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Datasheet for the decision of 21 July 2020

Case Number: T 1111/14 - 3.3.08

Application Number: 08770211.4

Publication Number: 2158321

IPC: C12N15/85

Language of the proceedings: EN

Title of invention:
METHOD OF EXPANDING HUMAN HEPATOCYTES IN VIVO

Applicants:
Oregon Health & Science University
The Board of Trustees of the Leland Stanford Junior University

Headword:
Human hepatocytes/OREGON UNIVERSITY

Relevant legal provisions:
EPC Art. 53(a), 54, 56, 83, 84, 123(2)
EPC R. 28(1)(c)

Keyword:
Main request - added matter - (no)
Claims - support in the description (yes)
Sufficiency of disclosure - (yes)
Exception to patentability - (no)
Novelty - (yes)
Inventive step - (yes)

Decisions cited:
T 0019/90, T 0409/91, T 0931/91, T 0659/93, T 0890/02, T 1020/03, T 0385/14

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 1111/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 21 July 2020

Appellants: (Applicants)

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Decision under appeal:

Decision of the Examining Division of the European Patent Office posted on 29 October 2013 refusing European patent application No. 08770211.4 pursuant to Article 97(2) EPC.

Composition of the Board:

Chairman B. Stolz
Members: M. R. Vega Laso
J. Geschwind

Summary of Facts and Submissions

I. The appeal of the applicants (appellants) lies from a decision of an examining division posted on 29 October 2013, refusing the European patent application No. 08770211.4 with the title "Method of expanding human hepatocytes in vivo", which was filed under the Patent Cooperation Treaty and published as WO 2008/151283 (in the following "the application as filed").

II. In the decision under appeal, the examining division found that claims 1 and 17 as then on file did not fulfil the requirements of Article 84 EPC, and that the claimed invention was not disclosed in the patent application as filed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Article 83 EPC). Moreover, the examining division found that the subject-matter of claim 14 then on file encompassed embodiments which were excepted from patentability pursuant to Article 53(a) and Rule 28(c) EPC.

III. Together with their statement of grounds of appeal, the appellants filed four sets of claims as their main request and first to third auxiliary requests in appeal proceedings. They requested refund of the appeal fee on the grounds that the examining division committed a substantial procedural violation, as well as oral proceedings if the board did not intend to grant their main request.

IV. Pursuant to their request, the appellants were summoned to oral proceedings before the board.

V. By letter dated 21 January 2020, the appellants submitted three sets of amended claims as new main request and new first and second auxiliary request, as well as further evidence, in particular a declaration by one of the inventors and three scientific articles.

VI. In a communication sent in preparation of the oral proceedings, the board expressed a provisional opinion on some substantive issues, raised a new objection under Article 84 EPC, and requested the appellants to submit a copy of documentary evidence referred to in the inventor's declaration.

VII. The appellants replied to the board's communication and submitted two sets of claims as new main request and first auxiliary request, as well as the requested evidence. They also withdrew the request for the refund of the appeal fee.

VIII. Following an indication by the board, the appellants submitted on 19 February 2020 an amended main request in which a typographical error had been corrected.

IX. The oral proceedings were cancelled.

X. Claims 1, 12 and 15 according to the main request read as follows:

"1. A method of expanding human hepatocytes *in vivo*, comprising:
i) transplanting isolated human hepatocytes into a *Rag2*$^{-/-}$*/Il2rg*$^{-/-}$ mouse, wherein the mouse is deficient for expression of Fah;
ii) allowing the human hepatocytes to expand for at least two weeks; and
iii) collecting human hepatocytes from the mouse,

wherein the mouse is homozygous for deletions or one or more point mutations in the *Fah* gene.

12. The method of claim 1, wherein the human hepatocytes were obtained or isolated from the liver of an organ donor, obtained or isolated from a surgical resection or derived from a stem cell, monocyte or amniocyte.

15. A genetically modified mouse whose genome is homozygous for deletions or one or more point mutations in the *Fah*, *Rag2* and *Il2rg* genes such that the deletions or point mutations result in loss of expression of functional FAH, RAG-2 and IL-2Rγ proteins, wherein the mouse is immunodeficient and exhibits decreased liver function."

Dependent claims 2 to 11, 13 and 14 are directed to embodiments of the method of claim 1. Dependent claims 16 to 18 relate to embodiments of the mouse of claim 15.

XI. In the present decision, reference is made to the following documents:

(1): H. Azuma *et al.*, August 2007, Nature Biotechnology, Vol. 25, No. 8, pages 903 to 910;

(2): D.A. Shafritz, August 2007, Nature Biotechnology, Vol. 25, No. 8, pages 871 and 872;

(3): K.-D. Bissig *et al.*, 18 December 2007, PNAS, Vol. 104, No. 51, pages 20507 to 20511;

(4): WO 00/17338, published on 30 March 2000;

(6): EP 1 496 110 A1, published on 12 January 2005;

(18): Declaration of Dr Markus Grompe, dated 5 February 2011;

(21): Declaration of Dr Markus Grompe, dated 16 September 2013; and

(34): K.-D. Bissig *et al.*, March 2010, The Journal of Clinical Investigation, Vol. 120, No. 3, pages 924 to 930.

XII. The submissions made by the appellants were essentially as follows:

Article 84 EPC

Support for the claims, under Article 84 EPC, was primarily a formal issue, requiring that the description corresponded to the scope of the claims (see decision T 1020/03, OJ EPO 2007, 204). The subject-matter of the independent claims 1 and 15 was reflected by the description, for example in the passage from page 2, line 22 to page 3, line 29. Therefore, the disclosure of the application corresponded to the scope of the claims and the requirement for support in Article 84 EPC was met.

Article 83 EPC

The examining division erred in finding that the claimed invention was not sufficiently disclosed in the application as filed. The whole argument of the examining division was based on the premise that the person skilled in the art would read the application questioning its teaching. This approach was

fundamentally incorrect. The examining division also used the wrong tests. It argued that the skilled person would not "unambiguously derive" from the application that the invention works in the absence of uPA pre-treatment, and that the skilled person would not be prompted to depart from the examples "with reasonable expectations of success".

The examining division's use of the documents (1) to (3) as evidence that the claimed invention did not work, was flawed. The documents did not prove this. In contrast, documents (18) and (21), which were declarations by the inventor, provided clear experimental proof that the invention worked, even omitting pre-treatment with uPA. Hence, the requirements of Article 83 EPC were met.

XIII. The appellants (applicants) requested that the decision under appeal be set aside, and that a patent be granted based on the claims according to the main request filed on 19 February 2020 or, in the alternative, based on the claims of any of the first auxiliary request filed on 4 February 2020 and the second and third auxiliary requests filed together with the statement of grounds of appeal.

Reasons for the Decision

The invention

1. The present application relates to a method for expanding human liver cells (hepatocytes) *in vivo*, and a genetically modified mouse in which human hepatocytes can be expanded (see section X above). According to the invention, the genome of the recipient mouse is

homozygous for deletions or point mutation(s) in the *Rag2* and *Il2rg* genes. As expression of functional RAG-2 and IL-2Rγ proteins is lost, the genetically modified mouse lacks functional T cells, B cells and natural killer cells and is thus immunodeficient. Hence, transplanted human hepatocytes are not rejected and can expand in the murine liver. Additionally, the genetically modified mouse is deficient for the expression of the *Fah* gene. According to the invention, this can be achieved by a deletion of the *Fah* gene (FRG mice) or one or more point mutation(s) ($F^{pm}RG$ mice) therein, both modifications leading to cell death of the murine hepatocytes without affecting the transplanted human hepatocytes.

Main request

Article 123(2) EPC

2. Basis for the method of claim 1 is found in claims 1, 2 and 4 of the application as filed. The feature "*one point mutation in the Fah gene*" has a basis in claim 32 of the application as filed, which is directed to the genetically modified mouse as such. It is clear from the application as a whole that the method of expanding human hepatocytes *in vivo* according to the invention is to be performed using genetically modified mice as described in the application.

3. Dependent claims 2 to 4, 6, 7, 9 to 11, 13 and 14 correspond to, respectively, claims 3, 5, 6, 13, 14, 22, 24, 25, 28 and 31 of the application as filed. The subject-matter of claims 5 and 8 has a basis in, respectively, claims 9 and 10, and claims 15 and 16 of the application as filed. Claim 12 corresponds to claim 26 of the application as filed which has been

amended by inserting "*obtained or* [isolated]". Basis for this wording is found in page 22, lines 22 and 23 of the application as filed.

4. Independent claim 15 corresponds to claim 32 of the application as filed which has been amended by deletion of the wording "*... and wherein human hepatocytes can be expanded in the mouse*". In view of the experimental evidence on file, it is accepted that this functional feature is an inherent feature of a mouse genetically modified as defined in present claim 15. Hence, the deletion of this feature does not contravene Article 123(2) EPC.

5. Dependent claims 16 and 18 correspond to claims 33 and 36 of the application as filed. Basis for the subject-matter of claim 17 is found in claims 34 and 35 of the application as filed.

6. Thus, the claimed subject-matter does not extend beyond the content of the application as filed.

Article 84 EPC

7. The claimed invention relies on genetic modifications of the murine genome resulting in loss of expression of functional FAH, RAG-2 and IL-2Rγ proteins. The genetically modified mouse, which is immunodeficient and exhibits decreased liver function, is used as a recipient in a method of expanding human hepatocytes *in vivo*.

8. In the decision under appeal, the examining division found that the claims then on file lacked support of technical nature in the description. In their view, "*... it is not unambiguously derivable* [from the

description] *that it may be possible to achieve the desired technical effect i.e. expanding human hepatocytes into FRG mice in absence of treatment with urokinase, without specific adaptation, such as an hypothetical depletion of the macrophages*" (see third paragraph on page 16 of the decision).

9. In the jurisprudence of the Boards of Appeal the requirement that the claims be supported by the description (Article 84 EPC, second sentence) is viewed either as a formal matter, which means that the requirement is considered to be met if the subject-matter of the claims is also apparent from the description (see, e.g., decision T 1020/03, OJ EPO 2007, 204), or as a substantive matter, i.e. as requiring that the claims reflect the actual contribution to the art in such a way that the skilled person is able to perform the invention in the entire range claimed (see, e.g., decisions T 409/91, OJ EPO 1994, 653; and T 659/93 of 7 September 1994).

10. In the present case, the requirement of support for the claims in the description is met not only from the formal, but also from the substantive point of view. The subject-matter of claim 1 is apparent from the passage on page 19, lines 3 to 20 of the description - which is quoted on pages 9 and 10 of the decision under appeal -, as well as from the passage from page 2, line 22 to page 3, line 5. A genetically modified mouse as defined in claim 15 is apparent from the passage on page 3, lines 23 to 27 of the description.

11. In the decision under appeal, the examining division admitted that the administration of a vector encoding human urokinase to the genetically modified recipient

mouse prior to injection of the human hepatocytes was not disclosed in the application as being an essential feature of the invention. But in its view, it was not clearly stated in the description that it would be possible to carry out the invention without expression of urokinase in the recipient mouse, and the description did not disclose "*... **how** it would be possible*" (see the first paragraph on page 11 of the decision under appeal).

12. Contrary to the examining division's view, the fact that it is not **expressly** stated in the description that the method of the invention can be carried out without administering a vector encoding urokinase to the recipient mouse, does not justify an objection of lack of support within the meaning of Article 84 EPC. As readily apparent from various passages of the description, e.g., the sentence bridging pages 2 and 3, as well as the passage on page 34, lines 12 and 13, a method that includes, in addition to the steps recited in claim 1, the step of administering a vector encoding urokinase to the mouse prior to injection of the human hepatocytes, represents only a particular embodiment of the method of claim 1.

13. As for the question whether (and how) the invention can be carried out without urokinase expression in the recipient mouse, it is disclosed in the application (see page 25, lines 10 and 11) that successful engraftment and expansion of human hepatocytes in murine liver requires an immunodeficient mouse with some degree of liver dysfunction. It is further stated that, as known in the art, liver dysfunction in mice can be achieved by expressing a gene encoding urokinase (also called urokinase-type Plasminogen Activator (uPA)) in the liver. Purportedly, this creates a growth

disadvantage for the murine hepatocytes which facilitates the expansion of transplanted human hepatocytes (see page 25, lines 15 to 19 of the application as filed and document (4) cited therein).

14. As apparent from the application as filed (see page 26, lines 13 to 22 read in the light of the passage on page 25, lines 21 to 23), according to the invention severe liver dysfunction is achieved by homozygous deletion of - or one or more point mutations in - the murine *Fah* (fumarylacetoacetate hydrolase) gene. Hence, while the extent of liver disease and the selective pressure towards human hepatocytes in FRG or $F^{pm}RG$ mice may be enhanced by administering a vector encoding uPA prior to transplantation, this is not absolutely required for carrying out the invention. In fact, as a further embodiment, the application discloses:

> "*In one embodiment, in FRG mice the extent of liver disease and selective pressure can be controlled by administering and withdrawing NTBC [...]. Withdrawal of NTBC provides a selective advantage for the transplanted human hepatocytes.*" (see the first two sentences of the passage bridging pages 36 and 37)

Further, it is stated in Example 1 of the application that:

> "*... NTBC withdrawal resulted in gradual hepatocellular injury in FRG mice and eventual death after 4-8 weeks ...*" (see last sentence in page 38)

15. In the decision under appeal, the examining division expressed the view that, in the passages quoted above

"*... the skilled person is not given sufficient technical information or incentive* [...] *and would not consider this as more than a 'try and see' possibility*" (see last sentence of the fourth paragraph on page 13 of the decision). The board disagrees with this view. The quoted passages disclose, clearly and unambiguously, an embodiment of the invention that does not require the administration of a uPA vector to the mouse prior to transplantation. The amount of technical details provided in the application for this particular embodiment might have to be considered for the assessment of sufficiency of disclosure, but it is of no relevance as regards the question whether claim 1 is supported by the description. Otherwise, the boundary between the requirements of Articles 83 and 84 EPC becomes blurred.

16. For these reasons, the board concludes that claim 1 is supported by the description, within the meaning of Article 84 EPC.

Article 83 EPC

17. According to the established jurisprudence of the boards of appeal, a finding of lack of sufficient disclosure should be based on serious doubts, substantiated by verifiable facts (see e.g. decision T 19/90, OJ EPO 1990, 476 and decision T 890/02, OJ EPO 2005, 497). In order to establish insufficiency of disclosure, it must be established, on the balance of probabilities, that a skilled person reading the patent, using his/her common general knowledge, would be unable to carry out the invention.

18. In the present case, the facts put forward by the examining division to substantiate the finding of lack

of a sufficient disclosure over the whole scope of claim 1 were based on statements made in Example 4 of the application, and on documents (1) to (3) and (18).

19. Example 4 of the application shows the repopulation of the liver of FRG mice with human hepatocytes. It is stated on page 42, lines 23 to 25 that "... [the] *experiments were performed to determine whether administration of a urokinase expressing adenovirus prior to transplantation of human hepatocytes would be* ***beneficial***" (emphasis added by the board). This statement is interpreted by the board as meaning that the purpose of the experiments was to try to **improve** a method as defined in claim 1 which does not require the administration of urokinase.

20. In the decision under appeal, the examining division relied for its adverse finding on, in particular, the following statements in Example 4:

> "*In three separate transplantations, primary engraftment of human hepatocytes was observed in FRG mice in recipients which had first received the uPA adenovirus. The uPA-pretreatment regimen was therefore used in most subsequent transplantation experiments.*
>
> *In total, human hepatocytes from nine different donors were used successfully and no engraftment failures occurred after introduction of the uPA adenovirus regimen.*" (see page 43, lines 13 to 18 of the application; emphasis as in the decision under appeal)

21. In the examining division's view, the remark that no engraftment failures occurred after introduction of the

uPA adenovirus regimen, implied that some engraftment failures occurred without introduction of the uPA adenovirus regimen. The examining division went on to conclude that "*... this information does not lead the skilled person to conclude that in absence of uPA treatment, there would be reasonable expectation of success to achieve the technical effect*" (see fifth paragraph on page 15 of the decision under appeal).

22. It is apparent from this conclusion and further statements in the decision under appeal (see, e.g., third and eight paragraph on page 16) that the examining division deviated from the legal and factual standards established in the jurisprudence of the Boards of Appeal for the assessment of sufficiency of disclosure. Contrary to its view, "some engraftment failures" cannot be equated to a failure to carry out the claimed method without administration of uPA. Occasional failure when testing a technical teaching does not impair its reproducibility, if the attempts are kept within reasonable bounds and do not require inventive skill (see decision T 931/91 of 20 April 1993). As a matter of fact, in the technical field at issue occasional failure is the rule, rather than the exception. For instance, it is reported in document (3) that transplantation success rate was between 45% and 100% for adult animals, and 73% for pups (see page 20509, last paragraph in the left-hand column, and page 20510, first sentence of the second full paragraph). Even when uPA adenovirus is administered to a genetically modified mouse according to the invention, at most 67% of the engraftments succeeded (see Table 1 in document (1)).

23. As further evidence that the claimed invention cannot be carried out without the administration of uPA, the

examining division referred to the passage on page 904, second paragraph in the left-hand column of document (1). This document is a scientific paper by the inventors which was published after the priority date. The relevant passage reads: "*... we were able to observe primary engraftment of human hepatocytes only in recipients that had first received the uPA adenovirus*".

24. In document (21), Dr Grompe, one of the authors of document (1), stated that the statement on which the examining division relied concerned only **primary** engraftment, whereas in secondary recipients (i.e., those transplanted with hepatocytes previously expanded in another mouse) which had not received any prior uPA adenovirus treatment, liver repopulation was highly successful, as Figure 3c of document (1) showed. Dr Grompe also pointed out that, as stated in the first sentence of the same paragraph as the passage quoted above, overexpression of urokinase in fact enhances hepatocyte engraftment, but that in the absence of such a treatment there is at least some level of engraftment, otherwise there was nothing to be "enhanced". In document (21), Dr Grompe provided also experimental results showing that, while liver repopulation was faster in those mice that had received the uPA pre-treatment, also mice without uPA pre-treatment showed repopulation after 5 months (in contrast to 3 months in the pre-treated mice).

25. In the decision under appeal, the examining division stated that these results, which had already been presented in document (18), an earlier declaration by Dr Grompe, "*... successfully addressed the issue whether uPA-pretreatment is essential to repopulate FRG mice with human hepatocytes*" (see page 18, last

sentence of the fifth paragraph). In view of the adverse decision, the wording "successfully addressed" in this passage can only have the meaning that the examining division considered the results to confirm that uPA treatment is essential. The results indicate, however, the opposite. Although delayed, in comparison to those transplanted to uPA pre-treated mice, human hepatocytes did in fact expand in mice without uPA pre-treatment. This is confirmed by document (34) published by an independent group that reports robust repopulation of the murine liver (up to approximately 95%) with human hepatocytes in FRG mice applying the method of the invention **without** uPA pre-treatment (see the sentence bridging the left and right-hand columns on page 925).

26. The examining division relied also on document (2) in which the scientific publication of Azuma et al. (document (1)) is commented. Like the examining division, the author of this document appears to have misinterpreted the passage of document (1) quoted in paragraph 23 above, as stating that pre-treatment of FRG mice with uPA-expressing adenovirus is required for repopulation with human hepatocytes (see last paragraph of the left-hand column on page 872, in particular the last sentence). As explained above, this interpretation is incorrect.

27. As regards document (3), also published after the priority date of the present application, the examining division admitted that the experiments described therein showed that pre-treatment with uPA was not essential for successful engraftment in FRG mice. It held, however, that additional specific adaptations of the methodology were required. In fact, in the experiments described in document (3) two further

components of the murine immune system (complement system and macrophages) were blocked by drug treatment. Depletion of macrophages is disclosed in the present application as a particular embodiment of the method of the invention (see page 21, first full paragraph). The use of a complement inhibitor is mentioned on page 37, lines 7 to 12, although it is stated that its administration to FRG mice is not required for liver repopulation with human hepatocytes. There is however no evidence on file showing that these two adaptations are essential for human hepatocytes to expand in FRG mice.

28. In view of the above, the board is persuaded that, on the balance of evidence, the requirements of Article 83 EPC are met.

Article 53(a) and Rule 28(1)(c) EPC

29. In the decision under appeal, the examining division held that the subject-matter of claim 14 then on file, which corresponds to that of present claim 12 (see section X above), was excepted from patentability under Article 53(a) and Rule 28(1)(c) EPC.

30. In view of the revised interpretation of Rule 28(1)(c) EPC (formerly Rule 28(c) EPC) by the European Patent Office (see decision T 385/14 of 11 September 2019), the examining division's objection cannot be upheld for claim 12 on file.

Article 54 EPC

31. Neither in the decision under appeal nor in its various communications did the examining division raise any objection concerning the novelty of the claimed

subject-matter. None of the documents presently on file describes the method of claim 1 or the genetically modified mouse of claim 15. Hence, novelty must be acknowledged.

Article 56 EPC

32. Since the application was refused on the grounds that claim 1 lacked support and sufficient disclosure, inventive step was not discussed in the decision under appeal. In the communication attached to the summons to oral proceedings in examination proceedings, the examining division regarded document (6) as the closest state of the art and formulated the problem to be solved as the provision of further means for expanding human hepatocytes in vivo in mice. In the last paragraph of section 5.2 of the communication, the examining division appears to object that this problem has not been plausibly solved over the whole scope of claim 1, in particular "*without pre-treatment with UpA*[sic] *and/or for two weeks expansion only*". However, in the last paragraph on page 10 of the communication under the heading "Clarity, support and disclosure: (Articles 84 and 83 EPC)", the examining division developed a further line of argument starting from document (4) as the closest state of the art, apparently coming to a similar conclusion (see the penultimate sentence of the last paragraph on page 10).

33. The board has some difficulties understanding the argumentation of the examining division in the communication attached to the summons. However, in the light of the evidence currently on file as outlined above in connection with the issue of sufficiency of disclosure, the board is persuaded that the technical problem of providing further – possibly improved -

means for expanding human hepatocytes *in vivo* in mice, which is the objective problem starting from either document (6) or (4), is solved by the invention over the whole scope of present claims 1 and 16. An objection that, in view of the prior art the claimed method and mouse were obvious to a skilled person has never been raised by the examining division and the board has no reason to raise it of its own motion.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent based on claims 1 to 18 according to the main request filed on 19 February 2020, and a description to be adapted thereto.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated