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Datasheet for the decision of 20 November 2014

Case Number: T 1526/11 - 3.3.08

Application Number: 02732970.5

Publication Number: 1399559

IPC: C12N15/13, A01K67/027, C07K16/00

Language of the proceedings: EN

Title of invention:
MOUSE LAMBDA LIGHT CHAIN LOCUS

Patent Proprietor:
Crescendo Biologics Limited

Opponents:
Harbour Antibodies /Erasmus Univ.Medical Center NL
Leeming, John Gerard
Merus B.V.

Headword:
Lambda light chain knock-outs/CRESCENDO BIOLOGICS LTD

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

Keyword:
Main request - requirements of the EPC met (yes)

Decisions cited:

Catchword:

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Case Number: T 1526/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 20 November 2014

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Appellant: (Opponent 3)	Merus B.V. Uppsalalaan 8 3584CT Utrecht (NL)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted on 10 May 2011 concerning maintenance of the European Patent No. 1399559 in amended form.**

Composition of the Board:

Chairman M. Wieser
Members: B. Stolz
J. Geschwind

Summary of Facts and Submissions

I. Three oppositions were filed against European patent No. 1 399 559. After oral proceedings, the opposition division decided that the patent could be maintained on the basis of the main request filed on 4 December 2009. Appeals against this decision were filed by all three opponents. Opponent 3, Merus BV, later withdrew its appeal.

II. Opponent 1 (appellant I) and opponent 2 (appellant II), respectively, filed their statements of grounds of appeal. Appellant II resubmitted document D65, and filed new documents D76 to D98.

III. With its response to the grounds of appeal, the patent proprietor (respondent) submitted new auxiliary requests I to III and new documents D99 to D101.

IV. Appellant II submitted further arguments.

V. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), annexed to summons for oral proceedings, informed the parties of the preliminary non-binding opinion of the board on relevant issues of the appeal proceedings.

VI. The respondent made further submissions. Opponent 3 informed the board that it would not attend the oral proceedings.

VII. Oral proceedings were held on 20 November 2014, in the absence of opponent 3. The respondent made auxiliary request III its new main request and withdrew all preceding requests.

VIII. Claim 1 of the main request reads as follows:

"1. A mouse in which the endogenous mouse λ light chain locus is functionally silenced, characterised in that the mouse has a deletion of λ light chain genes selected from the following part of the λ locus region:

(a) C2 and C3-C1; or

(b) C2-C1 (i.e. C2-C4-C3-C1)."

Claims 2 to 7 define specific embodiments of the mouse of claim 1, claims 8 to 10 define uses of the mouse of claim 1, and claims 11 and 12 define methods of producing the mouse of claim 1.

IX. The following documents are cited in this decision:

D1: Bruggemann et al., PNAS, 86, 6709 - 6713, (1989)

D2: WO 90/04036

D4: WO 98/24884

D6: Li et al., PNAS, 93, 12, 6158 - 6162 (1996)

D7: Schlake et al., Oncogene, 18, 6078 - 6082, (1999)

D8: Van Duersen et al., PNAS, 92, 16, 7376 - 7380, (1995)

D9: Sun & Storb, JEM 193, 6, 699 - 711, (March 2001).

D16: Sauer, Methods, 14, 381 - 392, (1998).

D18: Ramirez-Solis et al., Nature, 378, 720 - 724, (1995).

D19: Madsen et al., PNAS, 96, 10338 - 10343, (1999).

D22: Zou, Bruggemann et al., Eur. J. Immunol., 25, 2154 - 2162, (1995).

D24: Shinkai et al., Cell, 68, 5, 855 - 867, (1992).

D25: Mombaerts et al., Cell, 68, 5, 869 - 877, (1992).

D28: Kitamura et al., Nature 350, 423-426, (1991).

D29: Kitamura et al., Cell, 69, 623-631, (1992)

D42: Ren et al., Genomics 84, 686-695, (2004)

D43: Glaser et al., Nature Genetics, 37, 1187-1193, (2005)

D44: Mueller, Mech Dev 82, 3-21, 199

D52: Wu et al., Nature Protocols, Vol. 3(6), 1056-1076, (2008)

D59: Declaration by Dr Simon Andrews

D64: Weiss et al., Eur. J. Immun. 1985, 15, 765-768

X. The arguments of appellant I, as far as relevant for this decision can be summarized as follows:

Article 83 EPC

Claim 1 was not limited to the specific deletions disclosed in the examples. It encompassed deletions creating non-viable mice.

Article 54 EPC

As shown in Figure 58 and explained on page 224 of document D4, the lambda light chain locus could be inactivated by the generation of two small deletions in the C2-C4 and C3-C1 region. The fact that repetitive DNA sequences made knock-outs more difficult, did not mean that what was described in document D4 was not possible to achieve. Successive rounds of targeting in ES cells did not pose a real problem.

Article 56 EPC

Starting from document D4, disclosing the strategy, the technical problem consisted in providing a method for silencing the λ light chain locus. A possible solution was the creation of deletions using the CreLoxP system disclosed for instance in documents D5 to D8 or D16. The expectation of success was reasonable. Neither the size of the deletion, nor the successive targeting of two separate sites in ES cells posed real problems. Some sequence information was available from document D64 and only two small, well targeted deletions were required.

XI. With regard to the new main request (former auxiliary request III) Appellant II did not wish to add anything to appellant I's submissions.

XII. The arguments of the respondent, as far as relevant for this decision can be summarized as follows:

Article 83 EPC

The description and Figure 1 of the patent provided the necessary technical information to the skilled person to put the invention into practice across the full scope of the claim 1.

Article 54 EPC

The λ light chain locus was known to be different from other immunoglobulin loci. It had a complex, repetitive structure with a large intervening sequence between the C genes. The structure of the intervening sequence was unknown and any effect of deletions in this region was unpredictable. Several documents on file reported difficulties when targeting highly repetitive loci. Document D4 did not provide any structural information and merely suggested the construction of restriction maps from phage libraries in order to construct suitable vectors. The disclosure of document D4 was hypothetical and not enabling for the skilled person.

Article 56 EPC

Starting from document D4, the technical problem underlying the present invention was the provision of a mouse with a functionally silenced λ light chain locus. The prior art referred to by the appellants did not relate to knock-outs in the λ light chain region and did not assist in solving the technical problem. Since the construction of such large deletions in a part of the genome with highly repetitive structures was difficult per se and it was neither known whether the intervening sequence between the C2-C4 and C3-C1 loci contained essential chains nor whether surrogate light chain genes could take over the function of the deleted

genes, the skilled person had no reasonable expectation of success.

XIII. The appellants I and II requested that the decision under appeal be set aside and the patent be revoked.

XIV. The respondent requested that the decision under appeal be set aside and the patent be maintained on the basis of claims 1 to 12 of the new main request.

Reasons for the Decision

Admissibility of the main request

1. The main request, filed on the day of the oral proceedings, was originally filed as auxiliary request III with respondent's response to the grounds of appeal. Claim 1 of the new main request results from the combination of independent claim 1 with dependent claim 16 of the main request of the decision under appeal. Claims 2 to 10 correspond to claims 3 to 11, and claims 11 and 12 correspond to claims 13 and 14, respectively, of the main request of the decision under appeal. Previous claims 2, 12 and 15 have been deleted. The appellants did not object to the admissibility of this request. The board decides to admit it into the proceedings.

Admissibility of documents

2. Documents D1 to D64 formed the state of the art considered in the opposition proceedings and in the decision under appeal. The opposition division did not admit documents D65 to D75 into the proceedings (cf. point 1 of the decision under appeal). Documents D76 to D98 were filed by appellant II with its grounds of

appeal. Documents D99 to D101 were filed in the appeal procedure by the respondent.

3. Documents D65 to D75 were filed only one month before the oral proceedings and deemed not to be prima facie relevant by the opposition division. The board takes the view that the opposition division correctly exercised the discretion given to it under Article 114(2) EPC when deciding not to admit documents D65 to D75. Thus, these documents do not form part of the appeal proceedings.

4. The chairman informed the parties that it was very unlikely that all documents filed in the appeal procedure would be admitted into the procedure and asked them to indicate which of these documents had a prominent role in substantiating the parties' arguments. Appellant II referred to document D78 only. The respondent considered none of the documents cited in the appeal procedure to be absolutely necessary.

5. Document D78 is an extract from a textbook describing strategies for gene targeting in ES cells. This document is no more relevant to the case at issue than for instance documents D6, D18 or D19.

6. In view of the above, the board decides not to admit any of documents D65 to D101 into the proceedings.

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

7. The appellants did not raise any objections under these Articles and the board sees no need to raise any of its own motion.

Article 83 EPC

8. For the purpose of this decision, the gene arrangement of the relevant part of the λ light chain locus can be summarily described as "C2-J4-C4-(120kb intervening sequence)-C3-J1-C1".

9. The mouse of claim 1 is characterized by deletions of either the C2 and C3-C1 genes (two separate small deletions), or of the entire C2-C1 region of the λ light chain locus. All objections concerning insufficient disclosure, which were previously raised in relation to functional silencing by deletions in any other regions of the λ light chain locus, do no longer apply.

10. Appellant I submitted that the claim, in the absence of more specific definitions of the deletions, encompassed non-viable mice, i.e. non-working embodiments.

11. The patent discloses the screening of a phage λ library of mouse ES cell DNA with Cλ probes which identified Cλ genes, and the construction of vectors comprising the C2-C4 or the C3-C1 regions and loxP sites ([0029] and Figure 1). Viable knock-out mice according to claim 1 were created with these constructs ([0030-0031]).

12. Moreover, it is disclosed that deletion of the roughly 120 kb sequence interval between the C2 and the C1 genes (cf. Figure 1) does not affect viability of the mice (cf. [0037]).

13. The appellant has not provided any evidence or facts why deletions of the C1 to C4 genes using different vector constructs would affect viability of the claimed

mice and, therefore, has not discharged its burden of proof.

14. Accordingly, in view of the disclosure by the patent, the objection is dismissed.

Article 54 EPC

15. Document D4 describes a strategy for the targeted knock-out of the lambda light chain locus in mice (Example 28 and Figure 58). The lambda light chain locus is described as spanning approximately 200 kb with an interval of about 120 kb between two gene clusters (cf. Figure 58). As a first approach, a single targeted deletion of the entire 120 kb region flanked by the C2-C4 and C3-C1 genes was suggested but considered difficult to achieve (cf. page 223, lines 11-13). As an alternative, two smaller targeted deletions of the C2-C4 and C3-C1 gene regions were suggested. Some of the vectors proposed in Figure 58 are targeted to the same regions of the λ light chain locus as the vectors used in the patent in suit.

16. Targeted insertion by homologous recombination in many places of the mouse chromosome was known in the art and used to create knock out mice (cf. e.g. documents D1, D2, D4, D9, D22, D24, D25). The deletion of up to 200 kb of chromosomal DNA and the generation of mice was possible with the Cre/LoxP system (cf. e.g. documents D6, D44, D42). Double targeted insertions by two consecutive recombination events have been successfully performed in other parts of the mouse chromosome (cf. documents D18 and D19).

17. It was not disputed that document D4 conceptually disclosed methods for the functional silencing of the λ

light chain genes. The respondent submitted, however, that document D4 did not provide an enabling disclosure due to the many technical uncertainties the skilled person was confronted with.

18. As stated in document D4, the arrangement of the genes in the λ light chain locus was known to be different from the arrangement of the genes in the Ig heavy and k light chain loci (pages 222 and 223). It was known that the λ light chain locus, like other Ig loci, comprised repetitive homologous sequences rendering targeted deletions more difficult. Moreover, as stated in expert declaration D59, at the date of filing there was incomplete sequence information about the 120kb intervening sequence consisting of an unordered and incomplete set of partially overlapping sequences obtained from BAC genomic clones. It was therefore not known whether this region comprised further genes with vital functions or regulatory sequences affecting up- or downstream sequences.

19. Document D44, published before the filing date of the patent in suit, is entitled "*Ten years of gene targeting: targeted mouse mutants, from vector design to phenotype analysis*" and provides an overview of the technology available in the art. Regarding deletions, it is mentioned that replacement type targeting vectors up to about 20 kb may be routinely used without drastically lowering the targeting frequencies (page 5, right column, bottom). The creation of large deletions, up to 200 kb based on the Cre mediated recombination system is discussed (pages 8 and 9). With regard to the phenotype of targeted mouse mutants, it is stated that the phenotypes are not always those predicted from the presumed function of a given gene product. In some instances unexpectedly lethal phenotypes have been

observed whereas in other cases null mutants revealed either very minor or no apparent phenotypes presumably due to functional redundancies at the gene level (page 10, right column, top). Furthermore, the generation of large genomic deletions may also lead to the unintended loss of as yet unidentified genes or of regulatory elements governing the expression of unrelated genes (page 6, right column, 2nd paragraph).

Document D43, published after the present filing date, is entitled "*Current issues in mouse genome engineering*" and discusses several factors affecting successful genome engineering. Regarding gene targeting constructs, the authors state that targeting has not been without complications, mainly related to the way in which targeting constructs have been built (page 1188, left column, lines 1 to 3), that it is still unclear what lengths of targeting constructs are optimal, that such optima probably vary from locus to locus, and that targeting in mice remained unpredictably variable (page 188, left column, 2nd paragraph).

Document D52, published considerably after the present filing date, is entitled "A protocol for constructing gene targeting vectors: generating knockout mice for the cadherin family and beyond" and discloses a set of vectors to facilitate the construction of the targeting vectors. Regarding the design of targeting vectors, the authors note that the presence of excessive repetitive DNA can significantly reduce the targeting frequency (page 1058, left column, 2nd paragraph), and that if both arms contain an excess of repetitive DNA sequences, the targeting frequency will be low (page 1060, right column, 2nd paragraph).

20. To sum it up, while the tools for targeted gene knock-outs in mice had been available, and in many cases successfully used, each new target presented a new challenge even a long time after the present filing date.

In the present case, the primary factors contributing to this challenge were the expected repetitive structures, the incomplete sequence information and the unknown functions of the intervening sequence.

21. Since document D4 only contains an outline of the strategy to silence the λ light chain locus but leaves it to the skilled person to establish the conditions for successful completion, it does not provide an enabling disclosure and, therefore, cannot anticipate novelty of the claimed subject-matter .

Article 56 EPC

22. Starting from document D4, representing the closest state of the art, the technical problem underlying the claimed invention is the provision of a mouse with a silenced λ light chain locus.

23. As disclosed in paragraphs [0036-0040] of the patent, the λ light chain locus of mice with two separate deletions in the C2 and the C3-C1 regions, or a single deletion of the entire C2 to C1 region, is functionally silenced.

24. The board is therefore satisfied that the underlying problem has been solved.

25. Document D4 considered the creation of a single large deletion of the entire C2-C1 region difficult to

achieve and therefore unlikely to lead to success. This solution is therefore not derivable in an obvious way from the closest prior art document, either if taken alone or in combination with any other prior art document on file.

26. The second approach disclosed in document D4 proposes the introduction of two independent shorter deletions in the C2 and the C3-C1 regions, for obtaining a mouse with a functionally silenced λ light chain locus.

27. It has to be established whether the skilled person trying to implement this strategy had a reasonable expectation of success and, thus, would have arrived at the claimed subject-matter in an obvious way.

28. Appellant I submitted that this was so because two small deletions well targeted to conserved regions of the λ light chain locus, the genomic organization of which was known from e.g. document D64, and two successive insertions into the genome of ES cells, did not pose serious technical problems.

29. As already mentioned in points 16 and 17 above, the skilled person was in possession of the necessary technical tools for putting into practice what was proposed in document D4. The use of Cre recombinase based deletions was also generally known.

As mentioned in points 18-20, above, there were however a number of unknowns resulting from the size of the λ light chain locus, its structural complexity due to the expected presence of repetitive homologous sequences, and its to a large extent unknown nucleic acid sequence of the intervening sequence, which summed up to a low

expectation of success, when trying to implement the teaching given in document D4 at a theoretical level.

30. In order to substantiate its argument, appellant I pointed to document D6, which disclosed the targeted deletion of the amyloid precursor protein (APP) gene by the insertion of two loxP sites up and downstream of the DNA segment to be deleted of about 200 kB. The sequence of the APP locus is however different from the λ light chain locus. As shown in expert declaration D59, the APP locus, as opposed to the λ light chain locus, contained no significant regions of self similarity (repetitive elements) which would render the design of arms suitable for homologous recombination more difficult (cf. pages 5, 6). The complete DNA sequence of the APP locus was known. Since only incomplete and unordered sequence was available for much of the λ light chain locus, this left the skilled person with the task of designing and testing various targeting sequences. Even if some guidance could be derived from documents D64 or D9 about the creation of suitable vector elements, this could not take away the inherent uncertainty. Thus, although document D6 showed the successful deletion of about 200 kb of genomic DNA it could not increase the expectation of success in the present case.

31. In a similar way, documents D7 and D8 demonstrated targeted deletions in other loci which for the same reasons as stated in relation to document D6 could not help in increasing the skilled persons expectation of success in the present case.

32. A further reduction in the already low expectation of success resulted from the need to have two loxP sites inserted. As stated in document D16 (page 388, right

column), "*a nagging worry in all excision strategies in vitro is that multiple rounds of ES cell manipulation may result in a population of cells with reduced totipotency, thus jeopardizing chances that the gene-modification locus will be transmitted through the germline*". The document offers a solution to avoid a second round of manipulation of ES cells in relation to the removal of marker sequences but not in relation to the creation of a second deletion in a different site. This additional source of uncertainty is not mitigated in the present case by the fact that some successful double insertions have been described in the art (cf. documents D18, D19 and the above mentioned insertion in the APP locus in D6). None of these documents offers any guidance in the form of, for instance, a general teaching raising the expectation of success for a skilled person trying to solve the problem underlying the present invention.

33. Documents D28 and D29, disclose deletions of single repetitive immunoglobulin loci encoding the μ chain gene and the λ5 gene. They also do not provide any information that could be extrapolated to other loci and that would help a skilled person to increase its very low expectation of success when targeting the regions encoding the two constant gene regions of the λ light chain locus.

34. The same applies to a number of further documents relied upon by the appellants in support of their arguments. All of them disclose the targeting of loci different from the λ light chain locus.

35. The board concludes that in view of all the above mentioned difficulties, the skilled person did not have a reasonable expectation of successfully solving the

underlying technical problem based on the teaching of document D4 alone or in combination with any of the cited prior art documents.

36. Since the subject-matter of claims 1 to 12 cannot be derived from the prior art in an obvious way, the main request meets the requirements of article 56 EPC.

37. At the oral proceedings, the appellant submitted amended pages 3 to 5 of the description to bring it in line with the main request. The board is satisfied that this has been done in agreement with the requirements of the EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent on the basis of :

pages 3 to 5 of the description filed at the oral proceedings and pages 6 to 13 of the description of the patent as granted,

claims 1 to 12 of the main request filed at the oral proceedings,

figures 1 to 5 of the patent as granted.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated