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Datasheet for the interlocutory decision of 7 March 2007

Case Number: T 1262/04 - 3.3.04

Application Number: 99124640.6

Publication Number: 1016419

IPC: A61K 49/00

Language of the proceedings: EN

Title of invention:
Non-invasive localization of a light-emitting conjugate in a mammal

Applicant:
The Board of Trustees of the Leland Stanford Junior University

Opponent:
-

Headword:
Light detection in mammals/LELAND STANFORD

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
"Sufficiency of disclosure and novelty (yes) - documents D1 to D27 not detrimental to inventive step (yes)"

Decisions cited:
G 0010/93, T 0994/95, T 0157/03

Catchword:
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Case Number: T 1262/04 - 3.3.04

I N T E R L O C U T O R Y D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 7 March 2007

Appellant: THE BOARD OF TRUSTEES OF
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 13 April 2004 refusing European application No. 99124640.6 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chair: U. Kinkeldey
Members: B. Claes
R. Moufang

Summary of Facts and Submissions

I. This interlocutory decision of the board lies from the decision of the examining division to refuse European divisional application 99124640.6 with the title "Non-invasive localization of a light-emitting conjugate in a mammal" on the basis of Article 97(1) EPC for the reason that the subject-matter of the claims of both the main request filed with letter dated 25 July 2002 and the auxiliary request filed with letter dated 12 January 2004 lacked an inventive step.

II. The applicant (appellant) has appealed the decision of the examining division. The appellant has argued in favour of inventive step and has introduced, with the statement of the grounds of appeal, two further documents.

III. The board summoned the appellant to oral proceedings and issued a communication pursuant to Article 12 of the Rules of Procedure of the Boards of Appeal drawing the appellant's attention to the principles set out in decision G 10/93 of the Enlarged Board of Appeal (OJ EPO 1995, 172) and giving the board's preliminary opinion on a number of relevant issues.

IV. Oral proceedings were held on 7 March 2007 during which the appellant declared that the former second auxiliary request filed with letter of 7 February 2007, comprising a set of 27 claims, constituted the new main request.

The board drew the attention of the appellant to document D28, Contag *et al.*, Molecular Microbiology

(1995), 18(4), pages 593-603, which was the document first cited in the search report of the parent application PCT/US95/15040, published as WO 97/18841, although it was not cited in the search report established for the present divisional application. In an attempt to establish the exact publication date of document D28, the board presented a printout of Rightslink® of 7 March 2007 indicating 15 November 1995 as the date on which the document was published. The board announced that it considered the content of document D28 more relevant to the patentability of the claimed subject-matter, in particular inventive step, than any other document on file and comprised in the list of documents D1 to D5 as cited in the reasons of the decision under appeal or documents D6 to D27 as cited in the statement of grounds of appeal.

At the end of the oral proceedings the board announced the present interlocutory decision.

V. Claim 1 of the new main request read:

"1. A method for detecting tumour cells in a living non-human mammal, said method comprising:
(a) providing a living, non-human mammal comprising tumour cells, said tumour cells comprising a heterologous gene construct encoding at least one light generating protein;
(b) placing the mammal in the detection field of a photodetector device;
(c) maintaining the mammal within the detection field of the photodetector device; and

(d) measuring through opaque tissue, photon emission from said cells with said photodetector device, to detect said eukaryotic cells."

VI. The following further documents are relevant for the present decision:

D2: Contag *et al.* (1995), Pediatric Research, Vol. 37(4), part 2, Abstract 1017.

D3: Hooper *et al.* (1990), Journal of Bioluminescence and Chemiluminescence, Vol. 5, pages 123-130.

D4: Israel and Honigman (1991), Gene, Vol. 104, pages 139-145.

D5: WO 91/01305

D7: Rehemtulla *et al.* (2000), Neoplasia, Vol. 2(6), pages 491 to 495.

D8: Sweeney *et al.* (1999), Proc. Natl. Acad. Sci., Vol. 96(21), pages 12044 to 12049.

VII. The arguments of the appellant as far as they are relevant for the present interlocutory decision can be summarised as follows:

Sufficiency of disclosure

The appellant acknowledged that the description of the patent application did not contain a worked example of the claimed invention demonstrating the non-invasive

visualisation of (eukaryotic) tumour cells within the mammalian body by detecting light produced by these cells. However, the patent disclosed the invention in a manner sufficiently clear and complete for it to be carried out by the skilled person. Documents D7 and D8 provided ample evidence that the invention as claimed can be put into practice without any undue burden. The appellant has referred in this context to decision T 157/03 of 4 January 2005.

Novelty

The claimed subject-matter had not been made available to the public in the prior art.

Inventive step

The closest prior art was represented by document D2. The problem to be solved was the provision of non-invasive means for tracking tumour cells in a mammal.

Starting from document D2 the skilled person would not adapt the non-invasive method described therein for visualising prokaryotic cells to (eukaryotic) tumour cells seeing that there were a multitude of reasons why the skilled person would not have a reasonable expectation that such a method would be successful.

The prokaryotic *lux* operon was fundamentally different from the eukaryotic firefly *luc* gene. The encoded luciferases used different luciferin substrates and energy sources and, contrary to the prokaryotic system, the eukaryotic system required the administration or delivery of the luciferin substrate to the experimental

animal. The required administration of the luciferin substrate at a certain distance from the tumour occurrence raised questions whether or not the substrate would become available in the tumour cells associated with the luciferase. Further reasons were the fact that the method as described in document D2 was conducted with fast-growing bacteria transformed with high copy numbers of the light-generating gene. The expected levels of gene expression would be lower for eukaryotic tumour cells having a substantially lower rate of cell division and lower gene copy numbers.

VIII. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 to 27 of the new main request filed as second auxiliary request with letter of 7 February 2007.

Reasons for the Decision

1. Claim 1 is directed to a whole body imaging method for detecting tumour cells in a living non-human mammal whereby the light emitted from a light generating protein expressed in the tumour cells is measured through opaque tissue.

Sufficiency of disclosure

2. According to Article 83 EPC and the relevant established case law of the boards of appeal, the invention must be disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art over the entire scope and without undue difficulty.

3. The board notes that the description of the application does not disclose, by way of experimental report, methods which fall within the ambit of independent claim 1. The experimental examples contained in the application concern the rapid *in vivo* tracking or monitoring of bacterial infectious processes in animals, in particular mice, whereby *Salmonella* bacteria strains of varying virulence were transformed to contain the (prokaryotic) *lux* bioluminescence operon so as to express a bacterial luciferase capable of producing light when its substrate and oxygen are present.

4. The board however considers that the general part of the description of the application (see paragraphs [0022] to [0204]) discloses ample technical detail and measures which enable in a credible manner to work the invention as claimed.

5. Furthermore, it has been held in at least two decisions of the boards of appeal that in cases where the specification of the application or patent disclosed the invention merely at a general conceptual level but lacked any concrete or tangible proof that the claimed invention could be put into practice, post-published documents may constitute evidence that the invention was indeed reproducible without undue burden at the relevant date (see decisions T 994/95 of 18 February 1999, point 8; and T 157/03 of 4 January 2005, point 9). This board considers that this principle indeed applies at least to cases such as the present one where the technical teaching as disclosed in the application is credible (see point 4).

6. There are at least two post-published documents on file which disclose methods corresponding to the methods as claimed. Document D7, co-authored by one of the inventors, discloses the rapid and quantitative assessment of cancer treatment response in 9L gliosarcoma cells being engineered to express firefly luciferase using *in vivo* bioluminescence imaging with a cryogenically cooled CCD camera and magnetic resonance imaging (see e.g. title and abstract). Document D8, also co-authored by one of the inventors, discloses the visualisation by an intensified CCD camera of the kinetics of the clearance of human cervical carcinoma (HeLa) cells labelled by expressing firefly luciferase and engrafted in living immunodeficient mice. Furthermore, both documents describe the successful administration of the firefly luciferase substrate, i.e. luciferin, by means of injection in the peritoneal cavity, i.e. at a distance from the tumour cells, prior to visualisation.

7. The board considers that the technical details and measures implied for the methods as disclosed in post-published documents D7 and D8 do not go beyond the technical details and measures disclosed in the description of the patent application and is accordingly satisfied that the subject-matter of claim 1 was indeed reproducible without undue burden at the relevant date of the divisional application.

8. Accordingly, on the basis of the above considerations the board judges that the application discloses the subject-matter of claim 1 in compliance with the requirements of Article 83 EPC.

Novelty

9. The board is satisfied that none of the documents on file discloses an *in vivo* imaging method for detecting tumour cells in a living non-human mammal by measuring, through opaque tissue, the light emitted from a light generating protein expressed in the tumour.

10. For the above reason, the claimed subject-matter is novel pursuant to Article 54 EPC.

Inventive step

11. For assessing whether or not a claimed invention meets the requirements of Article 56 EPC, the boards of appeal apply the "problem and solution" approach, which requires as a first step, prior to the formulation of the technical problem to be solved by the invention as claimed, the identification of the closest prior art. In accordance with established case law of the boards of appeal the closest prior art is a teaching in a document conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, thereby requiring the minimum of structural modifications to arrive at the claimed invention.

12. The present application, in paragraph [0176], first sentence, indicates that "[t]*he bioluminescence technology is broadly applicable to a variety of hostpathogen systems and may also enable temporal and spatial evaluation of other biological events, as for example tumor progression and gene expression in living mammals, and have application in pharmaceutical*

development and screening." Indeed, the subject-matter of claim 1 concerns the non-invasive detection and imaging within the living mammal through opaque tissue such as skin of light (photons) emitted by tumour cells expressing a light-generating expressed protein. For the detection or measurement of the photon emission, the mammal has to be placed and maintained in the detection field of a photodetector device.

13. The board notes with reference to section IV above, that document D28 had been introduced into the proceedings by the board on the day of oral proceedings. Although it could be shown by the board that the publication of the document had occurred around the relevant date claimed for the present divisional application, the exact publication date of document D28 could not unambiguously be established. The board decided, in order to allow for more time to establish the precise publication date of document D28 and to safeguard the appellant's right to be heard, to take, for the assessment of inventive step for the purpose of this interlocutory decision, only those documents into account which had been on file before document D28 was introduced into the proceedings, i.e. documents D1 to D5 as cited in the reasons of the decision under appeal and documents D6 to D27 as cited in the statement of grounds of appeal.

14. With respect to these documents D1 to D27 on file, the board agrees with the appellant that, rather than document D5, which was identified by the examining division to represent the closest prior art for the assessment of inventive step of the methods claimed, document D2 represents this art. Contrary to the former,

the latter is the only document considered for the purpose of the present decision which discloses the non-invasive measurement and imaging within the living mammal and by means of a photodetector device of light emitted by cells which are associated with a light-generating protein through opaque tissue such as skin.

15. Document D2 is an abstract of a presentation made by one of the inventors before the filing date of the present application, is authored by all three inventors of the parent application and discloses experiments which correspond to the experimental examples as contained in the present patent application, namely rapid *in vivo* tracking or monitoring of bacterial infectious processes in animals.

15.1 In particular, *Salmonella* strains of varying virulence were transformed to contain the (prokaryotic) *lux* bioluminescence operon so as to express a bacterial luciferase capable of producing light when its substrate and oxygen are present. The bacterial glow could be detected through tissue, *in vitro* and *in vivo* whereby light detection required a low-light CCD-based imaging system (see abstract lines 9 to 11). Infection caused by intraperitoneal inoculation was reported to be easily visualized. In further experiments, mice of varying resistance were orally inoculated by virulent and avirulent strains of *Salmonella*. The virulent strains could be seen to spread widely, whereas for the less virulent strain no bioluminescence could be detected after 7 days (see abstract 12 to 17). Document D2 describes further that after inoculation and administration of an antibiotic to which the *Salmonella*

were sensitive, the bacteria optically disappeared within 5 hours (see abstract lines 16 to 17).

15.2 In the discussion section of document D2 the authors conclude that the technique allows the non-invasive tracking of bacterial infection *in vivo* registering only viable bacteria and that, seeing that bioluminescence is oxygen dependent, the engineered bacteria double as oxygen sensors. It is hypothesised that the approach allows for a real-time understanding of pathogenesis as well as the tracking of many processes in the body, such as infection or oxygen distribution in tumours (see abstract, last sentence, lines 18 to 24).

16. In the light of the closest prior art the objective technical problem to be solved by the invention as defined in independent claim 1 is hence the provision of a further application of the non-invasive method for detecting light-emitting proteins in a living, non-human mammal. The board is satisfied that the invention as disclosed in the patent application and claimed solves this problem (see point 4 above).

17. The board notes that document D2 itself does not suggest, neither implicitly nor explicitly, to apply the disclosed methodology to eukaryotic cells, let alone to tumour cells for their non-invasive imaging. Although the final sentence of document D2 mentions tumours (see point 15 above), the board is satisfied that this can be taken as referring to the use of the *Salmonella* bacteria as disclosed in the document as oxygen sensors and does not necessarily constitute a clear suggestion to the skilled person to apply the

disclosed bioluminescence technology in non-invasive detection methods for tumour cells in living mammals.

18. It therefore needs to be determined whether any of the other prior art documents taken into account for the present interlocutory decision (see above point 13) renders it obvious to the skilled person that the whole animal imaging methodology as applied to bacterial infection and disclosed in document D2, can be adapted so as to allow the non-invasive detection of tumour cells in a living, non-human mammal without requiring inventive skill.

19. Documents D3 and D4, both of which are comprised in the prior art, disclose the expression of (eukaryotic) luciferase (*luc* gene product) in eukaryotic cells and the *in vitro* detection of single cells expressing the marker or reporter protein. In document D3 the expression originated from a recombinant vaccinia virus and the *in vitro* imaging was conducted with an intensified CCD imaging system, whereas in document D4 the expression originated from a genomically located (eukaryotic) luciferase gene (*luc*) fused to a HIV-1 long terminal repeat and the *in vitro* imaging was conducted with a self-constructed device based on polaroid film.

20. The board considers that documents D3 and D4 may well describe the application of the *luc* expression system for the *in vitro* visualisation of eukaryotic cells in culture, the documents are however silent as to whether or not such cells are detectable within the body of a living mammal.

21. Similarly, document D5, the document which the examining division considered to represent the closest prior art, discloses so-called "rainbow" bioluminescence proteins which have been modified, e.g. in respect of intensity, colour or polarisation, such that their physical light-emitting properties are affected by their surroundings, i.e. their physical, chemical, biochemical or biological conditions. Document D5, in the paragraph bridging pages 7 and 8, states, in a very general and speculative manner, that the rainbow proteins may be used in the development of *inter alia* transgenic animals "*enabling gene expression, cell regulation, drug action, or cell damage to be located and measured in individual organs using the "rainbow effect".*" In the paragraph bridging pages 9 and 10, document D5 discloses, as one of the possible biological investigations in which the rainbow protein may be used, the detection and localisation of cancer cells.

22. The board notes, however, that document D5 does not disclose the detection and localisation of tumour cells as defined in claim 1 in mammals, by measuring through opaque tissue photon emission from the light generating protein associated with the tumour cells by a photodetector device. It notes furthermore that document D5 does not disclose the localisation of tumour cells in living, non-human mammals.

23. In view of the above considerations, the board concludes that none of the prior art considered for this decision renders it obvious to the skilled person that the whole animal imaging methodology as applied to bacterial infection and disclosed in document (2), can

be adapted so as to allow the non-invasive detection of tumour cells in a living non-human mammals.

24. The board adds that even if the skilled person, on the basis of the disclosure in the prior art, were to find it obvious to try adapting the methodology as described in document D2 in order to solve the objective technical problem and thereby arriving at the subject-matter as claimed, he would have been hampered by the following considerations from doing so.

25. Indeed, as has been argued by the appellant, and the board agrees herewith, the prokaryotic *lux* operon is fundamentally different from the eukaryotic firefly *luc* gene. The encoded luciferases use different luciferin substrates and energy sources and, contrary to the prokaryotic system, the eukaryotic system requires the administration or delivery of the luciferin substrate to the experimental animal. The required administration of the luciferin substrate at a distance from the tumour occurrence raised questions whether or not the substrate would become available in the tumour cells associated with the luciferase. The appellant has further argued that the method as described in document D2 was conducted with fast growing bacteria transformed with high copy numbers of the light-generating gene. The expected levels of gene expression would be lower for eukaryotic cells having a substantially lower rate of cell division and lower gene copy numbers.

26. In view of the above considerations the invention of claim 1 involves an inventive step, when considering only documents D1 to D27 (see point 13 above).

Order

For these reasons it is decided that:

1. The subject matter of claim 1 of the new main request (previous second auxiliary request filed with letter of 7 February 2007) complies with the requirements of Articles 54 and 83 EPC and with the requirements of Article 56 insofar as the documents D1 to D5 as cited in the reasons of the decision under appeal and documents D6 to D27 as cited in the statement of grounds of appeal are concerned.

2. Document 28 (Contag et al., Molecular Microbiology (1995) 18(4), pages 593-603) is introduced into the proceedings.

3. The proceedings are continued in writing.

The Registrar: The Chair:

P. Cremona U. Kinkeldey