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D E C I S I O N
of 13 October 2005

Case Number: T 0907/04 - 3.3.08

Application Number: 95911917.3

Publication Number: 773995

IPC: C12N 15/12

Language of the proceedings: EN

Title of invention:
DNA encoding the vertebrate homolog of hedgehog, VHH-1, expressed by the notochord, and uses thereof

Applicant:
The Trustees of Columbia University in the City of New York

Opponent:
-

Headword:
Rat sonic hedgehog/COLUMBIA

Relevant legal provisions:
EPC Art. 123(2), 56, 83
EPC R. 27(1)(e)

Keyword:
"Main request, auxiliary requests I to IV - added subject-matter (yes)"
"Auxiliary request V - inventive step (no)"
"Auxiliary requests VI to VII - sufficiency of disclosure (no)"

Decisions cited:
T 0002/83, T 0019/90, T 0315/03

Catchword:
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Case Number: T 0907/04 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 13 October 2005

Appellant: The Trustees of Columbia University
in the City of New York
West 116th Street and Broadway
New York, NY 10027 (US)

Representative: Wachenfeld, Joachim, Dr.
VOSSIUS & PARTNER
Postfach 86 07 67
D-81634 München (DE)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 2 March 2004 refusing European application No. 95911917.3 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Rennie-Smith

Summary of Facts and Submissions

I. The applicant (appellant) lodged an appeal against the decision of the examining division dated 2 March 2004 whereby the European patent application No. 95 911 917.3, which originated from an international application published as WO 95/23223 (to be referred to in the present decision as the application as filed), was refused pursuant to Article 97(1) EPC. The decision under appeal was based on a main and auxiliary requests filed on 7 January 2004 which were found, respectively, to contravene Articles 56 and 83 EPC.

II. On 12 July 2004, the appellant filed a statement of grounds of appeal wherein the requests before the examining division were maintained. The examining division did not rectify its decision and remitted the appeal to the board of appeal under Article 109(2) EPC.

III. The board sent a communication on 6 June 2005 pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal (RPBA) indicating its preliminary, non-binding opinion.

IV. In reply to the board's communication, the appellant filed observations and a new main request and auxiliary requests I to IV by letter dated 13 September 2005.

V. Oral proceedings took place on 13 October 2005 during which the appellant filed auxiliary requests V to VII.

VI. Claim 1 of the **main request** (claims 1 to 25) read as follows:

"1. A nucleic acid which encodes a rat vhh-1 protein, which protein comprises continuous amino acids having the amino acid sequence set forth in SEQ ID NO: 2 and comprises continuous nucleotides having the nucleotide sequence set forth in SEQ ID NO: 1 beginning with the nucleotide at position 315 and ending with the nucleotide at position 1625."

Claims 2 to 5 were directed to further embodiments of claim 1. Claims 6 to 9 related to vectors comprising the nucleic acid sequence of claim 1 and claims 10 to 12 concerned host vector systems for the production of a protein comprising the vector of any one of claims 6 to 9. Claim 13 was directed to a method of producing a protein comprising the host vector system of claim 10 and claim 14 concerned a rat vhh-1 protein as defined in claim 1 or a carboxy-terminal diffusible fragment thereof. Claims 15 to 17 related to a transgenic non-human mammal comprising the nucleic acid of claim 1. Claim 18 concerned a pharmaceutical composition comprising the protein as defined in claim 14 or a carboxy-terminal diffusible fragment thereof. Claims 19 to 20 and 21 to 25 were directed, respectively, to *in vitro* methods and to the use of the protein and fragment thereof as defined in claim 14 for the preparation of pharmaceutical compositions for treating different conditions in a (human) subject.

VII. Claim 1 of **auxiliary request I** (claims 1 to 4) read as follows:

"1. Use of a rat vhh-1 protein comprising continuous amino acids having the amino acid sequence set forth in SEQ ID NO: 2 or a diffusible carboxy-terminal fragment

thereof generated by autoproteolysis in an amount effective to generate a motor neuron from an undifferentiated motor neuron precursor cell for the preparation of a pharmaceutical composition for treating an abnormality associated with a lack of one or more normally functioning motor neurons in a human subject."

Claims 2 and 4 read as claim 1, wherein however the pharmaceutical composition was prepared for treating a neurodegenerative disease (claim 2) or an acute nervous system injury (claim 4) of motor neurons in a human subject. Claim 3 was dependent on claim 2 and defined the neurodegenerative disease as being Amyotropic Lateral Sclerosis.

VIII. Claims 1 to 3 of **auxiliary request III** read as claims 2 to 4 of auxiliary request I.

IX. Claims of **auxiliary requests II** (claims 1 to 4) and **IV** (claims 1 to 3) read, respectively, as claims of auxiliary requests I and III except for the deletion of all references to the diffusible carboxy-terminal fragment generated by autoproteolysis.

X. The only claim of **auxiliary request V** read as follows:

"1. A pharmaceutical composition comprising a rat vhh-1 protein comprising continuous amino acids having the amino acid sequence set forth in SEQ ID NO: 2 and a pharmaceutically acceptable carrier."

XI. The only claim of **auxiliary request VI** read as follows:

"1. Use of a rat vhh-1 protein comprising continuous amino acids having the amino acid sequence set forth in SEQ ID NO: 2 in an amount effective to generate a motor neuron from an undifferentiated motor neuron precursor cell for the preparation of a pharmaceutical composition for treating a neurodegenerative disease of motor neurons in a subject."

XII. The only claim of **auxiliary request VII** read as claim 1 of auxiliary request VI, wherein the pharmaceutical composition was prepared however for treating an acute nervous system injury of motor neurons in a subject.

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

D1: R.D. Riddle et al., Cell, 1993, Vol. 75, pages 1401 to 1416

D2: Y. Echelard et al., Cell, 1993, Vol. 75, pages 1417 to 1430;

D6: Database NCBI, NCBI no.: X76290, Echelard, Y. et al., 18 January 1994;

Exhibit A: press release by B. Mason, Contra Costa Times, Berkeley, California, 15 November 2004;

Exhibit B: M. Pines, HHMI Bulletin, December 2002, pages 22 to 27.

XIV. The appellant's arguments, insofar as they are relevant to the present decision, may be summarised as follows:

Main request and auxiliary requests I to IV
Article 123(2) EPC

The application as filed disclosed purified vertebrate vhh-1 proteins, in particular mammalian vhh-1 proteins, wherein a preferred embodiment was a rat vhh-1 protein. The application also disclosed methods of inducing the differentiation of motor neurons in a subject comprising the administration to the subject of a purified vertebrate vhh-1, in particular a rat vhh-1 protein. On page 66 of the application as filed it was stated that the invention specifically related to the isolation of a cDNA clone encoding a rat vhh-1 protein as well as to the characterization *in vivo* and *in vitro* of this gene and of the encoded vhh-1 protein so as to elucidate the role of the vhh-1 protein in inducing the developmental differentiation of motor neurons in embryos. In the same paragraph the likely use of the vhh-1 protein in the treatment of degenerative disorders of the central nervous system, particularly for motor neuron degeneration, was also cited. Thus, the use of the rat vhh-1 protein in the treatment of these degenerative diseases was directly and unambiguously disclosed in the application as filed.

Moreover, the set of claims as filed contained a first claim directed to a pharmaceutical composition comprising a purified vertebrate vhh-1 (claim 41) and then further claims directed to pharmaceutical compositions comprising a mammalian vhh-1 (claim 42) and a human vhh-1 protein (claims 43 and 44). In the

light of the description, it was clearly understood that a preferred embodiment of the broader claims 41 and 42 was a rat vhh-1 protein. The pharmaceutical compositions of these broader claims were explicitly referred to in all the methods of treating a human subject too (claims 45 to 50). Thus, the method claims were not limited to the use of pharmaceutical compositions comprising a human vhh-1 protein only but they contemplated the use of pharmaceutical compositions comprising other vertebrate vhh-1 proteins, in particular the rat vhh-1 protein. Therefore, the treatment of a human subject with a rat vhh-1 protein was directly and unambiguously disclosed in the application as filed.

Auxiliary request V
Articles 123(2) and 83 EPC

There was a formal support in the application as filed for the claimed subject-matter since the application as filed disclosed pharmaceutical compositions comprising a rat vhh-1 protein. Indications were also given in the application as filed that allowed the skilled person to formulate standard pharmaceutical compositions. Thus, the requirements of Articles 123(2) and 83 EPC were fulfilled.

Article 56 EPC

Document D2, which was identified as the closest prior art in the decision under appeal, disclosed the Sonic hedgehog (Shh) protein from mouse. The sequences of the Shh proteins from Drosophila, mouse, chick and zebrafish were shown in Figure 1. The mouse Shh protein

shared 84% amino acid identity with the chick Shh protein, being 99% identical in their amino terminal half. Document D2 referred to the striking Shh sequence conservation among cross-species. However, according to the "could-would approach" of the established case law (T 2/83, OJ EPO 1984, 265), it was not sufficient for denying inventive step that the person skilled in the art **could** have done something but evidence had to be provided showing that the skilled person **would** actually have done it. Thus, the key question in the present case was whether there was a motivation for the skilled person to look for the Shh protein from rat.

Starting from this closest prior art, there was no reason that could motivate the skilled person to clone the gene encoding the rat Shh protein and isolate the rat Shh protein. Since the Shh protein from mouse was known, the mouse was available to the skilled person as a suitable animal model for further scientific research. The information that could be derived from a rat Shh protein and from the use of the rat as an alternative animal model was clearly of no interest. Other animal models closer to a human situation, such as chimpanzees, were certainly of more interest since their diseases and disorders were expected to be more similar to the human ones. In fact, whereas chicken, mouse and frog embryos were routinely used as animal models for studies on the development of the embryo, that was not the case for rat, which was barely mentioned in the prior art. Thus, the skilled person lacked any motivation to look for the gene encoding the rat Shh protein. Although document D2 referred to the presence of floor plate-inducing activity in rat floor plate and to the fact that this activity was consistent with Shh

expression, this reference was nothing more than a mere speculation without a reliable basis in the prior art.

Since it was not obvious to clone the gene encoding the rat Shh protein, the question as to whether there was a reasonable expectation of success did not apply. Nevertheless, it was important to consider that the method for cloning the mouse Shh gene in document D2 was different from the method used for cloning the rat Shh gene in the application and that nothing except the disclosure of the present application taught the skilled person that the Shh proteins from mouse and rat were extremely homologous. Moreover, the claimed subject-matter was not directed to a rat Shh protein alone but to a pharmaceutical composition comprising this rat Shh protein. The application thus not only provided a gene encoding the rat Shh protein but it went a step further disclosing the use of this rat Shh protein in a pharmaceutical composition for treating neurodegenerative diseases in murine models.

None of the cited prior art documents suggested, let alone disclosed, a therapeutic use for the vhh-1 protein. This prior art was concerned only with scientific research intended to elucidate the biological activity and the mechanisms of action of the vhh-1 protein. There were references to the necessity of performing more scientific studies to answer these basic questions but there was no suggestion of a therapeutic use for the vhh-1 protein nor a motivation to formulate a pharmaceutical composition comprising this vhh-1 protein. Such a pharmaceutical composition was thus not obvious from the prior art.

Auxiliary requests VI and VII
Article 123(2) EPC

There was a formal basis in the application as filed for a pharmaceutical composition comprising a rat vhh-1 protein. The delivery of pharmaceutical compositions to sites of vhh-1 protein action so as to regenerate or differentiate motor neurons from undifferentiated motor neuron precursor cells for the purpose of alleviating abnormalities associated with acute nervous system injuries or chronic neurodegenerative diseases was also disclosed in the application as filed. Moreover, references to the use of the vhh-1 protein for the treatment of degenerative disorders of the central nervous system, particularly motor neuron degeneration, were also found in the application as filed. Since rat vhh-1 protein was disclosed as a particular embodiment of the invention, the use of a rat vhh-1 protein for the treatment of these disorders and diseases was also contemplated in the application as filed.

Article 83 EPC

The application as filed disclosed the preparation of pharmaceutical compositions comprising a rat vhh-1 protein and the use of these pharmaceutical compositions for inducing undifferentiated motor neuron precursor cells to differentiate into motor neurons for treating neurodegenerative diseases and acute nervous system injury of motor neurons. These therapeutic uses were not speculative but supported by experimental evidence provided in the examples of the application. These studies performed using neural plate explants clearly implicated the vhh-1 protein in the induction

of motor neurons and demonstrated that the vhh-1 protein initiated the differentiation of motor neurons in the neural tube of vertebrate embryos.

References were made in the application to standard methods of preparing pharmaceutical compositions for suitable administration and adequate therapeutic concentrations for achieving the desired therapeutic benefit were also referred to in the application. No particular difficulties or technical problems could arise in the preparation of these compositions. The skilled person was also in a position to determine the appropriate effective amount of vhh-1 protein with methods available in the prior art and nothing more than routine experimentation as shown by the application itself.

The possible problems referred to in the fourth series of experiments shown by the application as filed were only the result of a very specific experimental set up, i.e. the missexpression of the vhh-1 protein at ectopic locations. They were not representative for normal conditions, such as the ones described in the other series of experiments which demonstrated the production of motor neurons in a straightforward manner.

Post-published evidence demonstrated that the general teaching of the application was correct. The insertion of a rat vhh-1 gene into a harmless virus and then the injection into a rat's brain resulted in the delivery of the rat vhh-1 gene to brain stem cells stimulating them to divide three times faster than normal and in turn tripling the production of new neurons (Exhibit A). Exhibit B also mentioned the role of the vhh-1 protein

for inducing the differentiation of spinal progenitor cells into motor neurons. The application itself already referred to *in vivo* studies showing the effect of the (rat) vhh-1 protein to induce floor plate markers in the hindbrain and spinal cord. This post-published evidence clearly supported the results disclosed in the application and showed that the predicted therapeutic uses were successfully achieved.

According to the established case law (*inter alia* T 19/90, OJ EPO, 1990, 476), an objection under Article 83 EPC could only be raised if there were serious doubts substantiated by verifiable facts. In the present case, no serious doubts arose as to the correctness of what was taught in the application as filed and, as shown by the submitted post-published evidence, facts were on file showing that the skilled person was in a position to carry out the invention as claimed without departing from routine methods. It was also established case law that no reduction to actual practice was required and that valid evidence was only evidence demonstrating the state of affairs at the effective date (T 315/03 of 6 July 2004). In the present case, although the claimed subject-matter was not actually exemplified, it could easily be achieved following the teachings of the application. There was evidence was on file showing that these teachings were correct and that nothing more than routine skills were required at the effective date to carry them out. Thus, the requirements of Article 83 EPC were fulfilled.

XV. The appellant (applicant) requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request or alternatively one

of auxiliary requests I to IV filed on 13 September 2005 or one of auxiliary requests V to VII filed during the oral proceedings.

Reasons for the Decision

Main request and auxiliary requests I to IV
Article 123(2) EPC

1. Common to the main requests and auxiliary requests I to IV are claims centred on the use of a rat vhh-1 protein in an amount effective to generate a motor neuron from a undifferentiated motor neuron precursor cell for the preparation of a pharmaceutical composition for treating an acute nervous system injury or a neurodegenerative disease of motor neurons in a human subject (cf. points VI to IX *supra*). A key question common to all these requests is whether the specific combination of a **rat** vhh-1 protein for treating a condition in a **human** subject has a formal support in the application as filed.

2. Under the heading "Summary of the Invention" starting on page 6, the application as filed refers to several methods of generating motor neurons in a subject comprising the administration to the subject of a purified vertebrate vhh-1 protein (cf. page 7, lines 6 to 33). A pharmaceutical composition comprising a vertebrate vhh-1 protein is also provided by the application, wherein in one embodiment the vhh-1 protein is defined as a rat protein and, in another embodiment, as a human protein (cf. page 7, line 35 to page 8, line 4). It is in fact a pharmaceutical

composition comprising this latter embodiment, i.e. a human vhh-1 protein, which is proposed for use in a method for generating a motor neuron from an undifferentiated precursor neuron so as to treat acute nervous system injuries or chronic neurodegenerative diseases (cf. page 8, lines 6 to 22).

3. In a similar manner, under the heading "Detailed Description of the Invention" starting on page 47, the application as filed refers to the production of purified frog, mammalian, rat and human vhh-1 proteins (cf. page 60, lines 16 to 18) and to methods of inducing the generation of motor neurons in a subject comprising the administration to the subject of a purified vertebrate vhh-1 protein, wherein pharmaceutical compositions comprising a vertebrate, mammalian or human vhh-1 protein are also referred to (cf. page 61, line 13 to page 62, line 5). However, all methods for treating a **human** subject contemplate the use of a pharmaceutical composition comprising a **human** vhh-1 protein only (cf. page 63, line 34 to page 65, line 26). It is in this context that the paragraph cited by the appellant with the reference to a cDNA clone encoding a rat vhh-1 protein is found (cf. page 66, lines 5 to 22). However, when in this paragraph the role of the vhh-1 protein in inducing the differentiation of motor neurons and the likely use of this protein in the treatment of degenerative disorders are discussed, neither the specific source of the vhh-1 protein (human, rat, frog, etc.) nor the particular subject to be treated (human, rat, etc.) are mentioned.

4. The same situation is mirrored by the claims as filed. Claim 41 relates to a pharmaceutical composition

comprising a generic purified vertebrate vhh-1 protein, which is further defined in claim 42 as a mammalian vhh-1 protein and in claims 43 and 44 as a human vhh-1 protein. Claims 45 to 50 relating to methods for treating a **human** subject refer to the pharmaceutical compositions of claims 41 to 44, i.e. the generic vhh-1 proteins and the specific **human** vhh-1 protein. There is, however, no claim concerning the particular combination of the specific rat vhh-1 protein for treating a condition in a human subject.

5. In the light thereof, the board concludes that there is no explicit formal basis in the application as filed for this combination. It remains to be assessed whether an implicit disclosure of this combination is supported by the application as filed.

6. Whereas the application as filed discloses the use of a human vhh-1 protein for treating human subjects, which is a logical combination readily envisaged by the skilled person (protein to be used derived from the same species as the one to be treated so as to avoid disadvantageous immunological reactions, cross-species functional problems, etc.), it refers in general terms also to other vertebrate vhh-1 proteins, particularly mammalian vhh-1 proteins. In spite of this, the board fails to see any indication or suggestion leading the skilled person specifically to the selection of the rat vhh-1 protein (for the treatment of human subjects) among all possible vertebrate and mammalian vhh-1 proteins referred to in the application as filed and/or known from the prior art.

7. Thus, the use of a rat vhh-1 protein for treating a human subject is considered not to be supported by the application as filed and the requirements of Article 123(2) EPC are therefore not fulfilled.

Auxiliary request V
Articles 123(2) EPC, 84, 83 and 54

8. This request contains a single claim directed to a pharmaceutical composition comprising a rat vhh-1 protein (cf. point X *supra*). Formal support therefor is found in the description as originally filed, which refers to the rat vhh-1 protein as an embodiment of the generic pharmaceutical compositions (cf. page 7, line 35 to page 8, line 3). Thus, the requirements of Article 123(2) EPC are fulfilled.

9. The claim contains no deficiencies as regards to clarity and since the application characterizes and provides a purified vhh-1 protein derived from rat, the preparation of a pharmaceutical composition comprising this protein does not pose particular difficulties or require undue effort. The application itself refers to standard methods for obtaining these compositions (cf. page 62, lines 7 to 22). Thus, the requirements of Articles 84 and 83 EPC are fulfilled.

10. No objections under Article 54 EPC were raised in the decision under appeal for subject-matter related to a pharmaceutical composition comprising a rat vhh-1 protein (claim 18 of the main request then on file). There is also no discussion as to whether this subject-matter is entitled to the claimed priority (Articles 87 to 89 EPC) and as to the relevance of

documents pursuant to Article 54(3),(4) EPC. In view of the conclusions reached below as to the requirements of Article 56 EPC, the board refrains also from considering these points in more detail.

Article 56 EPC

11. Document D2, which is considered to be the closest prior art, refers to the cloning of a gene encoding the Sonic hedgehog (Shh, vhh-1 in the application) protein of mouse (cf. paragraph bridging pages 1417 to 1418). An alignment of the predicted amino acid sequence of the mouse vhh-1 protein with that of the vhh-1 proteins from Drosophila, chick and zebrafish sequences is shown in Figure 1 together with a cross-species comparison of amino acid identities of these sequences (cf. page 1418). Document D2 explicitly refers to a "*striking sequence conservation*", in particular the mouse and chick Shh share 84% of amino acid residues and, in their amino terminal half (positions 85 to 266), they are 99% identical. Therefore it is stated that "*the extreme interspecies conservation of the vertebrate Shh protein points to likely conservation of Shh function across vertebrate species*" (cf. *inter alia* page 1419, left-hand column, first and second full paragraphs). Document D2 also discloses several experiments showing the expression of mouse Shh (at the axial midline, in the limb and ectopic expression) and concludes that "*it will be interesting to explore the possible functions of related hh proteins in vertebrate development*" (cf. page 1427, right-hand column, last paragraph).

12. Starting from this prior art the problem to be solved can be defined as the provision of a related Shh protein in a form suitable for studying its function in the development. The solution is provided by a pharmaceutical composition comprising the rat vhh-1 protein (claim 1).

13. In fact, document D2 itself refers to the presence of floor plate-inducing activity "*in the chick and* ***rat*** *floor plate until late stages*" which is said to be *"consistent with Shh expression*" (cf. page 1426, left-hand column, full paragraph). In view of this clear hint towards the presence of Shh protein in rat, the board is convinced that the isolation of this protein from rat was obvious to the skilled person. Moreover, knowing the "*extreme interspecies conservation of the vertebrate Shh protein*" from document D2 itself (cf. point 11 *supra*), it appears that the skilled person had also a reasonable expectation of success, no matter which cloning method (and possible probes to be used accordingly) he or she decided to follow. Since a higher identity is normally expected for proteins of closely related species, a suitable probe would be one derived from the gene encoding the mouse vhh-1 protein (cf. document D6).

14. It is also important to note that all prior art documents on file are concerned with the biological role of the Shh protein. Document D1 discloses embryos implanted with ectopic retinoic acid beads which activate the Shh expression ***in vivo*** (cf. page 1411, right-hand column, last paragraph). Document D2 itself also refers to ectopic activation studies ***in vivo*** based on the direct injection of RNA encoding Shh into the

zebrafish egg (cf. page 1426, right-hand column). ***In vitro*** studies using neural plate explants in a medium conditioned by floor plate or notochord are also mentioned in document D2 (cf. page 1427, left-hand column). The use of neural plate explants in a medium comprising different growth factors or morphogens so as to study the ***in vitro*** effect of these products on the development of the neural plate explants is also reported in the prior art (cf. references on page 1427 of document D2, in particular Placzek et al., 1993, which is also cited in the application as filed). For all these ***in vitro*** and ***in vivo*** biological studies appropriate non-toxic compositions are required, i.e. compositions like the ones generally referred to on page 62 of the application as filed and defined therein as pharmaceutical compositions. In the context of this prior art, once the rat vhh-1 protein is made available to the skilled person, no inventive contribution can be seen in the provision of a pharmaceutical composition comprising this vhh-1 protein.

15. Thus, claim 1 of this request is considered not to fulfil the requirements of Article 56 EPC.

Auxiliary requests VI and VII
Article 123(2) EPC

16. As stated in point 8 *supra*, there is a formal basis in the application as filed for a pharmaceutical composition comprising a rat vhh-1 protein (cf. page 7, line 35 to page 8, line 3). There is also a formal basis for the delivery of generic pharmaceutical compositions to sites of vhh-1 protein action, in particular for the regeneration of motor neurons so as

to alleviate abnormalities associated with acute nervous system injury or chronic neurodegenerative diseases (cf. page 62, lines 24 to page 63, line 32). The combination of these teachings formally support the subject-matter claimed in the requests under consideration, which relates to the use of a rat vhh-1 protein for preparing a pharmaceutical composition for the treatment of a neurodegenerative disease (auxiliary request VI) or of an acute nervous system injury (auxiliary request VII) of motor neurons (cf. point XII *supra*).

17. Thus, the requirements of Article 123(2) EPC are fulfilled.

Article 83 EPC

18. It is undisputed that the application discloses the ability of a rat vhh-1 protein to induce, under certain conditions, the generation of motor neurons. In a first series of *in vitro* experiments, the application discloses the differentiation of chick neural plate explants into motor neurons by contact with COS cells transfected with a vhh-1 gene (cf. page 77, line 4 to page 78, line 11, page 81, line 14 to page 83, line 2). The involvement of vhh-1 in the patterning of the embryonic forebrain is shown in a second series of *in vitro* experiments (cf. page 101), which analyze the response of several neural plate explants (from telencephalic, diencephalic and rhombencephalic regions) to COS cells transfected with a vhh-1 gene, and conclude that vhh-1 might be implicated "*in the induction of ventral neuronal types along the entire rostrocaudal extent of the embryonic central nervous*

system" including "*motor neurons at spinal levels*" (cf. page 123, lines 18 to 24). A third series of *in vitro* experiments show that COS cells transfected with vhh-1 have a contact-dependent floor plate-inducing activity and a diffusible motor neuron inducing-activity. In fact, vhh-1 itself is shown to act on cells in neural plate explants to induce, independently, motor neurons and floor plate cells (cf. pages 136 to 141).

19. However, the claims of the auxiliary requests under consideration are not concerned with the use of a rat vhh-1 protein in an ***in vitro*** method for inducing the differentiation of motor neuron precursor cells into motor neurons but they are directed to the use of a pharmaceutical composition comprising a rat vhh-1 protein for treating a (neurodegenerative) disease or an (acute nervous system) injury of motor neurons in a subject, i.e. an ***in vivo*** use (cf. points XI and XII *supra*).

20. As regards sufficiency of disclosure there is no requirement in the EPC to submit results of *in vivo* experiments (animal models, clinical studies, etc.) for patent applications concerning biological substances, pharmaceutical compositions and/or uses thereof. It is in fact common to file applications with the results of *in vivo* experiments coming out some years later. However, the character of the examples required (where appropriate, cf. Rule 27(1)(e) EPC) to exemplify an invention depends on the very particular nature of the invention and the prior art relating thereto. Whilst for one invention results of *in vitro* experiments might fulfil the requirements of sufficiency of disclosure since a straightforward extrapolation to an *in vivo*

system is expected, for other inventions this extrapolation might not be feasible for a skilled person. Thus, the necessity of providing *in vivo* experiments as regards sufficiency of disclosure of a patent application must be assessed on a case-by-case basis.

21. Although in the present case the *in vitro* experiments demonstrate the ability of the vhh-1 protein to differentiate cells of neural plate explants into motor neurons, they also emphasize certain limitations of the process. In particular, differences found between an *in vivo* situation - with production of vhh-1 by the notochord - and the first series of *in vitro* experiments are suggested to "*reflect the onset of expression of notochord factors that inhibit the action of vhh-1 or the loss of expression of a required cofactor*" (cf. page 82, lines 19 to 21) and that "*differences in neural tube responses to vhh-1 and to the notochord could result from quantitative differences in vhh-1 levels ... Alternatively, the notochord may provide additional signaling molecules*" (cf. page 84, line 35 to page 85, line 5). In the second series of *in vitro* experiments it is emphasized that "*the repertoire of ventral neural cell types that can be induced by vhh-1/shh is defined by an earlier restriction in the rostrocaudal character of cells within the neural plate*" (cf. page 104, lines 3 to 5 and page 120, lines 2 to 7). Although the application refers to several possible mechanisms of action for the vhh-1 protein, it is stated - in the third series of *in vitro* experiments - that the actual mechanism of action remains unclear (cf. page 142, lines 1 to 31, Figure 17).

22. These limitations are also encountered in the fourth series of *in vivo* experiments showing the consequences of misexpressing the vhh-1 gene and the winged-helix gene HNF-3β in the neural plate and neural tube of frog embryos (cf. pages 149 to 176). The ability of vhh-1 to induce floor plate differentiation *in vivo* is spatially and temporally restricted or constrained by additional signals that specify the time and position of this differentiation (cf. page 149, lines 18 to 22, page 152, lines 11 to 16 and page 169, lines 13 to 16). The restrictions found in these (ectopic) experiments are said to operate during normal development too (cf. page 171, lines 12 to 26). Therefore, it is concluded that "*in vivo and in vitro studies have shown that neural cells have a limited period of competence to respond to floor plate inducing signals ... in vivo, therefore, there be constraints ... that act prior to and independent of the loss of competence of neural cells*" (cf. page 171, line 28 to page 172, line 5). Although these *in vivo* experiments do not describe the differentiation of neuron precursor cells to motor neurons, the results apply to these neurons too, since - as shown by the series of *in vitro* experiments - motor neurons are generated *in vivo* by both floor plate and notochord, the latter having similar constraints (cf. point 21 *supra*).

23. Notwithstanding these limitations associated with a possible *in vivo* use of vhh-1, the appellant has argued that a skilled person could carry out the claimed uses in a straightforward manner without requiring any inventive skill and without an undue burden. In support thereof, post-published documents have been filed,

namely exhibits A and B (cited as expert opinions), which allegedly confirm that the *in vivo* uses as claimed in the requests under consideration are realised.

24. However, exhibit B, which bears a publication date of December 2002, reports only on *in vitro* experiments that show the differentiation of mouse embryonic stem (ES) cells into motor neurons by exposure to developmentally relevant signals. The fact that all changes are achieved by only using two signals (acid retinoic and Sonic hedgehog protein) is qualified as "*a huge chunk of luck*" (cf. page 24, left-hand column, last full paragraph) since three steps are required for an ES cell to become a motor neuron: first it has to become "*a generic neural progenitor cell*", then "*a spinal cord progenitor cell*" and finally "*a particular progenitor cell that gives rise to a motor neuron*" (cf. page 24, paragraph bridging left- and right-hand columns). It is this third step which is not so simple "*because different concentrations, or grades, of sonic hedgehog have different effects. Hedgehog's graded actions normally produce* ***at least five different classes of neurons*** *... typically, only 25-30 percent of the cells ... are motor neurons*" (cf. page 24, right-hand column, first full paragraph, bold by the board). Moreover, for a cell-based treatment of neurodegenerative diseases "*simply having generated a motor neuron is not sufficient*" and "*big hurdles*" are ahead since "*the motor system relies heavily on the precision of connections and circuits*" (cf. page 26, left-hand column, full paragraph) and it is highly important to develop an homogeneous mixture of desired cell types only (cf. page 24, right-hand column, the

three last paragraphs). Thus, although intended for a different type of treatment (cell-based vs. vhh-1 based), exhibit B only confirms - eight years after the priority date of the application - the limitations referred to in the application, i.e. the relevance of having suitable undifferentiated motor neuron precursor cells and of knowing the appropriate concentrations of vhh-1 protein, for achieving a successful differentiation to the desired motor neurons.

25. Exhibit A, published in 2004, does not help the appellant's case further. The document refers to the injection into the rat's brain (hippocampus) of a rat Sonic hedgehog gene inserted into a harmless virus, which delivers the gene to brain stem cells stimulating them to divide and tripling the production of new neurons (cf. page 3, second paragraph). However, neither the type of stem cells stimulated to divide nor the type of the resulting neurons (motor, intermediate, dopaminergic, etc.) are characterized. Moreover, a possible treatment of brain diseases is said to be "*a decade or more away*" (cf. page 3, third paragraph), the next step being "*to try to learn how to control the development of stem cells into neurons*" (cf. page 3, penultimate paragraph), which it is understood to mean, although expressed in other words, as trying to learn how to overcome the limitations and constraints disclosed in the application as filed (ten years after the claimed priority date).

26. It follows from the foregoing that, although the application itself identifies these limitations and constraints, it provides no helpful teachings how to overcome them nor are these found in the prior art or

in the documents on file. Thus, at the filing date of the application, the use of a rat vhh-1 protein according to the claims of the requests under consideration placed an undue burden on the person skilled in the art (the more so when considering that the application fails to identify the actual mechanism of action of the vhh-1 protein, cf. point 21 *supra*).

27. It is worth noting at this point that the serious doubts as to whether the skilled person at the effective (filing or priority) date was in a position to perform the claimed use without an undue burden actually arise from the restrictions and constraints identified in the application itself. These limitations were also referred to and acknowledged in the prior art, the submitted post-published documents (exhibits A and B) only supporting them further. No contradiction can be seen with the established case law of the Board of Appeals that requires verifiable facts to substantiate the serious doubts (cf. T 19/90, OJ EPO, 1990, 476, point 3.3. of the Reasons) and the evidence to demonstrate only the state of affairs at the effective date (cf. T 315/03 of 6 July 2004 to be published at the OJ EPO, points 9.5 and 9.6 of the Reasons).

28. Thus, the board considers that auxiliary requests VI and VII do not fulfil the requirements of Article 83 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani