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Datasheet for the decision of 21 June 2012

Case Number: T 1156/09 - 3.3.08

Application Number: 05734077.0

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Language of the proceedings: EN

Title of invention:
Method of cell transdifferentiation

Applicants:
KANEKA CORPORATION
Satomura, Kazuhito

Headword:
Osteoblast-Neuronal cell transdifferentiation/KANEKA

Relevant legal provisions:
EPC Art. 56
RPBA Art. 12(4), 13(1)

Keyword:
"Admissibility of the Main Request and of Auxiliary Requests 1 and 2 (no)"
"Admissibility of Auxiliary Request 3 (yes)"
"Auxiliary Request 3 - inventive step (no)"

Decisions cited:
T 0390/07, T 2196/09, T 0922/08

Catchword:
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Case Number: T 1156/09 **-** 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 21 June 2012

Appellants:

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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted on 9 January 2009 refusing European patent application No. 05734077.0 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: T. J. H. Mennessier
Members: P. Julià
J. Geschwind

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division dated 9 January 2009 whereby European patent application No. 05 734 077.0 (published as EP 1 775 340) was refused for lack of inventive step (Article 100(a) EPC, Article 56 EPC). Basis for the refusal was a main and sole request filed on 15 September 2008 at the oral proceedings before the examining division. The examining division did not admit the reintroduction into the examining proceedings of a first auxiliary request originally filed on 2 September 2008 and withdrawn at the beginning of the oral proceedings.

II. The applicants (appellants) filed a notice of appeal and paid the appeal fee. On 13 May 2009, they filed a statement setting out their grounds of appeal with an amended set of claims 1 to 5, their sole claim request, different from that underlying the appealed decision.

III. The examining division did not rectify the contested decision and referred the appeal to the board (Article 109 EPC).

IV. With the summons to oral proceedings, the board sent a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) informing the appellants of its preliminary, non-binding opinion on substantive matters.

V. On 16 May 2012, the appellants replied to the board's communication and filed a Main Request, identical to that filed with their grounds of appeal, and Auxiliary

Requests 1 to 3. Auxiliary Request 3 was identical to the request underlying the decision under appeal.

VI. Claim 1 of the Main Request, which was identical to the sole request filed with appellants' grounds of appeal, read as follows:

"1. A method for transdifferentiating mature osteoblast cells into nerve-cell-like cells which express the nerve cell markers neurofilament and glial fibrillary acidic protein (GFAP), comprising changing culture medium for the osteoblast to medium containing bFGF, FGF-8, EGF and BDNF."

Claims 2 and 3 were embodiments of claim 1 and referred to a selection of markers characterizing mature osteoblast cells (claim 2) and compounds staining mature osteoblast cells (claim 3). Claims 4 and 5 were also directed to embodiments of the method of claim 1 with the culture medium for osteoblasts containing particular compounds (claim 4) and with a given type of specific osteoblast cells (MC3T3-E1) in a culture medium identical to that of claim 4 (claim 5).

VII. In Auxiliary Requests 1 and 2, claims 2 and 3 of the Main Request had been deleted. Claim 1 of Auxiliary Request 1 combined claims 1 and 4 of the Main Request. In Auxiliary Request 2, claim 4 of the Main Request had been deleted and claim 1, the sole claim of this request, combined claims 1 and 5 of the Main Request.

VIII. Claim 1 of Auxiliary Request 3, which was identical to the request underlying the decision under appeal, read as follows:

"1. A method for morphologically changing MC3T3-E1 osteoblast cells into nerve-cell-like cells which express the nerve cell markers neurofilament and glial fibrillary acidic protein (GFAP) by changing culture conditions for the MC3T3-E1 cells, wherein culture conditions suitable for culturing the MC3T3-E1 cells are changed to culture conditions suitable for culturing the nerve-cell-like cells, which comprises exchanging the medium from a medium containing ß-glycerophosphate, ascorbic acid and Glutamax to a medium containing bFGF, FGF-8, EGF and BDNF."

IX. Oral proceedings were held on 21 June 2012, at which the appellants maintained their previous requests and further requested the board to admit two further documents into the appeal proceedings. This latter request was withdrawn at the end of the oral proceedings.

X. The following documents are cited in this decision:

D1: J. Kohyama et al., Differentiation, 2001, Vol. 68, pages 235 to 244;

D2: K. Jin and D.A. Greenberg, Experimental Neurology, 2003, Vol. 183, pages 255 to 257;

D6: P.C. Schiller et al., J. Biol. Chem., 2001, Vol. 276, No. 17, pages 14133 to 14138;

D8: WO 01/88104 (publication date: 22 November 2001).

XI. The arguments of the appellants, insofar as they are relevant to the present decision, may be summarized as follows:

Admissibility of the Main Request and of Auxiliary Requests 1 and 2

Claim 1 of the Main Request was almost identical to claim 1 of the request underlying the appealed decision. The terms "*transdifferentiating*" and "*morphological changing*" were substantially identical and both terms were used to express the conversion of one type of differentiated cells into another type. Although the expression "*mature osteoblast cells*" in claim 1 of the Main Request was broader than the reference to "*MC3T3-E1*" cells in the request before the examining division, these specific cells were typical, well-known examples of mature osteoblasts. Thus, except for a generalization of some features, the essential features and the subject-matter of the claims before the examining division were maintained in the Main Request and all arguments put forward in the first instance proceedings for the request underlying the appealed decision applied similarly to the Main Request. The Main Request did not make a fresh case but was only somehow broader in comparison to the claims considered by the first instance. Claims 2 and 3 of the Main Request were dependent on claim 1 and their subject-matter was clearly supported by the description.

Auxiliary Requests 1 and 2 were filed in direct reply to the board's concerns expressed in its communication pursuant to Article 15(1) RPBA regarding the admissibility of the Main Request. Claims 2 and 3 of

the Main Request had been deleted in these Auxiliary Requests which, except for a justified generalization of some features, intended to be as similar as possible to the request underlying the appealed decision.

Admissibility of Auxiliary Request 3

Auxiliary Request 3, identical to the request underlying the appealed decision, was filed in direct reply to the objections raised in the board's communication concerning the admissibility of other requests that broadened the subject-matter upon which the examining division had decided in the first instance proceedings. Although Auxiliary Request 3 was not filed together with the statement of grounds of appeal, it did not represent a change of subject-matter or make a fresh case in comparison to the grounds of appeal because it was only a limitation to the subject-matter already present in the request filed with the grounds of appeal, i.e. it was only a preferred embodiment of the broader subject-matter filed with the grounds of appeal. Thus, all arguments put forward therein also applied to the subject-matter of Auxiliary Request 3.

Auxiliary Request 3
Article 56 EPC

Claim 1 defined a method characterized by two steps. The first step was the culture of (pre)osteoblastic MC3T3-E1 cells in a specific osteoblast medium. As a result thereof, these cells differentiated into mature osteoblast cells. Regardless of how far the differentiation was carried out, i.e. whether the

MC3T3-E1 cells were completely (terminally) differentiated mature osteoblast cells or only mature osteoblast cells, this first step was essential to the claimed method. Both the type of cells and the osteoblast medium used to culture them provided an advantageous effect for starting the second step, namely the transdifferentiation of these osteoblast cells into nerve-cell-like cells, by exchanging the osteoblastic medium for a neuronal medium as defined in claim 1.

Document D2, the closest prior art, referred to neuronal transdifferentiation studies that used, as a starting material, bone marrow stem cells (BMS), in particular marrow stromal cells (MSC), and a culture medium with several factors. Although other neuronal transdifferentiation studies that used heterologous skin, adipocytes and fibroblasts cells were also cited, there was no mention of (pre)osteoblast cells let alone of the MC3T3-E1 cells used in the method of claim 1. Moreover, there was no disclosure in document D2 of a method comprising two different steps but only of a method consisting, exclusively, of a sole step, namely the culture of the source cells in a neuronal transdifferentiation medium.

Starting from the closest prior art, the technical problem to be solved was the provision of an improved, easier method for transdifferentiating differentiated cells into nerve-cell-like cells. The method of claim 1 solved this problem. In contrast to the stem cells cited in document D2, which were difficult to culture and not well-characterized, the MC3T3-E1 cells were standard, well-characterized, easily available and,

when using the osteoblastic medium defined in claim 1, easy to culture, thereby providing an advantageous culture for starting the second step of the method of claim 1. In this second step, the mature osteoblast cells were easily transdifferentiated into nerve-cell-like cells by using the neuronal transdifferentiation medium defined in claim 1, as opposed to the cumbersome and difficult neuronal transdifferentiation known in the prior art.

There was no indication in document D2 that could have led a skilled person to use (pre)osteoblast cells, let alone MC3T3-E1 cells. The use of these cells could not be derived from document D2 in an obvious manner. Likewise, it was not obvious to derive a method with two steps as that of claim 1 from a method with a single step as that disclosed in document D2. The deficiencies of document D2 were not remedied by other prior art documents on file, such as documents D6 and D1.

Document D6 did not disclose a two-step method and was concerned only with transdifferentiation of MC3T3-E1 (pre)osteoblast cells into adipocyte cells but not into neuronal cells. This transdifferentiation was different from that of claim 1, since both osteoblasts and adipocytes were known to have a same mesoderm origin as opposed to neuronal cells that had an endoderm origin. There was nothing in the combination of documents D2 and D6 that would have led a skilled person to the method of claim 1 in an obvious manner.

Document D1, which did not even refer to the MC3T3-E1 cells used in the method of claim 1, disclosed a

neuronal transdifferentiation method completely different from that of claim 1, since it essentially relied on demethylating agents or on the genetic transformation of mature osteoblast cells for achieving neuronal transdifferentiation. Nothing in the combination of documents D2 and D1 would have led a skilled person to the method of claim 1 in an obvious manner.

Indeed, nothing in the prior art would have led a skilled person to the method of claim 1 that combined two different steps, namely a first step with a specific type of cells and a specific medium which resulted in an advantageous expanded differentiation of osteoblast cells for starting the second step in which these cells were neuronal transdifferentiated by using a specific culture medium. These two specific and advantageous steps were not derivable from the prior art in an obvious manner, let alone their combination and the advantageous results obtained therefrom.

XII. The appellants (applicants) request the board to set aside the decision under appeal and to grant a patent on the basis of the Main Request or, in the alternative, on the basis of one of the first to third Auxiliary Requests filed with letter of 16 May 2012.

Reasons for the Decision

Admissibility of the Main Request

1. According to the case law of the Boards of Appeal, the purpose of appeal proceedings is to give a judicial

decision upon the correctness of a separate earlier decision taken by a department of first instance, i.e. to review the appealed decision in order to give the losing party the possibility of challenging this decision on its merits. It is not the purpose of appeal proceedings to give an appellant a further opportunity to create a fresh case by, for instance, recasting the claims as it sees fit and to have all requests admitted into the proceedings (cf. "Case Law of the Boards of Appeal of the EPO", 6th edition 2010, VII.E.1 and VII.E.16.1, pages 821 and 888). In line with this case law, Article 12(4) RPBA states that it is within the power of the board to hold inadmissible facts, evidence or requests which could have been presented or were not admitted in the first instance proceedings.

2. Claims 2 and 3 of the Main Request relate to subject-matter that was never present in the first instance proceedings. These claims are entirely new in the proceedings and do not intend to address any of the objections raised by the examining division in the appealed decision. No reasons have been given by the appellants for their introduction at this stage of the proceedings. Thus, claims 2 and 3 of the Main Request constitute by themselves a fresh case on appeal.

3. In these circumstances, the board, in the exercise of its discretion pursuant to Article 12(4) RPBA, decides not to admit the Main Request into the appeal proceedings.

Admissibility of Auxiliary Requests 1 and 2

4. According to the appellants, these Auxiliary Requests were filed in direct reply to the board's concerns regarding the admissibility of the Main Request - as expressed in the board's communication pursuant to Article 15(1) RPBA. In these Auxiliary Requests, claims 2 and 3 of the Main Request have been deleted and, thus, the concerns of the board on the admissibility of the Main Request do not apply anymore for these Auxiliary Requests (cf. Section XI, *supra*).

5. However, claim 1 of Auxiliary Requests 1 and 2 is directed, as claim 1 of the Main Request, to a method for "*transdifferentiating*" mature osteoblast cells and not to a method for "*morphologically changing*" these cells as in the request underlying the decision under appeal (cf. Sections VI and VIII, *supra*).

5.1 The term "*transdifferentiating*" was also present in other requests filed before the examining division and later withdrawn in the course of the examining proceedings. According to the "*Minutes of the oral proceedings before the examining division*" (hereinafter "*the Minutes*"), a Main Request objected under Article 123(2) EPC was replaced by a new Main Request directed to a method for "*transdifferentiating*" MC3T3-E1 osteoblast cells (cf. page 2, paragraph 9 and Annex I of the Minutes). According to the Minutes, the first examiner stated that the feature "*morphological change*" was still missing from that request and "*(a)fter agreeing that the claim should read a method for morphologically changing MC3T3-E1 osteoblast cells ...*" the discussion further continued (cf. page 2,

paragraphs 10 and 11 of the Minutes). As a result thereof, the applicants "*submitted a new request containing one claim with the necessary changes ...*", namely the request underlying the decision under appeal (cf. page 2, paragraph 12, page 3, paragraph 15 and Annex II of the Minutes). It is noted that the Minutes were not contested and that it has not been argued that the examining division committed a procedural violation.

6. In the light thereof, the board understands that, in the course of oral proceedings before the examining division, the applicants/appellants withdrew all their requests that were directed to a method for "*transdifferentiating*" mature osteoblast cells and that the sole request remaining on file, i.e. that underlying the decision under appeal, was directed to a method for "*morphologically changing*" these cells.

Accordingly, there is no reference in the decision under appeal to the term "*transdifferentiating*", neither to the applicants/appellants' arguments - in written or at the oral proceedings - put forward during the examination proceedings nor to the reasons for the examining division not to accept this term in the applicants/appellants' requests. Thus, as a consequence of the withdrawal of these previous requests, the board has been effectively deprived of the opportunity to examine the substantive merits of applicants/appellants' arguments and the reasons of the examining division for not accepting that term.

The introduction of the term "*transdifferentiating*" in appeal proceedings does not address, let alone overcome, any of the objections raised by the examining division

in the appealed decision but only reverts the claimed subject-matter to that examined at an earlier stage of the examination proceedings. This is not in line with the purpose of appeal proceedings as established in the case law of the Boards of Appeal (cf. point 1, *supra*, and, *inter alia*, decisions T 390/07 of 20 November 2008, points 1 to 3 of the Reasons, T 2196/09 of 1 December 2011, points 4 and 5 of the Reasons and T 922/08 of 13 October 2011, point 2.1 of the Reasons).

7. Therefore, the board, exercising its discretion pursuant to Article 12(4) RPBA, decides not to admit Auxiliary Requests 1 and 2 into the appeal proceedings.

Admissibility of Auxiliary Request 3

8. Article 13(1) RPBA states that any amendment to a party's case after it has filed its grounds of appeal may be admitted and considered at the board's discretion. The discretion shall be exercised in view of *inter alia* the complexity of the new subject matter submitted, the current state of the proceedings and the need for procedural economy.

9. Auxiliary Request 3 has been filed, as Auxiliary Requests 1 and 2, in direct reply to the board's communication pursuant to Article 15(1) RPBA. These requests were filed within the time limit set out by the board for receipt of any written submissions in response to this communication. Auxiliary Request 3 is identical to the sole request underlying the decision under appeal and its subject-matter does not arise any new issues or objections other than those considered by the examining division in the appealed decision.

10. As stated by the appellants (cf. Section XI, *supra*), the subject-matter of Auxiliary Request 3 represents a preferred embodiment of the broader subject-matter of the sole claim request filed with the appellants' grounds of appeal (cf. Sections VI and VIII, *supra*). Thus, all arguments put forward by the appellants in their grounds of appeal were also similar for, and applied to, the subject-matter of Auxiliary Request 3.

11. For these reasons, the board, in the exercise of its discretion pursuant to Article 13(1) RPBA, decides to admit Auxiliary Request 3 into the appeal proceedings.

Auxiliary Request 3
Articles 123(2), 83, 84 and 54 EPC

12. In the decision under appeal, the examining division acknowledged that the request under consideration fulfilled the requirements of Articles 123(2), 83, 84 and 54 EPC (cf. page 3, points 1.1 to 1.3 of the decision under appeal). The refusal of the application by the examining division was exclusively based on the objections of lack of inventive step (Article 56 EPC) (cf. pages 3 to 6, point 1.4 of the decision under appeal). In view of the conclusions reached below as to the requirements of Article 56 EPC (*infra*), the board sees no necessity to examine these other Articles of the EPC and to decide thereon.

Article 56 EPC
The closest prior art, the technical problem and its solution

13. As stated by the appellants, the method of claim 1 is characterized by two steps. The first step comprises the culture of (pre)osteoblastic MC3T3-E1 cells in an osteoblast medium, and, in the second step, this medium is changed to a neuronal medium so as to morphologically change the MC3T3-E1 osteoblast cells into nerve-cell-like cells (cf. Section VIII, *supra*).

14. Document D2, identified as the closest prior art, reviews several studies of the prior art concerned with the transdifferentiation of non-neuronal to neuronal cells. According to this document, "*(m)ost work in this area has focused on bone marrow cells (BMC), especially the marrow stromal cells (MSC) ...*" which are cultured in a neuronal differentiation medium containing neuronal factors, such as brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), etc. (cf. page 255, left-hand column, second paragraph of document D2). Document D2 also refers to other neuronal transdifferentiation studies in which the starting material was not undifferentiated stem cells but more differentiated cells, such as cells isolated from the dermis of mouse skin, cells from mouse or human adipose tissue, isolated human fibroblast-like preadipocytes, etc. The culture of those cells in a medium with neuronal factors results in neuronal transdifferentiation and production of nerve-cell-like cells (cf. page 256, left-hand column, second and third paragraphs of document D2).

15. The appellants argue that, starting from this prior art, the technical problem to be solved is the provision of an improved, easier method for transdifferentiating, or morphologically changing, differentiated osteoblast

cells into nerve-cell-like cells (cf. Section XI, *supra*). However, apart from the use of the well-known, commercially available MC3T3-E1 cells - which in the board's view cannot contribute to inventive step, in the absence of any experimental data (*infra*), the board considers that the objective technical problem to be solved can only be seen in the provision of a mere alternative method for morphologically changing differentiated cells into nerve-cell-like cells (cf. page 4, third paragraph from the bottom of the decision under appeal). The method of claim 1, as shown by the Examples of the application, provides a solution to this technical problem.

Is the method of claim 1 obvious?

16. Since, as to the issue of inventive step, the appellants have argued that each step of the method of claim 1, as well as their specific combination, are important (cf. Section XI, *supra*), the first question to be asked is whether the first step of this method, namely the culture of (pre)osteoblastic MC3T3-E1 cells in an osteoblast medium for obtaining osteoblasts, provides an inventive contribution.

16.1 Contrary to appellants' assertions, there is no definition in claim 1 of the specific culture conditions for the (pre)osteoblastic MC3T3-E1 cells and of the particular osteoblast medium used in the first step of the method of this claim. Whereas several components of this medium are cited in claim 1 (ß-glycerophosphate, ascorbic acid and Glutamax), there is no indication of their concentration, the presence of other compounds, culture time and other conditions

of culture (for a comparison see, for instance, paragraphs [0046] to [0048] of the application). Moreover, osteoblast media were known in the art as shown in paragraph [0033], point (1) of the application. Indeed, document D6, concerned with transdifferentiation studies from (pre)osteoblast cells to adipocyte cells, discloses the culture of (pre)osteoblastic MC3T3-E1 cells in an osteogenic medium with compounds similar to those used in the first step of the method of claim 1 (cf. page 14134, left-hand column, Section entitled "Cell Cultures and Treatments" of document D6). It is also worth noting that MC3T3-E1 cells were well-known in the art and, as stated in the application, MC3T3-E1 "*... is a typical osteoblast cell line that is recognized and widely used throughout the world*" (cf. page 4, paragraph [0026] of the application).

16.2 The osteoblast medium used in the Examples of the application is disclosed therein as being only "*(a)n example of a composition of a medium for osteoblasts*" (cf. page 4, column 6, lines 14 and 15) and not associated with any technical advantage or effect, i.e. it is not identified as an essential feature of the claimed method. There is no evidence whatsoever on file showing that the particular osteoblast medium used in the Examples of the application, let alone the broader one indicated in claim 1, may provide an advantageous effect over other osteogenic media known in the prior art (cf. "Case Law", *supra*, I.D.9.9, page 221).

16.3 It follows from these considerations, that no inventive skill is required to select the well-known, commercially available **(pre)osteoblastic MC3T3E1 cells**

and culture them in an osteogenic medium, as that referred to in claim 1, for obtaining osteoblast cells. Thus, the first step of the method of claim 1 - by itself alone - does not provide an inventive contribution and, in the light of this prior art, it is considered to be obvious for a skilled person.

17. It is now to be assessed whether the second step of the method of claim 1, namely to morphologically change osteoblast cells into nerve-cell-like cells by culturing these osteoblast cells in a neuronal medium, requires an inventive contribution.

17.1 As stated in point 14 *supra*, document D2 refers to neuronal transdifferentiation studies in which neuronal media similar to the neuronal medium defined in claim 1 are used. As argued above for the first step of claim 1 (cf. point 16.1 *supra*), the board does not consider that in the second step of claim 1 the specific culture conditions of the MC3T3-E1 osteoblast cells are defined other than by the presence of the neuronal factors mentioned therein and which are disclosed in the application as being not associated to any technical advantage or effect (cf. paragraph [0033], point (2) of the application). These neuronal transdifferentiation studies referred to in document D2 report the use of differentiated cells, such as fibroblasts and adipocytes, as starting material. Adipocyte cells are known in the art to have the same mesoderm-derived origin as osteoblasts (cf. page 235, left-hand column, Abstract of document D1), both types of cells are known to be related and to show a high degree of plasticity - as stated in document D6 (cf. page 14133, left-hand column, Abstract of document D6), which discloses the

use of MC3T3-E1 cells in transdifferentiation studies from osteoblasts to adipocytes (cf. point 16.1 *supra*).

17.2 Likewise, although using other methods than that disclosed in the application, osteoblasts were employed as starting material in the neuronal transdifferentiation studies of document D1, showing thereby the production of ectoderm-derived cells (neurones) from mesoderm-derived cells (osteoblasts) (cf. point 18.1 *infra*). It is worth noting here that reference is made in document D1 to MC3T3-PA6 stromal cells (cf. page 240, right-hand column, second line from the bottom of document D1), a MC3T3 cell line having adipogenic potential and related to the MC3T3-E1 cells with osteoblast potential used in document D6.

17.3 In view of this prior art, the board considers that the selection of osteoblasts, in particular of **MC3T3-E1 osteoblasts**, as an alternative type of differentiated cells to those indicated in document D2 for carrying out neuronal transdifferentiation studies - or for morphologically changing osteoblast cells into nerve-cell-like cells - using the neuronal medium indicated in claim 1, does not require any inventive contribution from a skilled person. Thus, the second step of the method of claim 1 - by itself alone - does not provide an inventive contribution and, in the light of this prior art, it is considered to be obvious for a skilled person.

18. As a last point, it remains to be assessed whether the combination of the first and second steps of the method of claim 1 requires any inventive skill and/or provides

an unexpected advantageous effect, as argued by the appellants.

18.1 There is prior art on file, namely document D1, describing a method of neuronal transdifferentiation which comprises a first and a second step. The first step is the culture of a (KUSA/A1) cell line derived from bone marrow to induce osteogenesis by known differentiation protocols (cf. page 236, left-hand column, third paragraph from the bottom and page 237, right-hand column, second paragraph of document D1). In the second step, the resulting isolated osteoblasts are transdifferentiated into neuronal cells - albeit admittedly by different methods than that used in the application (cf. page 235, left-hand column, lines 11 to 14 of the Abstract and page 236, right-hand column, first and second paragraphs of document D1).

18.2 As stated above, neither the selection of (pre)osteoblastic MC3T3-E1 cells to obtain osteoblasts by culture in an osteoblast medium nor the use of MC3T3-E1 osteoblasts, as starting material for neuronal transdifferentiation in a neuronal medium, is considered to require an inventive contribution from a skilled person (cf. points 16.3 and 17.3 *supra*). In the light of the cited prior art, a straightforward combination of these two steps is considered to be, in itself, obvious and not to require an inventive contribution unless, as argued by the appellants (cf. Section XI, *supra*), this combination provides an unexpected, advantageous or surprising effect.

18.3 According to the established case law, if a surprising effect is advanced as an indication of the presence of

an inventive step, it needs to be supported by evidence or experimental data, such as comparative tests (cf. "Case Law", *supra*, I.D.9.9, page 221). In the present case, apart from the selection and advantageous use of the well-known, commercially available MC3T3-E1 cells (*supra*), there is no evidence on file showing any unexpected, advantageous or surprising effect. There is no comparison of a culture of (pre)osteoblastic MC3T3-E1 cells and other known (pre)osteoblast cells in both the osteoblast medium defined in claim 1 and in other known osteoblast media. Likewise, there is no comparison of the degree, yield, efficiency, etc. of the morphological change of MC3T3-E1 osteoblasts into nerve-cell-like cells and that of other known osteoblast cells in the neuronal medium indicated in claim 1 and/or in other known neuronal media. There is no evidence whatsoever on file showing that a combination of the first and the second steps of the method of claim 1 results in an unexpected, advantageous or surprising effect. In the absence of such evidence, appellants' argument cannot be followed by the board.

18.4 Thus, the board considers that, in the light of the prior art, in particular of documents D2 and D6 - with the knowledge of document D1, the combination of the first and second steps of the method of claim 1 is also obvious to a skilled person.

Reasonable expectation of success

19. According to the established case law, when a course of action or technical approach is considered to be obvious, it remains to be assessed whether the skilled

person would have carried it out with a reasonable expectation of success (cf. "Case Law", *supra*, I.D.6, page 177).

19.1 It is known in the art that the adjectives "*differentiated*" and "*transdifferentiated*" are relative terms since they refer to a continuous process starting from a source material of more or less undifferentiated or progenitor cells, such as (pluripotent embryonic) stem cells or as in the present case (pre)osteoblastic cells, which progresses toward a population of end-stage or terminally (trans)differentiated cells, in the present case, morphologically changed nerve-cell-like cells (see, for instance, page 6, lines 26 to 39 of document D8; cited here as representative of the common general knowledge).

19.2 Indeed, as already stated in point 16.1 *supra*, claim 1 does not indicate the specific culture conditions of the (pre)osteoblast MC3T3-E1 cells in the osteoblast medium indicated in that claim. Thus, the population of MC3T3-E1 osteoblast cells referred to in claim 1 may well comprise pre-mature, mature and/or terminally, completely mature MC3T3-E1 osteoblast cells, i.e. there is a certain ambiguity in claim 1 with regard to the actual degree of differentiation of the MC3T3-E1 osteoblast cells.

19.3 Important is, however, that document D6 shows that MC3T3-E1 osteoblast cells have a high degree of (trans)differentiation plasticity (cf. points 16.1 and 17.1 *supra*) and that in document D1 nerve-cell-like cells are obtained by neuronal transdifferentiation of osteoblasts (cf. point 17.2 *supra*). This is in line

with the disclosure of document D2 in which neuronal transdifferentiation and production of nerve-cell-like cells are reported for both undifferentiated and differentiated cells (cf. point 14 *supra*).

19.4 In view of this prior art, the board is convinced that, in the present case, a reasonable expectation of success was given. It is also noted that, neither in appeal proceedings nor in the first instance proceedings, reference has ever been made to the presence of particular technical difficulties encountered when carrying out the method of claim 1.

Conclusion

20. In the light of all the above considerations, the board sees no reason to deviate from the decision of the examining division as regards Article 56 EPC and thus, considers the subject-matter of Auxiliary Request 3 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 T. J. H. Mennessier