mammalian epithelial cells and did not provide for the claimed generalisation.

*Claim 1; auxiliary requests 10 or 11*

In accordance with the case law (T 279/93 of 12 December 1996), a use claim had to be construed as a process claim. Claim 1 of both requests thus related to a process for making a vehicle or delivery system comprising using a cell strain comprising transfected cells. This claim was not one of the granted claims and, thus the requirements of Article 123(3) EPC were not fulfilled.

***Article 54 EPC; novelty***

*Claim 1 of auxiliary requests 8 and 9*

Document (12) was detrimental to the novelty of claim 1 as it disclosed on pages 12 and 13 the transfection of normal human diploid skin fibroblasts with a plasmid vector comprising the gene encoding dihydrofolate reductase (DHFR) as well as the expansion of the transfected cells. The fact that the DHFR gene was a gene encoding a therapeutic product was known from document (77), page 273 which taught that "protection of normal marrow from methotrexate toxicity might allow higher doses of methotrexate in chemotherapy for cancer".

***Article 56 EPC; inventive step***

*Claim 1 of auxiliary requests 8 and 9*

- The closest prior art was document (2) as it disclosed transfection of primary human skin

fibroblasts with a plasmid comprising a selective marker (CAT) by electroporation. The same steps were disclosed as steps (a) to (c) in claim 1 of auxiliary requests 8 or 9 and the cell strain was expanded for three weeks after transfection i.e. for about 35 doublings.
The difference with the claimed process was that the latter was directed to transfection with a DNA encoding a therapeutic product.

Starting from document (2), the problem to be solved could be defined as devising an alternative implementation of the transfection method.

The proposed solution was to transfect the human cells with a gene encoding a "therapeutic product". As the nature of the transfected gene would not be expected to affect the transfection process, the above mentioned difference did not impart inventive step.

As for the appellant's argument that inventive step resulted from the fact that for the delivery of a therapeutic product *in vivo*, an important number of cells had to be available, i.e. from the fact that the necessary number of *in vitro* doublings would be so high that it would be perceived by the skilled person as incompatible with obtaining cells which would still be normally metabolically active (i.e. not senescent), it was based on the false premise that the feature "for delivering a therapeutic product in vivo" necessarily brought with it a limitation to a high number of available cells. This was not correct as it could readily be understood from the patent itself (page 12, lines 5 to 8).

Even if step (c) of the claimed process was carried out for at least 20 doublings (claim 1 of auxiliary request 9), this did not mean that in the overall time necessary for carrying out (a) to (c), the cells would necessarily have become senescent. It would depend on the type of human cells which were used as well as on the origin of the primary cells. Thus, inventive step could not be justified on the basis that the skilled person would have refrained from using a population of senescent cells for delivery of a therapeutic product *in vivo*.

XI. The appellant requested in writing that a decision be taken on the written material in the case, that the decision under appeal be set aside and the patent be maintained on the basis, in order of preference, of the main request or auxiliary requests 1 to 11, all filed on 15 October 2004.

The respondents requested that the appeal be dismissed.

## Reasons for the Decision

*Main request and auxiliary requests 1 to 3; claim 15*
*Articles 123(2) EPC and 84 EPC*

1. Claim 15 of these requests corresponds to originally filed claim 65 (see Sections I and VII, *supra*). It differs therefrom in particular:

– in step (a), by the fact that the DNA construct may comprise a DNA encoding a therapeutic product and,

– in step (b), by the fact that the transfected cells express an endogenous gene at a higher level than occurs in the corresponding non-transfected cells because of the presence of the DNA construct.

2. In its preliminary communication, the board expressed the opinion that the claim wording was unclear because step (a) did not mention the presence in the DNA construct of a DNA which would be causative of an elevated level of endogenous gene expression when inserted in the genome of the transfected cells (cf. step (b)). To this, the appellant answered that the skilled person would have no difficulties in understanding that this DNA and the DNA encoding the therapeutic product were not the same part of the DNA construct. This may well be but in that case it remains that step (a) fails to mention an essential part of the DNA construct, i.e. the "regulatory" element. The board, thus, confirms its opinion that the claim is unclear (Article 84 EPC). Furthermore, accepting the appellant's argument implies that DNA constructs are comprised within the claim, which carry at the same time a gene encoding a therapeutic product, a DNA acting on the expression of an endogenous gene and homologous DNA sequences. The appellant did not point out to any basis in the application as filed for such constructs, nor was the board able to find any. For this reason, the claim fails to fulfil the requirements of Article 123(2) EPC.

3. As claim 15 is present in the main request and auxiliary requests 1 to 3, said requests are all refused for failing to fulfil the requirements of Article 123(2) EPC and/or Article 84 EPC.

*Auxiliary requests 4 to 7; claim 15*
*Article 123(2) EPC*

4. Claim 15 of the auxiliary requests 4 to 7 corresponds to originally filed claim 78 (see Sections I and VII, *supra*). Although not identically worded, step (a) (1) of the process of claim 78 comprises the same DNA sequences as step (a) of the process of claim 15. However, the process of claim 78 does not comprise a step such as step (b) of claim 15 insofar as over-expression of an endogenous gene is concerned. In fact, the board was unable to find a disclosure in the application as filed as a whole, of the sequences (a)(i) and (a)(iii) of claim 15 in relation to the over-expression of an endogenous gene, i.e. was unable to find a basis for the combination of steps (a) and (b). The appellant did not point out where such a basis could be found, simply arguing that the sequences listed in step (a) would be expected to lead to an increase in gene expression, which, in the board's judgment, may be true for some of them under specific circumstances. However, pursuant to Article 123(2) EPC, the question is not whether or not the generically claimed sequences may sometimes lead to an increase in endogenous gene expression but rather whether or not they have originally been disclosed as leading to an increase in gene expression. As already above mentioned, it is the board's opinion that they have not and, therefore, it is concluded that there is no basis in

the application as filed for the process of claim 15 comprising steps (a) and (b).

5. The auxiliary requests 4 to 7 are rejected for failing to fulfil the requirements of Article 123(2) EPC.

*Auxiliary request 8; claim 1*
*Articles 123(2)(3) and 84 EPC*

6. The application as filed discloses on pages 12 and 13 the production of transfected primary and secondary cells expressing an exogenous DNA encoding a therapeutic product, as well as the expansion of said transfected cells. Their use "to provide a variety of products to an individual" is mentioned, for example, on page 15. Examples are shown of the production of EPO or human growth hormone by transfected human primary fibroblasts and human mammary epithelial cells respectively (examples 15 and 20). The board is, thus, satisfied that the application as filed discloses a process for producing a cell strain starting from human primary or secondary cells.

7. Step (c) of claim 1 is not disclosed *expressis verbis* in the application as filed. Yet, since human cells do not immortalize *in vitro*, it is an intrinsic characteristic of the human transfected cells that they will be non-immortalized and, thus, step (c) is *de facto* accomplished by isolating any human cell strain from the transfected cells. That, as argued by respondent V, the primary or secondary cells used for transfection **may in some instances** comprise some tumorigenic (immortalized) cells which **may** become transfected and thereafter, **may** be retained for

expansion appears to be such an exceptional circumstance that it can be ignored as *de minimis*, with the consequence that step (c) need not be explicitly disclosed in the application as filed for said application to give a proper basis to the whole process.

8. For these reasons, the Board concludes that claim 1 of this request fulfils the requirements of Article 123(2) EPC.

9. Claim 1 corresponds to granted claim 6, point (b) when dependent on granted claim 1. The requirements of Article 123(3) EPC are fulfilled.

10. Lack of support in the description for the delivery of a therapeutic product *in vivo* (i.e. to humans) was argued to render the claim deficient pursuant to Article 84 EPC in the event that the feature "for delivering a therapeutic product *in vivo*" was considered an essential feature of the claimed process and so, because no such delivery had been exemplified. In view of the findings of lack of inventive step (see point 17 below) this objection of lack of support need not be taken into account.

*Article 54 EPC; novelty*

11. Document (12) was argued to be detrimental to novelty. It is concerned with providing a process for producing mammalian proteins in cell cultures. As a first step in this process, normal human diploid skin fibroblasts are transfected with a plasmid carrying the DHFR gene, a DNA fragment encoding part of the human tpA gene and the hygromycin B phosphotransferase gene (hph) as a

resistance marker (pages 12 and 13). Expression of this last gene enables the selection of the transfectants on a medium comprising hygromycin B. These transfectants are then checked by PCR for having inserted the DHFR gene and tpA DNA in the tpA region of the chromosome (pages 13 and 14). At no point in this first step of the method is the DHFR gene expressed. This last gene is only made use of in the second step of the process which is not relevant to the process of claim 1 as it does not take place in the human cells but in Chinese hamster ovary cells (which have been transformed by the human recombinant chromosome).

12. In the board's judgment, the skilled person would never understand the first step of the process, firstly as an independent process and secondly, as a process for making transfected human cells for delivering a therapeutic product *in vivo*, taking into account that the only gene which is expressed (hph) is not encoding a therapeutic product and that the unexpressed DHFR gene is not at any point identified as a gene encoding a therapeutic product, nor is it used in this quality.

13. Respondent II argued that the DHFR gene (the expression of which causes resistance to methotrexate) was a gene encoding a therapeutic product and, that therefore the process described in document (12) was a process for producing a human cell strain for delivery of a therapeutic product *in vivo* albeit not explicitly described as such. This argument was based on the suggestion in document (77) that "Protection of normal marrow from methotrexate toxicity might allow higher doses of methothrexate in chemotherapy for cancer", which suggestion originated from data showing that

primary and secondary recipients marrow cells of mice which were resistant to methothrexate were partially protected from methotrexate toxicity (page 273, left-hand column). There is, however, no evidence on file that the DHFR gene was ever used in the context of a therapy. This, of course, takes away the relevance of respondent II' argument.

14. For the reasons given in point 12 *supra*, the board concludes that document (12) does not affect the novelty of claim 1. As there are no other documents on file which would be detrimental to novelty, it is concluded that the subject-matter of claim 1 fulfils the requirements of Article 54 EPC.

*Article 56 EPC; inventive step*

15. The appellant identified the technical effect which imparted inventive step to the subject-matter of claim 1 as being that the cells obtained by the claimed *in vitro* process may **subsequently** be used in a **further** process for delivering a therapeutic product *in vivo*. In his view, the feature "for delivering a therapeutic product *in vivo*" provided the **absolute** requirement for the claimed *in vitro* process that it be carried out for a number of cell doublings suitable to obtain the amount of cells necessary for said delivery. The skilled person would consider this feature to be incompatible with the cells retaining a normal metabolism and, therefore, would not think that the cells could be "fit for delivery". This meant that he/she would not have thought it worthwhile to start developing the claimed process.

16. Before starting the problem-solution approach to inventive step, it is thus necessary to assess whether the absolute requirement argued for is, in fact, a true limitation of the claimed process because, if it is not, it cannot be taken into account in the reasoning on inventive step.

17. The board notices that:

- it is not an explicit requirement of present claim 1 that the cells should be grown *in vitro* for a minimum number of cell doublings;

- the only granted claim specifying a minimal length of time of *in vitro* culturing i.e. granted claim 13 is a dependent claim. This indicates that the minimal number of cell doublings has to be an optional feature.

- there is no requirement in the application as filed with regard to a minimal time of *in vitro* culturing. In fact, it is mentioned on page 32: "*The number of required cells in a transfected clonal or heterogeneous cell strain is variable and depends on a variety of factors, which include but are not limited to, the use of the transfected cells, the functional level of the exogenous DNA in the transfected cells , the site of implantation of the transfected cells (...), and the age, surface area, and clinical condition of the patient*."

Thus, it is not a *sine qua non* characteristic of the claimed process that the cells must be grown to a

minimum number of doublings which would be thought incompatible with them retaining their normal metabolic capacities. Otherwise stated, the feature "for delivering a therapeutic product *in vivo*" does not bring with it the alleged implicit limitation of the claimed *in vitro* process in terms of a minimum amount of cell doublings which, according to the appellant, would justify an acknowledgement of inventive step. Inventive step must be assessed on the merits of the process as defined by the four steps which are explicitly mentioned.

18. The closest prior art is document (2) which discloses the transfection of primary human skin fibroblasts by electroporation. Normal human skin fibroblasts are provided from skin biopsies and transfected with a DNA encoding chloramphenicol acetyl transferase (CAT) as a selective marker. The transfectants are left to grow on G418 selective medium for approximately four weeks and are then transferred to a non-selective medium i.e. they are still alive and expressing the transfected selective gene after four weeks.

19. Starting from the closest prior art, the problem to be solved can be defined as devising an alternative implementation of the process of document (2).

20. The solution is to carry out said process with a gene encoding a therapeutic product.

21. This solution can be put into practice as a matter of routine. There is no evidence that the nature of the transfected gene would have a negative impact on obtaining transfectants and growing them *in vitro*.

Therefore, the process of claim 1 is derivable in a straightforward manner from the teachings of document (2). The subject-matter of claim 1 is not inventive.

22. The auxiliary request 8 is rejected for failing to fulfil the requirements of Article 56 EPC.

*Auxiliary request 9; claim 1*
*Article 123(2) EPC*

23. This claim only differs from claim 1 of auxiliary request 8 in that step (d) involves expanding the cell strain for a sufficient time for at least 20 doublings. At oral proceedings, respondents III objected that there was no basis in the application as filed for this feature. Claim 1 is, in fact, granted claim 13. At the opposition stage, claim 13 was never objected to for failing to comply with the requirements of Article 123(2) EPC although Article 100(c) EPC was a ground of opposition. It may be possible to consider the late-submitted objection as a new argument which, in accordance with the Enlarged Board decision G 4/92 (OJ EPO 1994,149), could, in principle, be taken into account in the absence of the appellant. Yet, the board found it more expedient at oral proceedings to deal directly with inventive step and in view of the conclusion which was then reached (point 30 below), the objection under Article 123(2) EPC needed not be treated.

*Article 56 EPC*

24. Inventive step will now be assessed taking into account that the feature of the transfected cells (i.e. for

delivering a therapeutic product *in vivo*) which was argued by the appellant (point 15 *supra*) to confer to the claimed process the absolute requirement that it be carried out for a length of time allegedly incompatible with a normal metabolism has now been rendered explicit by inserting into step (d) a lower limit of time expansion of at least 20 cell doublings.

25. The closest prior art is document (2) which, as mentioned in point 18 above, discloses a process for producing transfected human fibroblasts. These cells express the transfected CAT gene for at least three weeks (approximately four weeks; page 169) after they have been transfected, this length of time being enough for 35 cell doublings (as understood by the board, from respondent III' submissions at oral proceedings).

26. The problem to be solved can be defined in the same manner as for claim 1 of auxiliary request 8 ie as devising an alternative implementation of the process described in the closest prior art.

27. The solution provided is to carry out the process with a gene encoding a therapeutic product for a time sufficient for more than 20 cell doublings, as this amount of doublings is the minimum amount to be performed during the expansion step, step (d). In fact, the parties appear to be in agreement that it will take at least 31 doublings but most likely from 34 to 37 doublings, for the claimed process to be completed (steps (a)-(d)).

28. At the priority date, the onset of senescence had been reported to be "at around 23 doublings" (eg. document

DB4 as annex to document (76)), alternatively, as "well before 35 doublings" (document DK3 as annex to document (98)). It was also known that senescence was accompanied by a great number of metabolic changes in the senescent cells (document DB2 as annex to document (76)). For the board, taking together these data and the fact that 34 to 37 doublings were necessary for the claimed process to be completed, implies for the present claimed subject-matter that the cells must indeed have gone past the onset of senescence, i.e. that in the cell population not all cells have retained their full metabolic capacities.

29. The question which is, thus, to be answered is whether as argued by the appellant, the skilled person aware of these data would have refrained from implementing the claimed process because he/she would not have seen a reasonable expectation of success of obtaining cells which would still be healthy enough to express the transfected "therapeutic" gene after the required time of *in vitro* culturing.

30. In the board's judgment, this question must be answered by the negative for the simple reason that document (2) provides evidence that the transfected cells still express the transfected CAT gene after 35 cell doublings as can be deduced from their ability to grow on selective medium. Moreover, as already mentioned in point 21 *supra*, the nature of the transfected gene would not be expected to be relevant. Hence, while aware of the changes in the natural metabolism which occur from the onset of senescence, the skilled person would nonetheless be very strongly encouraged to attempt to develop the claimed process, by the results

already achieved in the most pertinent prior art. As putting the process into practice could be done in a routine manner on the basis of the teachings of document (2), the conclusion is reached that the subject-matter of claim 1 is not inventive.

31. For these reasons, auxiliary request 9 is rejected for failing to fulfil the requirements of Article 56 EPC.

*Auxiliary requests 10 and 11; claim 1*
*Article 123(3)*

32. Claim 1 of both these requests relates to the use of a cell strain in the manufacture of a vehicle or delivery system. There is no counter-part to this claim in the granted claims. The one which most closely corresponds is granted claim 14 which relates to a barrier device containing a cell strain (see Sections I and VII, *supra*). Compared to this granted claim, claim 1 manifestly extends the scope of protection as a barrier device is just one form of delivery system, which is itself a much broader concept. Thus, the requirements of Article 123(3) EPC are not fulfilled.

33. Auxiliary requests 10 and 11 are rejected for failing to comply with the requirements of Article 123(3) EPC.

## Order

**For these reasons it is decided that:**

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani