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Datasheet for the decision of 9 November 2015

Case Number: T 2220/14 - 3.3.08

Application Number: 02709544.7

Publication Number: 1360287

IPC: C12N15/00

Language of the proceedings: EN

Title of invention:
METHODS OF MODIFYING EUKARYOTIC CELLS

Patent Proprietor:
REGENERON PHARMACEUTICALS, INC.

Opponents:
Kymab Limited
Merus B.V.
Novo Nordisk A/S

Headword:
VelocImmune mouse/REGENERON

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 123(2), 123(3)
RPBA Art. 12(4), 13(1), 13(3)

This datasheet is not part of the Decision.
It can be changed at any time and without notice.

Keyword:
Postponement of the oral proceedings - (no)
Admission of documents (139), (161), (169) to (172) and (175) - (no)
Admission of documents (156), (158) and (168) - (yes)
Reopening debate - (no)
Confidentiality of documents (169) to (172) - (yes)
Admission of main request filed at the oral proceedings - (yes)
Requirements of the EPC fulfilled - (yes)

Decisions cited:
G 0007/93, T 0012/81, T 0056/87, T 0158/91, T 0452/91, T 0612/92, T 0694/92, T 0639/95, T 1149/97, T 1119/05, T 1621/09, T 0161/11

Catchword:
Sufficiency of disclosure - see sections 55 to 72

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Case Number: T 2220/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 9 November 2015

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Respondent III: (Opponent 03)	Novo Nordisk A/S Novo Allé DK-2880 Bagsvaerd (DK)
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Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 28 November 2014 revoking European patent No. 1360287 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman	M. Wieser
Members:	M. R. Vega Laso
	D. Rogers

Summary of Facts and Submissions

I. The appeal from the patent proprietor (appellant) lies from a decision of an opposition division of the European Patent Office under Article 101(3)(b) EPC posted on 28 November 2014, by which the European patent No. 1 360 287 with the title "Methods of modifying eukaryotic cells" was revoked. The patent had been granted on the European patent application No. 02709544.7 filed as international application under the PCT and published as WO 02/066630 (in the following "the application as filed").

II. The patent in suit was granted with 32 claims. Claims 1, 14 and 15 read as follows:

"1. A method of modifying an endogenous immunoglobulin variable region gene locus in an isolated mouse embryonic stem (ES) cell by an *in situ* replacement of the endogenous locus with an orthologous human gene locus or by an *in situ* replacement of one or more V and J, or V, D, and J gene segments of the endogenous locus with orthologous human V and J, or V, D and J gene segments, said method comprising:

a) obtaining a large cloned genomic fragment greater than 20kb containing orthologous human V and J, or V, D, and J gene segments;
b) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (a) to create a large targeting vector for use in a mouse ES cell (LTVEC);
c) introducing the LTVEC of (b) into a mouse ES cell to replace said endogenous immunoglobulin variable gene locus or said one or more V and J, or V, D, and J segments thereof *in situ* with the orthologous human

gene locus or the orthologous human V and J, or V, D and J gene segments; and
d) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (c) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced *in situ* with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.

14. A genetically modified eukaryotic cell or a mouse comprising a genetically modified immunoglobulin variable region locus obtainable by the method of any one of the preceding claims *in situ* in place of the endogenous immunoglobulin variable region gene locus.

15. A mouse embryonic stem (ES) cell containing a genetically modified immunoglobulin variable region gene locus obtainable by the method of any one of claims 1 to 12 *in situ* in place of the endogenous immunoglobulin variable region gene locus."

III. Two oppositions to the grant of the patent were filed based on the grounds for opposition under Article 100(a) in conjunction with Articles 54 and 56; Article 100(b) and (c) EPC. An intervention pursuant to Article 105(1) EPC based on the same grounds for opposition was filed during the proceedings before the opposition division.

IV. In the decision under appeal, the opposition division found that the subject-matter of the claims according to each of the main request and first to fourth auxiliary requests then on file extended beyond the content of the application as filed (Articles 100(c) and 123(2) EPC; see pages 7 and 8 under the headings

"*Homology arms greater than 20 kb*" and "*one or more V and J or V, D and J gene segments*", and page 10 of the decision). The subject-matter of the claims according to the fifth auxiliary request then on file was considered to lack novelty in view of documents (4), (8) and (9) (see page 15, first full paragraph of the decision). Furthermore, the opposition division found that the subject-matter of the sixth to sixteenth auxiliary requests did not involve an inventive step within the meaning of Article 56 EPC (see section 3 starting on page 18, and section 2 bridging pages 23 and 24 of the decision). As *obiter dictum*, the opposition division stated that it considered the requirements of Article 83 EPC to be met (see section 1 bridging pages 24 and 25 of the decision).

Among the evidence submitted by the parties after the deadline for making submissions in preparation for the oral proceedings, the opposition division admitted document (130) into the proceedings. Documents (129) and (142) were not considered to be *prima facie* relevant and, therefore, were not admitted (see the passage under the heading "Admissibility of late filed documents" in section 4 starting on page 11 of the decision). As regards document (139), the opposition division considered that, even though the humanized mouse described therein was intended to solve the same problem as that of the patent in suit, it represented a different solution to this problem than that proposed in the patent in suit and was therefore *prima facie* irrelevant with regard to Articles 54, 56 and 83 EPC. Hence, the document was not admitted into the proceedings (see section 2 on page 18 of the decision under appeal).

V. By letter dated 23 December 2014, the appellant requested accelerated processing of the appeal on the basis both that the proceedings before the opposition division had been accelerated and that the patent was the subject of on-going infringement litigation. Respondents I, II and III (opponents 01, 02 and 03, respectively) were given the opportunity to comment. Upon consideration of the parties' submissions, the board decided to grant the appellant's request and suggested a timetable for the parties' submissions and oral proceedings to be held on 27 to 29 October 2015.

VI. Together with its statement setting out the grounds of appeal, the appellant filed 22 sets of claims as main request and auxiliary requests 1 to 21, respectively, as well as additional evidence. On 26 March 2015, the appellant replaced the sets of claims according to auxiliary requests 1, 6, 10, 11 and 15 by amended sets of claims in which typographical errors had been corrected.

VII. The respondents replied to the statement of grounds of appeal and submitted further evidence.

VIII. In a communication under Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) attached to the summons to oral proceedings, the board commented on some procedural and substantive issues to be discussed at the oral proceedings.

IX. On 8 September 2015, respondent I submitted additional evidence (documents (169) and (170)) and requested that this evidence be kept confidential. Respondent II addressed the board in this respect and filed further evidence (document (171)).

X. In reply to the board's communication, the appellant submitted 12 sets of claims as main request and auxiliary requests 1 to 11 that replaced its previous requests. The appellant also made submissions on procedural and substantive issues.

XI. By communications dated 22 September and 2 October 2015, the board informed the parties that the admission into the appeal proceedings and the status of the documents filed by respondent I on 8 September 2015 would be discussed at the scheduled oral proceedings, and that, in the meantime, the documents in question and the submissions relating to them would not be available for public inspection.

XII. On 12 October 2015, the appellant filed additional confidential evidence (document (172)).

XIII. Further evidence (documents (173) and (175); see section XIX below) was submitted by respondent II on 25 October 2015.

XIV. Oral proceedings were held on 27, 28 and 29 October 2015. With the agreement of the parties, the oral proceedings were adjourned until 9 November 2015.

XV. On 4 November 2015, respondent II submitted arguments in support of its request to admit document (175) into the proceedings, and requested to re-open the debate on the admittance of documents (169) and (172) as well as on issues under Articles 83 and 56 EPC.

XVI. On the same day, the appellant filed a set of claims as a new first auxiliary request that replaced the corresponding previous request.

XVII. The oral proceedings were resumed on 9 November 2016. At 16:00 hours, the appellant replaced the claims according to its previous main request by amended claims 1 to 6.

XVIII. Claim 1 of the main request differs from claim 1 of the patent as granted (see section II above) in that the wording "*... by an in situ replacement of the endogenous locus with an orthologous human gene locus or ...*" and "*one or more* [V and J, or V, D and J gene segments]" has been deleted from the preamble of the claim, and the additional feature "*... to create a modified immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions, ...*" has been introduced at the end of the preamble. Moreover, the wording of steps c) and d) has been amended to read:

"...

c) introducing the LTVEC of (b) into a mouse ES cell to replace said V, D, and J segments *in situ* with the orthologous human V, D and J gene segments; and

d) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (c) to identify a mouse ES cell in which said V, D and J segments have been replaced *in situ* with the orthologous human V, D and J gene segments."

Claims 2, 3 and 4 are identical to, respectively, claims 5, 6, and 8 of the patent as granted, except that the dependencies have been adapted. Claims 5 and 6 are identical to claims 14 and 15 of the patent as granted (see section II above), except that claim 6 now refers to any one of claims 1 to 4.

XIX. The following documents are referred to in the present decision:

(1): US 6,114,598, published on 5 September 2000;

(3): Y.-R. Zou et al., 1994, Current Biology, Vol. 4, No. 12, pages 1099 to 1103;

(4): WO 99/45962, published on 16 September 1999;

(7): S. Taki et al., 19 November 1993, Science, Vol. 262, pages 1268 to 1271;

(8): WO 91/00906, published on 24 January 1991;

(9): WO 90/04036, published on 19 April 1990;

(10): Y. Thang et al., October 1998, Nature Genetics, Vol. 20, pages 123 to 128;

(18): WO 98/24893, published on 11 June 1998;

(46): Statement of Andrew Murphy, dated 27 January 2014;

(77): US 6,069,010, published on 30 May 2000;

(79): M. Kingzette et al., September 1998, Proc. Nat. Acad. Sci. USA, Vol. 95, pages 11840 to 11845;

(81): Declaration of Victor L. J. Tybulewicz, dated 15 July 2014;

(83): A. E. Karu et al., 1995, ILAR Journal, Vol. 37, No. 3, pages 132 to 141;

(94): Gene Targeting, A practical Approach, 2nd edition, 2005, ed. by A. L. Joyner, Oxford University Press, New York, pages i to xviii and 1 to 175;

(103): Statement of Prof. Dr. Hendriks, dated 15 July 2014;

(104): K. J. Seidl et al., March 1999, Proc. Nat. Acad. Sci. USA, Vol. 96, pages 3000 to 3005;

(112): K. R. Thomas and M. R. Capecchi, 6 November 1987, Cell, Vol. 51, pages 503 to 512;

(114): A. P. Monaco and Z. Larin, July 1994, TIBTECH, Vol. 12, pages 280 to 286;

(116): Statement of Craig H. Bassing, Ph. D., dated 16 July 2014;

(120): T. Blankenstein and U. Krawinkel, 1987, Eur. J. Immunol., Vol. 17, pages 1351 to 1357;

(121): C. M. Johnston et al., 2006, J. Immunol., Vol. 176, pages 4221 to 4234;

(126): A. J. Murphy et al., 2014, PNAS Early Edition, pages 1 to 6;

(127): L. E. Macdonald et al., 2014, PNAS Early Edition, pages 1 to 6;

(129): R. Wuerffel et al., November 2007, Immunity, Vol. 27, pages 711 to 722;

(130): K. J. Seidl et al., 1998, International Immunology, Vol. 10, No. 11, pages 1683 to 1692;

(139): E.-Ch. Lee et al., 16 March 2014, Nature Biotechnology, Advance online publication, pages 1 to 8;

(142): Excerpts from transcript of a Markman hearing held in the United States;

(156): M. Brüggemann, "The Preparation of Human Antibodies from Mice Harbouring Human Immunoglobulin Loci", in: Transgenic Animals, Generation and Use", 1997, ed. by L. M. Houdebine, pages 397 to 402;

(158): H.-L. Dougier et al., 2006, Eur. J. Immunol., Vol. 36, pages 2181 to 2191;

(161): Opinion & Order of of District Judge Katherine B. Forrest, dated 21 November 2014 in *Regeneron Pharmaceuticals, Inc. -v- Merus B.V.*, United States District Court, Southern District of New York;

(168): R. M. Gerstein et al., 1990, Cell, Vol. 63, pages 537 to 548;

(169): "VelocImmune History narrative - from Drew's memory", neither signed nor dated;

(170): Approved judgement of Mr Justice Arnold, dated 7 September 2015 in *Regeneron -v- Kymab*, Patents Court, London;

(171): Declaration of Ellen Broug, not dated;

(172): First witness statement of Andrew Joseph Murphy in *Regeneron –v- Kymab*, Patents Court, London, dated 2 October 2015;

(173): Notice of Electronic Filing, dated 22 October 2015 in *Regeneron Pharmaceuticals, Inc. –v- Merus B.V.*, United States District Court, Southern District of New York;

(175): Memorandum, Decision & Order of District Judge Katherine B. Forrest, dated 6 August 2015 in *Regeneron Pharmaceuticals, Inc. –v- Merus B.V.*, United States District Court, Southern District of New York.

XX. The submissions made by the appellant concerning issues relevant to this decision, were essentially as follows:

Request for postponement of the oral proceedings

There was no reason to postpone the oral proceedings. The evidence filed by respondent II at a late stage of the appeal proceedings, in particular documents (173) and (175) should not be admitted into the proceedings.

Admission of document (139) into the proceedings

In document (139) respondent I confirmed the usefulness of loci, cells, and mice of the patent in suit. There was a procedural deficiency in the opposition division's treatment of this document. The opposition division held it inadmissible for lack of sufficient relevance for the issue of sufficiency of disclosure, and refused to make a fresh decision on its admission in relation to the issue of inventive step. The board

should overturn the opposition division's decision on the admissibility of this document and admit it for all purposes, or admit it for the purpose of the second issue in particular.

Admission and confidentiality of documents (169) to (172)

Documents (169) to (171) should not be admitted into the proceedings and, in any case, they should be kept confidential in the sense of not being placed in the publicly accessible part of the file. If document (169) were admitted into the proceedings, so had document (172) to be admitted.

Articles 123(2)(3) and 84 EPC

The findings in the decision under appeal on these issues were correct.

Article 83 EPC - Sufficiency of disclosure

The opposition division had been correct in finding that the invention was disclosed in the application as filed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

Article 54 EPC - Novelty

The claimed subject-matter was novel, in particular with regard to documents (1) and (4). Document (1) described neither a method nor transgenic mice that produced hybrid antibodies containing human variable regions and mouse constant regions. Document (4) taught that some hybrid antibodies might be produced after a trans-switching event; however, this event represented

an unintended and unplanned for consequence of the introduction of a fully human immunoglobulin heavy chain locus which occurred naturally, rather than arising from any purposive engineering of mice in a particular way to improve antibody production. Thus, such antibodies were not the inevitable result of the teachings of document (4).

Article 56 EPC - Inventive step

The subject-matter of claims 1, 5 and 6 involved an inventive step. The adverse findings in the decision under appeal were severely flawed due to a misunderstanding of the claimed invention and the state of the art. The state of the art in production of antibodies in transgenic mice at the priority date had been clearly focused on making fully human antibodies directly in mice. In contrast, in the claimed invention improved antibody generation was obtained by allowing the process of recombination and class switching to occur in its native (murine) genomic context. This approach was particularly advantageous for the heavy chain because of the functionality of the murine constant region. As shown in document (46), even in transgenic mice having a chimeric heavy chain locus with only 3 human V segments spleenic B cell levels had been found to be near normal.

Contrary to the opposition division's view, document (1), which disclosed an embodiment in which a fully human immunoglobulin locus (human variable and constant regions) replaced and inactivated the endogenous locus, was to be considered the closest state of the art for the subject-matter of claims 5 and 6. Starting from this document, the problem to be solved was the provision of an improved platform for

antibody discovery, in particular as regards the development of human therapeutics. This problem was solved over the whole scope of the claims and the solution provided in the patent was not obvious to a person skilled in the art.

Document (4), which had been incorrectly regarded by the opposition division as the closest state of the art, described a "straight to human" mouse model. Document (4) did not teach that human variable regions should be operably linked to mouse constant regions in a DNA construct for expression of an antibody repertoire, and that the construct should be used for targeted integration *in situ* at the endogenous locus. The skilled person at the priority date would not have modified the transgene approach of document (4) based on the disclosure of document (7), which related to a very early exploration of gene targeting with a single rearranged human variable region gene. However, ES cells or mice having a rearranged immunoglobulin heavy chain locus did not fall under the scope of the claims.

XXI. Respondent I's submissions on issues relevant to this decision were essentially as follows:

Admission of documents (168) and (175) into the proceedings

Document (175) was a decision of a US District Court setting out many findings of fact relevant to the assessment of sufficiency (Article 83 EPC) and inventive step (Article 56 EPC) of the claimed invention. Although document (175) was late-filed in terms of the appeal procedure before the boards of appeal, it had been filed as early as possible, only

one day after it was issued. Because of its relevance, document (175) should be admitted.

Admission and confidentiality of documents (169) to (172)

Document (169), although late filed, had been filed as early as possible, as it was only following the decision of Arnold J in document (170) on 7 September 2015 that it was possible to present this document to the board. Document (169) showed that one of the inventors encountered difficulties putting the invention into effect and was therefore relevant to the issue of sufficiency under Article 83 EPC. Document (169) thus supported the view that it would involve an undue burden for the skilled person to put the invention into effect.

Document (170) was a procedural decision from the Patents Court in London in parallel proceedings involving the appellant and respondents I and III. In the English litigation these parties were subject to confidentiality undertakings which covered, inter alia, document (169). Document (170) was a decision of Arnold J, giving permission to respondent I to seek the permission of the board to admit document (169) into the current proceedings. Respondent II argued that this decision of Arnold J should be admitted as it contained an admission by the appellant that a "one step" cloning procedure was not technically possible (see paragraph 15 of document (170)).

Article 123(2) EPC

Re: "large cloned genomic fragment greater than 20 kb/ 100 kb" - Claims 1 and 4

The actual use of a cloned genomic fragment greater than 20 kb/100 kb in the method of claim 1 was not disclosed in the application as filed, neither was it supported by the Examples. The passage on page 6, lines 4 to 6, which the opposition division had considered to be the basis for this feature, taught only that the LTVEC was "*capable of accommodating*" a large DNA fragment greater than 20 kb/100 kb. This said something about the capacity of the LTVEC but nothing about the actual size or nature of the fragment actually used or accommodated within the LTVEC. The passage on page 8, lines 10 to 25, on which the appellant relied, disclosed a large cloned genomic fragment greater than 20 kb only in the context of a specific preferred embodiment of the invention, which was a mouse genomic fragment modified by deletion. However, deletion was different from *in situ* replacement as required in claim 1. The combination of features which were disclosed in a different context added subject-matter and offended against Article 123(2) EPC.

Re: "in situ replacement" - Claims 1, 5 and 6

Contrary to the opposition division's view, the term "*in situ*" was not a general feature of the invention. The opposition division had ignored the context of the passage on page 43, lines 14 to 19, which was the only teaching of "*in situ*" replacement in the application as filed and required that there is replacement of all mouse variable region VDJ/VJ genes with their human

counterparts. Moreover, since in the application as filed the term "*in situ*" was used only in the context of direct replacement, it could not be combined with elements of the general description which were taught in the context of a deletion, as it was the case with the large cloned genomic fragment of greater than 20 kb. Therefore, the combination of at least these two features was not permissible. Additionally, *in situ* replacement was only taught in the context of a mouse in which all VDJ and VJ genes were replaced by their human counterparts. Since this limitation was not in the claims, Article 123(2) EPC was contravened.

Re: "V, D and J gene segments" - Claim 1

Even though the wording "*one or more*" had been deleted from claim 1, the amended claim still contained added matter as it covered *inter alia* the insertion of one V, one D and one J gene segment. The functional feature included in claim 1 ("*... to create a modified ...*") was only disclosed in the application as filed in the context of a mouse that had all three chimeric loci (one heavy locus and two light chain loci) in homozygous form, and in which all of the V, D and J regions have been replaced. Therefore, the functional language could not be equated to a disclosure of only some V, D and J gene segments.

Re: "to create a modified immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions" - Claim 1

There was no basis in the application as filed for claiming the creation of a modified immunoglobulin locus that produced hybrid antibodies containing human variable regions and mouse constant regions, wherein

the locus did not have the entire murine variable region replaced with the entire human variable region. Second, while the passage on page 43, lines 14 to 15 and 27 to 28 referred to the resultant immunoglobulin loci (plural), claim 1 related only to the heavy chain immunoglobulin variable gene locus. The disclosure on page 43 was made only in respect of mice and not in respect of isolated ES cells. It was part of an example and required many additional elements, for instance loci rearrangement during B-cell development. Since these elements were disclosed only when operating in combination with other features, it was not permissible to delete them.

Re: Selection from lists - Claim 1

In order to arrive at the present claim 1, multiple selections had to be made. The claim required the replacement of the heavy chain variable region (selection of one of three possible lists) by orthologous human DNA (selection of one of two possibilities) contained in a cloned fragment greater than 20 kb (100 kb being a further possibility). As regards which parts of the variable region were replaced, the claimed subject-matter was selected from six different possibilities.

Article 84 EPC – Clarity and support

It was not clear what the term "*hybrid*" antibodies containing human variable regions and mouse constant regions meant.

Article 83 EPC - Sufficiency of disclosure

The opposition division was incorrect to conclude that the VelocImmune transgenic mice described in documents (126) and (127) could support the improved production of useful antibodies, because the disclosure in Example 3 of the patent in suit did not relate to those mice.

As far as the claims covered an insertion of human V, D and J gene segments without concomitant deletion of the mouse V, D and J gene segments, the method of claim 1 was not sufficiently disclosed because the MOA method was not informative.

The 5' location of the mouse Ig loci was not conclusively known at the priority date. Not knowing the 5' end of the locus, the skilled person did not know how to completely replace the mouse V region. Thus, the feature "*in situ replacement of V, D, and J gene segments of the endogenous locus with orthologous human V, D and J gene segments*" was unclear and the invention claimed in claim 1 was not sufficiently disclosed in the patent.

Claim 1 required that the modified Ig locus "*produces hybrid antibodies containing human variable regions and mouse constant regions*", and claim 6 was directed to a mouse ES cell containing such a locus. ES cells did not naturally produce antibodies, yet there was no teaching of any steps that should be taken to ensure that they did. Thus, the invention claimed in claim 6 was not sufficiently disclosed, within the meaning of Article 83 EPC.

To the extent that claim 1 was not limited to long LTVEC arms greater than 20 kb, the claim covered a method that cannot work, and thus the locus could not be produced. Moreover, since the claims did not specify the complete removal of the endogenous mouse locus, it was possible that antibodies that included a murine V segment were produced.

Article 54 EPC - Novelty

The subject-matter of claims 5 and 6 lacked novelty over document (1). The opposition division was wrong to conclude that the general teaching of document (1) was limited to only fully human antibodies. This conclusion ignored the specific wording of columns 5 and 10 which clearly taught hybrid antibodies containing human variable regions and mouse constant regions. In the passage from column 10, line 50 to column 11, line 2 it was taught that the variable region of the human immunoglobulin heavy chain locus (including V, D, and J gene segments), or portions thereof, was isolated and then this variable DNA ("*the human locus*") was flanked with sequences from the murine locus. The reference to "*the human immunoglobulin locus*" in column 10, line 63 did not mean the <u>full</u> human Ig locus, but rather the variable region part of the human locus. This reading was entirely consistent with the interpretation of the passage on column 5, lines 48 to 54. The wording "*and/or*" in this passage directly and unambiguously disclosed antibodies having xenogeneic, i.e. human regions.

Document (1) taught, in Example VII (see column 39, lines 14 to 26) and shown in Figure 16C, targeted insertion at one allele of a human heavy chain, including human C_μ and C_δ constant region gene segments

at the endogenous locus, as well as the Sμ switch region, to form a heterozygous mouse. The other, unmodified allele contained all the native murine switches and constant region gene segments. In a heterozygous mouse, the modified locus would inevitably trans-switch between alleles (as evidenced by documents (79), (158) and (4)), and thus would express rearranged human V, D and J gene segments upstream of mouse constant regions provided by the other allele. Thus, an ES cell comprising the modified immunoglobulin locus as shown in Figure 16C produced hybrid antibodies that had a human variable-mouse constant heavy chain structure.

Article 56 EPC - Inventive step

Document (4) as the closest state of the art

Document (4) was to be considered as the closest state of the art. This document taught a randomly inserted transgene, which required the host endogenous loci to be separately inactivated. A chimeric HVMC heavy chain locus was produced from a fully human locus *via* trans-switching, and hybrid antibodies useful for human therapeutic use were generated by immunization of mice. Document (4) explicitly contemplated the use of such a mouse as a platform for discovering antibodies against a predetermined antigen, isolating and testing those antibodies and humanizing the constant region to generate fully human antibody therapeutic products. The advantages of a repertoire of trans-switched antibodies were apparent from the passage on page 85, lines 10 to 32. The skilled person would be motivated by the teaching of document (4) to look for alternative, particularly more reliable and/or direct methods for generating the useful hybrid antibodies.

Starting from document (4), the objective technical problem was to provide alternative means for the production of antibodies comprising human variable-mouse constant heavy chains. The problem could not be to provide an improved means, because there were no data comparing the means proposed in the patent in suit and those in document (4). In particular, there was no evidence of an improved repertoire.

The solution proposed in the claims was obvious in view of document (7). The approach described therein was well-known and would be consulted by the skilled person looking at methods of modifying immunoglobulin loci. The techniques of documents (4) and (7) were clearly compatible. The skilled person would appreciate from document (7) the immediate benefits of using the targeting techniques already described in document (4) for a different purpose, in a single method step of inserting human V, D and J gene segments into the murine heavy chain immunoglobulin locus, concomitant with host locus inactivation, resulting in benefits from the correct chromosomal location of B-cell development, rearrangement, cis-switching and hypermutation. The skilled person would therefore use the method of document (7) to replace the J_H region of the endogenous murine locus with a human V, D and J gene segment, thus arriving at an ES cell and a mouse falling within the scope of claims 5 and 6.
Document (1) also supported the recognised advantage of carrying out simultaneous insertion and inactivation by targeting the endogenous loci directly, rather than using random insertion (see column 8, lines 19 to 50). Moreover, the passage bridging pages 1269 and 1270, and page 1270, middle column of document (7) was to be

regarded as an incentive to provide a less restricted antibody repertoire.

The skilled person would have a strong expectation of success that the inserted human V, D and J gene segments at the endogenous locus, in proper location relative to the constant region gene would function to produce hybrid antibodies. Document (156) encouraged the skilled person to use the methodology of document (7) to produce the hybrid antibodies of document (4). The role of the constant region in antibody maturation was known from document (18) (see page 25, lines 1 to 9).

Also the subject-matter of claim 1 was obvious in view of a combination of documents (4) and (7). Starting from document (4) as the closest state of the art, it was obvious to make the HVMC locus using standard techniques which were available in the art and even from the common general knowledge, either individually or in combination, as apparent from document (77). Document (4) taught the same methods used in claim 1 for targeting the endogenous locus, but for the purpose of inactivation. However, it would be obvious in view of document (7) to target human DNA into the endogenous locus to simultaneously insert and inactivate. Indeed, document (7) also disclosed the use of targeting vectors, and the use of quantitative methodology (Southern blot analysis) to assess insertion at the immunoglobulin locus (see page 16298, left hand column, last three lines).

Document (1) as the closest state of the art

Document (1) disclosed that "*<u>at least portions</u> of the human heavy and light chain immunoglobulin loci*" could

be used to "*directly replace the corresponding endogenous immunoglobulin loci by homologous recombination in embryonic stem cells*" (see column 6, lines 30 to 34).

Starting from document (1), the technical problem was to determine which portions of the human heavy and light chain immunoglobulin loci should directly replace the corresponding endogenous Ig loci. The solution proposed in claims 5 and 6 was obvious in view of document (4), in which the advantages of human variable-mouse constant hybrid antibodies were recited (see page 85, line 32 to page 86, line 24). Document (83) was fully supportive of the well-recognised value of the insertion at the endogenous locus, namely, regulation, stability and transmission. This was confirmed by document (156).

Also the subject-matter of claim 1 did not involve an inventive step over a combination of document (1) and document (4). The method of claim 1 differed from the method described in document (1) in that homologous recombination methodology was used to arrive at the targeted insertion vector. Thus, the objective technical problem was the provision of an alternative method for constructing a vector for targeted insertion. The use of homologous recombination as a technique for modification of nucleic acids was well known in the art at the priority date, as apparent from document (10) (Abstract). Homologous recombination methods were also disclosed in document (4) (see Example 33 on page 240).

XXII. Further arguments submitted by respondent II on the relevant issues were as follows:

Request for postponement of the oral proceedings

The oral proceedings should be postponed in order to give the board and the parties the opportunity to consider facts that had appeared in the US discovery procedure (documents (173) and (175). Otherwise, the right to be heard would be violated.

Admission and confidentiality of documents (169) to (172) - Article 13(3) RPBA

The requests of the parties, that certain documents not be placed upon the publicly accessible part of the EPO's file, derived from the fact that the appellant and respondents I and III were subject to confidentiality undertakings in parallel English proceedings. Respondent II stated that as it was not a party to this English litigation it was not subject to these undertakings.

Admission of claims 1 - 6 of Main Request filed at 16:00 hours on 9 November 2015 during the oral proceedings

The submission of a new claim request at such a late stage of the appeal proceedings constituted an abuse of procedure. There was no justification for the late-filing of the claims. The main request should not be admitted into the proceedings.

Article 123(2) EPC

The feature "*replacement of V, D, and J gene segments*" in claim 1 had no basis in the application as filed. Although the wording "*one or more*" had been deleted from the claim, the wording "*V, D, and J segments*"

still included single V, D, and J segments and combinations of specific, single V, D, and J segments, as well as rearranged V, D, and J gene segments. However, such embodiments were not disclosed in the application as originally filed.

There was no basis in the application as filed for replacing mouse endogenous segments by their orthologous segments. In fact, there was no disclosure in the application as to what was meant by an orthologous segment.

The introduction of the feature "*to create a modified immunoglobulin locus that produces hybrid antibodies ...*" into claim 1 offended against Article 123(2) EPC because there was no basis in the application as filed for a modified immunoglobulin locus that produces an antibody.

Article 123(3) EPC

Since none of the claims as granted had a hybrid antibody as the final product of the claimed methods, the scope of the patent as granted did not comprise hybrid antibodies. Thus, the introduction of the feature "*to create a modified immunoglobulin locus that produces hybrid antibodies ...*" into claim 1 contravened Article 123(3) EPC.

Article 84 EPC - Clarity and support

The meaning of the wording "*V, D, and J gene segments*" in claim 1 was not clear and this lack of clarity resulted from the deletion of the wording "*one or more*" which also rendered unclear the meaning of the wording "*orthologous human V, D, and J gene segments*". The

skilled person was left in doubt about which endogenous sequences were to be replaced by which orthologous sequences.

Although the term "*locus*" was included in different features of claim 1, it was not defined in the original specification. The skilled person would not know whether the "*locus*" refers to the whole locus or a part thereof, and whether the wording "*heavy chain variable region gene locus*" included the heavy chain constant region.

Also the meaning of the wording "*orthologous human*" was not clear. The definition provided in the passage on page 22, lines 22 and 23 of the application as filed, did not specify what "*functional equivalent*" meant with respect to a part of an orthologous human locus, i.e. which human V, D, or J gene segment was functionally equivalent to which mouse V, D, or J gene segment.

Apart from the fact that the term "*hybrid antibody*" was not clearly defined, the use of this term in the context of modification of the heavy chain variable region gene locus rendered claim 1 unclear because essential features, namely the requirement of a light chain locus were missing. The same applied to claim 5.

Article 83 EPC – Sufficiency *of disclosure*

The opposition division erred when coming to the conclusion that the requirements of Article 83 EPC are met. At the priority date, it was not known which specific V, D, and J segment is orthologous to a given, specific mouse V, D, and J segment. This was also the case today. This information, however, would be essential to practice the claimed invention.

The preamble of claim 1 required that a modified locus was created that produced hybrid antibodies. There could be no doubt that a locus could not produce antibodies, let alone a heavy chain locus which lacked the corresponding light chain.

There was no MOA assay available which could be used to determine the correct replacement of the gene locus. In Example 3, a MOA assay was described only on a theoretical level. However, no results were shown. The burden of proof lay with the appellant to show that the MOA assay could be performed.

Article 54 EPC - Novelty

Document (1)

The content of document (1), in particular the disclosure in column 5, lines 48 to 57; column 10, line 50 to column 11, line 2, Example VIII and Figure 16c in combination with the common general knowledge (as regards the bacterial homologous recombination specified in step b) of the method of claim 1), was novelty-destroying for the subject-matter of claim 1. This was corroborated by document (81).

The opposition division erred in interpreting the wording "... *that produces ...*" in claim 1 to require that the modified immunoglobulin locus may, under specific, not defined circumstances, result in a hybrid antibody. Since the chimeric locus specified in claim 1 did not inevitably produce hybrid antibodies (at least not in the absence of a locus encoding a light chain), it was also not necessary that a locus described in the

prior art inevitably led to the production of such antibodies.

It was undisputed that in Example VII and Figure 16c the production of a modified heavy chain locus was described. This locus included human variable regions, human constant regions and mouse constant regions, the human and mouse constant regions being separated by a *neo* sequence. Contrary to the opposition division's view, the *neo* sequence would have no effect on the downstream constant regions, as apparent from document (104) (and explained in document (103), sections 19 and 20). As apparent from documents (4) and (79), in a given percentage of the antibodies produced in mice, sequences encoding variable regions became linked to constant regions from the other allele by trans-switching.

The wording "*hybrid antibodies*" could be interpreted as referring to an antibody having a fully human heavy chain paired with a mouse light chain. The heterozygous mice described in Example VIII and Figure 17 also produced such hybrid antibodies and was therefore novelty-destroying.

Document (1) disclosed PCR for determining that homologous recombination has occurred. Thus, also the subject-matter of claim 2 was anticipated by document (1).

Since document (1) disclosed all steps of the method of claim 1, the locus shown in e.g. Figure 16c must be obtainable by the method of claim 1. Even if it were considered that step b) was not part of the common general knowledge, this would have no impact on the

structural features of the modified locus and had to be disregarded when assessing novelty.

Documents (4), (8) and (9)

The opposition division erred in finding that the subject-matter of claim 5 was novel over document (4) or (9). The wording "*in situ*" was to be interpreted to mean that one sequence is found at the position of another sequence. By the trans-switching event described in document (4), human variable regions were placed in front of mouse constant regions, resulting in hybrid antibodies. Contrary to the opposition division's finding, the chimeric locus was found in place of the gene encoding the endogenous constant region, as shown in Figure 4d of document (168). The same arguments applied with respect to document (9).

The subject-matter of claims 5 and 6 was anticipated also by document (8) which disclosed hybrid antibodies where a human variable region was placed before a mouse constant region (see claims 10 and 11), i.e. at the position of the mouse variable region.

Article 56 EPC - Inventive step

Document (1) as the closest state of the art

If the board considered that the difference between the method of claim 1 and that described in document (1) lies in step b) and/or the feature "*to create a modified immunoglobulin locus that produces hybrid antibodies*", in assessing inventive step the two features cannot be considered together because they had a different technical effect. If step b) were the difference, the problem to be solved was the provision

of an alternative method for modifying an immunoglobulin locus. The solution proposed in claim 1 would be obvious because bacterial homologous recombination was part of the common general knowledge. If the difference was the production of hybrid antibodies, the problem would be the provision of hybrid antibodies. Since the patent did not provide any experimental evidence showing that this problem had been solved, an inventive step could not be asserted.

Moreover, the method of claim 1 was obvious in view of a combination of document (1) with document (4) or (3), both disclosing that hybrid antibodies may be desirable under certain circumstances.

The features specified in claims 2 to 4 and the structural features of claims 5 and 6 were apparent from document (1). If at all, only the method steps could contribute to inventive step, as regards the product-by-process claims. However, the reasons put forward in connection with claim 1 applied also to claims 5 and 6.

Document (4) as the closest state of the art

Starting from document (4), the problem to be solved was the provision of an alternative method for the production of hybrid antibodies. At the priority date, it was known from e.g. document (1) that homologous recombination could be applied to produce fully human or chimeric antibodies. Furthermore, document (7) disclosed that immunoglobulin sequences could be replaced *in situ* by other immunoglobulin sequences. The skilled person would have used homologous recombination to replace the mouse variable immunoglobulin sequences

by their human orthologous sequence, thus arriving at the subject-matter of claim 1.

Document (8) as the closest state of the art

Document (8), by reference to document (112), disclosed the production of hybrid antibodies by homologous recombination. The problem to be solved was the provision of an alternative method for the production of hybrid antibodies. As correctly stated in the decision under appeal, the steps of the method of claim 1 were well-known in the art. Thus, claim 1 lacked inventive step in view of a combination of document (8) with document (112) and the common general knowledge.

Document (3) as the closest state of the art

The secreted antibodies described in document (3) had a mouse variable region and a human constant region. Document (3) taught that the murine portion "minimizes the danger of disturbing membrane expression and signalling". The problem to be solved was to provide hybrid antibodies with human variable and mouse constant regions. The solution proposed in the patent in suit was obvious from a combination of document (3) with document (1).

XXIII. Respondent III endorsed the arguments brought forward by respondents I and II.

XXIV. The appellant (patent proprietor) requested that the decision under appeal be set aside and the patent be maintained upon the basis of claims 1-6 of the main request submitted to the board at the oral proceedings on 9 November 2015 at 16:00 hours.

XXV. The respondents (opponents) requested that the appeal be dismissed

Reasons for the Decision

Request for postponement of the oral proceedings

1. Respondent II requested in its fax dated Friday 25 October 2015, and sent at 18:51 hours, that the oral proceedings starting on Tuesday 27 October 2015 be adjourned.

2. The reason for the request for such an adjournment was that a decision of a US court in litigation between the appellant and respondent II could be expected to be issued in a few weeks and that this decision, "*... may have a serious impact on sufficiency of disclosure and inventive step of the subject matter of the opponent patent ...*".

3. The board notes that the claims of the patent in the US litigation are not the same as the claims of the patent in suit in this appeal. The board does not consider that findings of fact made by another tribunal relating to different claims will assist the board in arriving at its decision. The board therefore rejects respondent II’s request for an adjournment of the oral proceedings.

Admission of evidence into the appeal procedure

Document (139) - Article 12(4) RPBA

4. Document (139) was filed roughly two weeks before the oral proceedings before the opposition division. The board therefore agrees with the opposition division that this document was late filed. Pursuant to Article 114(2) EPC, the opposition division therefore had a discretion to admit this document or to disregard it.

5. Such a discretionary decision should only be overruled in appeal if the discretion of the first instance department has been exercised according to the wrong principles, or without taking into account the right principles, or in an unreasonable way (G 7/93, OJ EPO 1994, 775, point 2.6 of the Reasons; T 1119/05 of 8 January 2008, point 3.2 of the Reasons).

6. The opposition division gave as its reasons for not admitting document (139) that it disclosed a humanized mouse for the production of therapeutic antibodies. The opposition division considered that if the mouse disclosed in document (139) was intended to solve the same problem as that of the patent in suit, it disclosed a different solution to this problem than that of the patent in suit and was therefore *prima facie* irrelevant for the consideration of Articles 54, 56 and 83 EPC.

7. The board therefore considers that the opposition division took its decision by taking into account the right principles in a reasonable way, and that this exercise of discretion should therefore not be overturned (see T 161/11 of 27 August 2014, points 9

to 12 of the Reasons). Document (139) is therefore not admitted into the appeal procedure.

Documents (156), (158) and (161) - Article 12(4) RPBA

8. Documents (156), (158) and (161) were submitted by respondent I together with its reply to the statement of grounds of appeal.

9. Pursuant to Article 12(4) RPBA, everything presented by the parties in, *inter alia*, the notice of appeal and statement of grounds of appeal, or any written reply thereto filed in due time shall be taken into account by the board if and to the extent it relates to the case under appeal and meets the requirements of Article 12(2) RPBA.

10. Document (156) was submitted as evidence that the results of document (7) had become part of the common general knowledge of the skilled person at the priority date of the patent in suit. Document (158) was filed as evidence for the frequency of trans-switching events in mice. Both documents are considered to be relevant to the assessment of novelty and inventive step and are therefore admitted into the proceedings.

11. Document (161), which was submitted to support respondent I's line of argument on Article 83 EPC, is entitled "Opinion and Order (Claim Construction)", authored by District Judge Katherine B. Forrest of the US District Court, Southern District of New York and dated 21 November 2014. The document concerns a counterclaim by respondent II for invalidity and non-infringement in relation to the appellant's US patent and contains the Court's determinations as to claim construction.

12. Document (161) relates to a US patent granted with claims that differ from those being considered by the board. Thus, its content cannot be considered to relate to the case under appeal, as required by Article 12(4) RPBA. Moreover, the board notes that the conclusions reached by Judge Forrest are based on evidence, in particular declarations and depositions by witnesses that have not been presented in the present appeal proceedings. Thus, if the board were to admit this document into the proceedings and consider its content, it would not be able to scrutinize the evidence on which Judge Forrest based her conclusions and would have to either consider or disregard them without much basis for either approach.

13. In view of the above, document (161) is not admitted into the proceedings.

Document (168) – Article 12(4) RPBA

14. Document (168), which was filed by respondent II with its response to the statement of grounds of appeal, relates to interchromosomal DNA recombination (trans-switching) and is relevant to the assessment of novelty. The board does not see any cogent reasons why this document should not be considered. Thus, document (168) is admitted into the proceedings.

Document (175) – Article 13(1)(3) RPBA

15. The board's discretion to admit and consider documents such as the late filed document (175) filed at a very late stage of the proceedings is codified in Article 13(1)(3) RPBA. This article also codifies the

power of the boards to ignore material unrelated to a case (see point 26 of T 1621/09 of 22 September 2011).

16. In procedures before the boards of appeal questions of patentability are to be decided solely in accordance with the EPC. As regards decisions made on patentability in Contracting States to the EPC, these are not to be cited as if they were binding upon the boards of appeal, and claims should not be refused because their patentability cannot be upheld under the jurisdiction of one member state (T 452/91 of 5 July 1995, point 5.4.1). Such considerations also apply to decisions of courts of non-member states such as the USA.

17. Document (175) is a decision of the US District Court, Southern District of New York by District Judge Katherine B. Forrest, dated 2 November 2015. The document is entitled "Opinion and Order", is 114 pages long and concerns an action in the United States between the appellant and respondent II that is described as being a counterclaim by respondent II for "inequitable conduct or affirmative egregious misconduct" in relation to the appellant's US patent (see bottom of page 2 of document (175)).

18. The board notes that document (175) concerns a patent with different claims than those before the board, that the US court heard witnesses that have not been heard by the board, that the decision concerns in part the withholding from the US court and patent office by the appellant of documents, all of which are before the board, and that the focus of the decision is on a US legal doctrine of "inequitable conduct" in the US patent granting process.

19. The board is thus unable to see the relevance of document (175) to the issue of the compliance of the claim requests before it with the requirements of Articles 56 and 83 EPC. The board therefore does not admit this document into the proceedings.

Documents (169) to (172) - Article 13(3) RPBA

20. Document (169) is late filed. Respondent I has explained the reasons for this late filing. The board's discretion to admit and consider such documents is codified in Article 13(1) RPBA. This article also codifies the power of the boards to ignore material unrelated to a case (see point 26 of T 1621/09, *supra*).

21. The board has read document (169) and has the impression that this document:

- Was produced for purposes other than the present dispute.
- Was produced from memory by one man many years after the events in question.
- Is neither signed, nor sworn.
- Refers to facts, events and persons in a vague way; for example, several individuals are named only by their first name.

22. The board is therefore of the opinion that document (169) is a document of a type and quality to which little weight can be given.

23. Respondent I submitted document (169) to support its case under Article 83 EPC. Article 83 EPC requires that a European patent application shall disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the

art. Such a requirement needs to be fulfilled at the date of filing of the application. The "person skilled in the art" is a notional figure, not a real person. Document (169) is written from memory by one of the inventors. It describes in an informal way the difficulties that the inventors had in getting the invention to work. The respondents seem to be suggesting that document (169) is relevant to Article 83 EPC upon the basis that if the inventors had problems putting the invention into effect, then this is evidence that the "person skilled in the art" would have such problems. The board is not convinced by this argument. The key issue is the disclosure of the European patent application and how the notional skilled person would understand this disclosure. A document describing what the inventors did in order to make their invention, even if such a document did not suffer from the weaknesses of document (169) enumerated above, would not assist on this point. The board therefore finds that document (169) lacks relevance as regards sufficiency of disclosure and the board therefore declines to admit document (169) into the proceedings.

24. Given its position on document (169), the board sees no reason to admit any of documents (170) to (172) into the proceedings, all of these documents being even less relevant than document (169) and deriving their significance, if any, from document (169).

Reopening debate on admission of documents (169) and (172), and reopening debates on Articles 56 and 83 EPC

25. The above requests are set out in respondent II's letter of 4 November 2015. That is they were made during the adjournment period between the first three

days and the last, and fourth day, of the oral proceedings. They were linked by respondent II to its request to admit document (175) into the proceedings, made in its letters of 3 and 4 November 2015

26. Given that the board has decided not to admit document (175), there is no reason to reopen the already completed debate on the admission of documents (169) and (172).

27. Further, as document (175) was not admitted into the proceedings, there is no new fact, evidence or argument before the board that would justify the reopening of the already completed debate on Article 83 EPC and the independent claims of the new main request filed at 13:15 hours on 28 October 2015. The same conclusion applies to the request to reopen the debate on Article 56 EPC for independent claims 5 and 6 of the new main request with respect to document (1).

28. The board therefore rejects these requests.

Confidentiality of documents (169) to (172)

29. At this point it is also convenient to consider the question of whether, as requested by the appellant, to maintain documents (169), (171) and (172) as confidential, which means that they will not be stored in the publicly accessible part of the EPO’s file.

30. Respondent I requested that document (169) be maintained as confidential in its letter of 8 September 2015. None of the other parties have opposed this request, respondent II has remarked, however, that it does not consider itself bound by any confidentiality

obligations arising from the English litigation to which it is not a party.

31. Document (171) was filed on 1 October 2015 by respondent II without any request that this document be kept confidential. The appellant in its 12 October 2015 letter, requests that document (171) be treated as regards confidentiality in the same way as document (169).

32. Document (172) was filed by the Appellant on 12 October 2015 with a request that it be kept confidential.

33. None of these documents have been admitted into the proceedings, the board therefore sees no good reason not to maintain the confidentiality of these documents, given that requests to this effect have been filed by the appellant, and respondent I. The board notes that no party has made any positive case that these documents should be placed on the publicly accessible part of the file. The board therefore directs that these documents are not to be made publicly accessible.

Admission of claims 1 - 6 of Main Request filed at 16:00 hours on 9 November 2015 during the oral proceedings

34. Article 13(1) RPBA sets out the board's discretion to admit and consider late filed claim requests. The board is to exercise its discretion according to the complexity of the new subject-matter submitted, the current state of proceedings, and the need for procedural economy. In addition, Article 13(3) RPBA provides that if amendments to a party's case are made after oral proceedings have been arranged, (the case here), then such amendment shall only be admitted if

the board and the parties can reasonably be expected to deal with it at the oral proceedings.

35. In this case the main request consists only of claims that have already been considered by the board and subject to argument from the parties and represent a set of claims that in the opinion of the board comply with the requirements of the EPC. They thus clearly fulfil all of the criteria for the board to admit them. The board therefore admits the appellant's main request into the proceedings.

Article 123(2) EPC

Re: "large cloned genomic fragment greater than 20 kb/100 kb" - Claims 1 and 4

36. In the decision under appeal, the opposition division found that page 6, lines 4 to 6 of the application as filed provided a basis for the use of a large cloned genomic fragment greater than 20 kb or 100 kb, as specified in, respectively, claim 1, step a) and claim 8 of the main request then on file (see page 5, lines 11 to 31 of the decision). In the board's view, this applies also in respect of claims 1 and 4 of the set of claims presently on file.

37. The board shares the opposition division's view that the wording "*... capable of accommodating ...*" used in the relevant passage on page 6 does not require that the vectors indeed accommodate fragments greater than 20 kb (or 100 kb, as specified in present claim 4). However, a person skilled in the art reading the application as filed understands from the passage on page 6 of the application as filed that a requirement of a certain capacity of the LTVEC vectors used in the

method of the invention can only mean that the size of DNA fragments to be inserted into the vectors will be greater than 20 kb, and in particular greater than 100 kb. Otherwise, such a requirement would make no technical sense.

38. The disclosure in the application as filed as a whole does not call this understanding in question. In particular, as regards the *in situ* replacement embodiment exemplified in Example 3 of the application as filed the human DNA fragments cloned into the LTVEC1 and LTVEC2 vectors are in fact greater than 20 kb, even greater than 100 kb (see Figure 4A in which human inserts of approximately 200 to 300 kb are illustrated).

Re: "in situ replacement" - Claims 1, 5 and 6

39. The opposition division found that the introduction of the wording "*in situ replacement*", "*in situ in place*" or "*replaced in situ*" in claims 1, 9, 14, 15 and 16 of the main request underlying the decision under appeal did not violate Article 123(2) EPC (see page 4, first full paragraph of the decision). The board holds that the reasons given by the opposition division for this finding apply also to claims 1, 5 and 6 as presently on file. Although the literal wording "*in situ replacement*" appears in the application as filed only in the context of replacement of heavy chain V, D and J (or light chain V and J gene segments) with their human counterparts, a person skilled in the art understands from the disclosure in the application taken as a whole that "*in situ*" replacement is a feature of the invention as it relates to a general method for modifying a gene or a locus, and as it relates in particular to the method for modifying the endogenous immunoglobulin

heavy or light chain locus. This is directly and unambiguously derivable from the technical teaching in the application as filed as a whole, in particular from the teaching that the LTVECs used in the replacement method include flanking sequences derived from the murine locus which will recombine with the endogenous sequences by homologous recombination. This can only mean that the modification of a gene or locus by insertion, deletion or replacement is intended to occur at the site of the targeted endogenous gene or locus. Like the opposition division, the board has no reason to believe that a person skilled in the art would regard the feature "*in situ*" as inextricably linked to the specific replacement of <u>all</u> V, (D) and J gene segments of the immunoglobulin heavy and light chain locus.

Re: "V, D and J gene segments" - Claim 1

40. In the decision under appeal, the opposition division found that the objection under Article 123(2) EPC to the deletion of the wording "*one or more*" in claim 1 of the auxiliary request 5 then on file, was not justified (see page 11, first full paragraph of the decision). This objection has been raised again in appeal proceedings.

41. The board shares the opposition division's view that the disclosure in pages 46 to 48 of the application as filed provides a basis for the replacement of V, D and J gene segments. It is apparent from the section starting on page 46 taken together with Figures 4A and 4B of the application as filed that, in a first step of the method some V gene segments and the D and J gene segments of the human gene locus contained in the cloned fragment replace some of the endogenous V gene

segments as well as the endogenous D and J gene segments *in situ*. Thus, contrary to the respondents' allegation the *in situ* replacement of V, D, and J gene segments is directly and unambiguously derivable from the application as filed. The board is also of the view that the amended claim 1 as presently on file in which the wording "*one or* more" has been deleted, cannot be construed to encompass the replacement of single V, D and J gene segments, either in rearranged or unrearranged form.

Re: "to create a modified immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions" - Claim 1

42. In the board's view, the skilled person would not understand the general disclosure in the passage on page 43, lines 14 to 15 and lines 27 and 28 of the application as filed, to be restricted to embodiments of the claimed method in which the entire human heavy and light chain variable region replaces the corresponding murine endogenous sequence. It is apparent from the sections under the headings "a.Introduction" and "b.Brief Description" of Example 3 of the application as filed that the aim of the invention is to produce hybrid antibodies containing human variable regions and mouse constant regions. The advantages of such hybrid antibodies are described in the latter section. The skilled person derives from this section that the described advantages are associated with the linkage of the human V, D and J gene segments to the murine constant region genes in the genuine murine genomic environment (i.e. "*in situ*"), rather than to the presence of the entire variable region of the human immunoglobulin locus. While the section "c.Materials and Methods" starting on

page 46 describes specific methods for replacing the entire endogenous V, D and J region by the entire V, D and J region of the human heavy chain locus, the general part of the description in pages 43 to 45 is not restricted to these embodiments.

43. It can be unambiguously derived from the passage from page 10, line 33 to page 12, line 8 and claims 7 to 10 of the application as filed that the large cloned genomic fragment inserted into the targeting vector may contain the whole or part of the human immunoglobulin variable region gene locus and may replace the endogenous gene locus in whole or in part (see page 10, line 33 to page 11, line 15; and claim 7). Further fragments of the variable region gene locus can be inserted as specified in page 11, lines 17 to 31, and claim 8. According to claim 9 and the passage from page 11, line 33 to page 12, line 2, replacement of the whole gene locus could be achieved by repeating the same steps.

44. As regards respondent I's objection that claim 1 related only to the heavy chain immunoglobulin variable locus, the board draws attention to page 12, lines 4 to 8 and claim 10 of the application as filed, which is dependent from claim 7 and specifies that the immunoglobulin variable (region) gene locus can be, *inter alia*, a variable (region) gene locus of the heavy chain. The board also notes that, according to claim 7 of the application as filed, the replacement is carried out in a mouse ES cell. A transgenic mouse is then generated by introducing the modified ES cell into a blastocyst and subsequently introducing the blastocyst into a surrogate murine mother for gestation (see steps e) and f) in claim 31 of the application as filed).

45. The board does not believe that, for compliance with Article 123(2) EPC to be acknowledged, further elements necessary for the production of the hybrid antibodies mentioned in the passage on page 43, for instance loci rearrangement during B-cell development must be specified in the claim. The person skilled in the art reading the application as filed understands that these elements are in fact required for the production of the hybrid antibodies, but do not constitute the core of the invention disclosed in the application.

Re: Selection from lists - Claim 1

46. Respondent I's objection that the subject-matter of present claim 1 is the result of multiple selections from lists disclosed in the application as filed, is without merit. The alleged "lists" are in fact alternative embodiments which are clearly and unambiguously derivable from the application as filed. With regard to the segments to be replaced, the board is unable to read the application as filed as disclosing the alleged "list" of six different possibilities, from which the subject-matter of claim 1 would be only one.

Conclusion

47. For the reasons given above, the objections raised under Article 123(2) EPC in appeal proceedings fail. Further objections upon which the opposition division decided, have not been pursued in appeal or became obsolete after deletion of the claims in question.

Article 123(3) EPC

48. The board believes that the introduction of the feature *"... to create a modified immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions* ..." into the preamble of claim 1 does not extend the scope of protection conferred by the patent as granted. The amendment to claim 1 introduces a functional requirement for the modified gene locus, and therefore limits, rather than extends the scope of the claim.

49. Contrary to respondent II's view, amended claim 1 is not directed to a method for the production of hybrid antibodies, but to a method of modifying a murine endogenous immunoglobulin heavy chain variable region gene locus to obtain a modified gene locus that, when expressed in a suitable host and under suitable conditions, produces hybrid antibodies containing human variable regions and mouse constant regions. The actual production of these hybrid antibodies is not being claimed in present claim 1.

50. The board concludes that Article 123(3) EPC is not contravened.

Article 84 EPC - Clarity and support

51. Respondent II contended that, with regard to the introduced wording "*hybrid antibodies*", amended claim 1 lacks clarity within the meaning of Article 84 EPC. The board disagrees. It is clear from the feature "*containing human variable regions and mouse constant regions*" which characterises the hybrid antibodies specified in claim 1 that, in the context of this claim, "*hybrid*" means that the antibodies produced by

the modified gene locus contain regions which originate from different organisms, namely human variable regions and mouse constant regions.

52. Moreover, while it is true that, in principle, a heavy chain gene locus alone does not produce antibodies, because also a light chain is required for such production, a person skilled in the art understands that claim 1 specifies only features which characterize the invention. Further requirements for the production of antibodies - like the presence of an immunoglobulin light chain – are immediately evident to the person skilled in the art.

53. In respondent II's view, also the meaning of wording "*orthologous V, D, and J gene segments*" is vague. This objection is not justified. The board holds that a person skilled in the art in the technical field of the invention has a good knowledge of the structure of the human and murine immunoglobulin heavy chain gene locus and understands that, although there may be differences in the number of V, D or J gene segments between the two loci, the murine gene segments are to be replaced by human gene segments with the same function as regards their contribution to the structure of the immunoglobulin heavy chain. With a mind willing to understand, claim 1 cannot be construed as requiring that single, specific endogenous V, D, or J gene segments are replaced by single, specific human counterparts.

54. Hence, in the board's view none of the respondents' objections under Article 84 EPC is justified.

Article 83 EPC - Sufficiency of disclosure

55. In the decision under appeal, the opposition division did not decide on the objections raised by the opponents under Article 83 EPC. However, it expressed the view – as *obiter dictum* – that, as regarded the seventh auxiliary request then on file, the requirement of sufficiency of disclosure was fulfilled.

56. Having considered the objections raised by the respondents in appeal proceedings and the evidence on file, the board sees no reason to doubt that the invention as claimed in claims 1, 5 and 6 is disclosed in the application as filed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art, as required by Article 83 EPC.

57. It is a well-established principle in the case law of the boards of appeal that, for the disclosure of an invention to be sufficiently clear and complete within the meaning of Article 83 EPC, the skilled person, on the basis of the information provided in the application itself, supplemented by the common general knowledge at the filing date (or the priority date, if applicable), must be able to carry out the invention without undue burden of experimentation or inventive skill (see e.g. decisions T 694/92 OJ EPO 1997, 408 and T 612/92 of 28 February 1996).

58. The assessment of sufficiency of a disclosure has to be conducted in each case on its own merits, and it depends on the correlation of the facts of the case to certain general parameters, e.g. the amount of reliable technical details disclosed in the application, the time when the disclosure was presented to the public and the corresponding common general knowledge, as well

as the character of the technical field and the average amount of effort necessary to put into practice a certain written disclosure in that technical field (see decision T 158/91 of 30 July 1991, point 2.3 of the reasons; and T 639/95 of 21 January 1998).

59. In the present case, the notional person skilled in the art for the purpose of assessing sufficiency of disclosure must be regarded as being a team of specialists with knowledge and capabilities in the fields of genetic engineering, immunology and mouse embryology and genetics, and in particular ample experience in the fields of bacterial homologous recombination, targeted recombination in eukaryotic cells and generation of transgenic mice from embryonic stem cells containing xenogeneic, in particular human DNA, these techniques being - undisputedly - well-established at the priority date. The team would have a good knowledge of the genetic structure of the murine and human immunoglobulin gene loci.

60. Since the technical field to which the present invention relates is highly complex, the average amount of effort necessary to put into practice a written disclosure in this field would be rather high and involve a considerable amount of trial and error.

61. As regards the disclosure content of the application as filed, the board does not share the respondents' view that the pertinent disclosure for the purpose of assessing sufficiency of disclosure is restricted to Example 3. Rather, it is the technical content of the application as a whole, which relates to methods of modifying eukaryotic cells that can be applied to modify any endogenous gene or chromosomal locus, which is to be considered. In Example 3 the methods of the

invention are exemplified for the modification of specific loci, but when trying to carry out the invention - which, in the board's view, cannot be equated to repeating Example 3 - the skilled person would resort to the entire technical information provided in the application as filed.

62. In their pleadings at the oral proceedings, the respondents based their objection of lack of sufficient disclosure mainly on documents (126) and (127), two scientific articles co-authored by the inventors and published in 2014. In the respondents' view, document (127), in particular Figure 1 was a clear evidence that a person skilled in the art could not have carried out the invention applying the technical teachings contained in Example 3 of the application as filed.

63. The board disagrees with this view. First, the fact that the approach followed by the appellant itself to humanize the mouse heavy chain variable gene as described in document (127) differs from the approach suggested in Example 3 of the application as filed does not necessarily mean that the latter cannot be carried out without an undue burden of experimentation and/or inventive skills. The respondents have not presented convincing evidence that this would be the case, their main argument being that Example 3 is a "prophetic" example. However, there is no requirement in the EPC that, either at the priority or filing date, the applicant must have carried out the claimed invention. The requirement of Article 83 EPC is that a person skilled the art, following the teachings in the application as filed supplemented with his/her common general knowledge and with a reasonable amount of experimentation, including some trial and error, would

be able to carry out the invention as claimed at the relevant date.

64. Second, the board observes that the approach followed in document (127), in which a total 2.6 Mb of the murine heavy chain variable region gene locus was replaced by 1 Mb of the human orthologous locus, does in fact make use of the methods disclosed in the application as filed. The board is persuaded that any required modifications could be devised by a person skilled in the art at the relevant date using his/her common general knowledge with a reasonable amount of trial and error, but without applying any inventive skills. In fact, this approach makes use of the targeted integration/deletion of large cloned genomic fragments in murine ES cells by homologous recombination using LTVECs as described in detail in the application as filed (the so-called *VelociGene* method referenced as (7); see page 3, left-hand column, first full paragraph of document (127)). Applying this method, a 144 kb fragment of human immunoglobulin DNA containing 3 V_H, all 27 D_H, and 9 J_H was inserted using about 75 kb of mouse homology arms (Figure 1B, step A). Concomitantly, 16.6 kb of the mouse sequence were deleted. The fact that this transgene still contains murine V gene segments which may be involved in rearrangement and give rise to antibodies which contain, at least in part, a murine variable region is, in the board's view, not prejudicial to Article 83 EPC. The presence of 3 V_H ensures that antibodies containing (entirely) human variable regions and mouse constant regions are produced.

65. The approach in document (127) also makes use of a quantitative assay to detect modification of allele, which is designated MOA in the application as filed

(see step d) in present claim 1) and LONA ("loss-of-native-allele") in document (127) (see page 3, left-hand column, lines 19 to 24). The board does not share the respondents' view that the MOA step would not work if orthologous human sequences were inserted without concomitant deletion of the corresponding murine sequences. First, the board does not construe the wording "*to replace*" in step c) of the method of claim 1 as meaning that all murine V, D and J gene segments must be deleted concomitantly with the insertion of the human segments. As apparent from document (127), it is not necessary to delete all the murine sequences to be replaced by the corresponding human sequences. Deletion of as little as 16.6 kb murine sequence is sufficient to detect a modification of allele, i.e. a loss of murine sequences at the site where the human sequences are inserted.

66. The approach described in document (127) also makes use of site-specific recombination, in particular the loxP/Cre tool disclosed in Example 3 of the application as filed to insert further human V gene segments and delete murine sequences (see Figure 1B, step B and LTVEC1 and LTVEC2 in Example 3 of the application). According to document (127) (see Figure 1C, steps D to H), insertion of additional V gene segments was achieved by several rounds of sequential targeting by homologous recombination. This has also been described in the application as filed (see passage from page 11, line 17 to page 12, line 2 and claims 8 and 9). It has not been disputed that, at the priority date, there was information available in the art that would allow a person skilled in the art to design suitable homology arms for inserting in a sequential fashion fragments of the human DNA containing additional V gene segments, without applying any inventive skills.

67. Concerning the homology arms, the sole argument put forward by the respondents was that the 5' end of the murine immunoglobulin variable region locus was not conclusively known at the priority date and that, therefore, the skilled person could not have created LTVEC2. To support their objection, the respondents relied on documents (116) and (121). Document (116) is a statement filed by the present appellant which includes allegations as to the lack of information on the genomic fragment to be used as 5' homology arm in Example VII of document (1).

68. In the board's view, these allegations are without merit. Although the 5' end of the murine heavy chain variable region locus was fully characterized only in 2006 (see document (121)), at the relevant date BAC libraries of the murine immunoglobulin genes were available. Hence, a person skilled in the art could have found by, e.g., chromosome walking with the aid of the technical information provided in document (120) a suitable DNA fragment containing the 5' end of the locus for use as a homology arm. In the board's view, this would not have required an undue amount of experimentation, because a detailed mapping as reported in document (121) was not required.

69. The respondents based a further objection under Article 83 EPC on documents (93) and (94), arguing that, at the relevant date, only deletions of up to approximately 30 kb had been achieved. Deleting 200-300 kb of the murine locus, as suggested in Figure 4A of the application as filed, would have been impossible, because the capacity of BAC vectors was limited. The respondents relied also on document (127) in which the appellant admitted that only 114 kb of the

proximal end of the human heavy chain locus had been inserted, and 16.6 kb of the mouse sequence deleted. The board is of the view that this evidence is not conclusive. First, the relatively "poor" gene targeting results reported in documents (93) and (94) were achieved with "conventional" gene targeting techniques, eventually adding a site-specific recombination step, but not with vectors containing large homology arms, as in the application. Second, as apparent from document (114) the capacity of a BAC vector is about 300 kb; thus, the "theoretical" disclosure in Figure 4A of the application is not called in question. Thirdly, the board holds that, if the skilled person trying to carry out the invention with the guidance provided in the application as filed would have failed to replace 200-300 kb of the murine sequence by the human sequence, the next logical step would have been to try again with a shorter DNA fragment. The skilled person would not have thought that it was absolutely necessary to use a fragment of 200-300 kb to insert the human J, D, and at least some V gene segments into the murine locus and concomitantly would have deleted at least part of the murine sequence in order to be able to detect modification of allele (MOA), as taught in the application.

70. With respect to the transgene with 144 kb of human immunoglobulin sequence described in document (127) (see page 3, left-hand column, first full paragraph), the respondents disputed that 3 V_H would be sufficient for producing hybrid antibodies. The evidence in Appendix A1 of document (46) (see "Velocimmune 3hVH:137 mVK"), which has not been plausibly questioned, shows that this objection is not justified.

71. Finally, the respondents pointed to the passage on page 4, right-hand column, second full paragraph of document (127) and argued that the skilled person trying to carry out the invention would have been confronted with unexpected fertility problems of the transgenic mice, and would have found no guidance in the application as filed to solve this problem. In the board's view, this difficulty cannot be seen as insurmountable because, as the appellant plausibly argued, only male mice were affected and fertility was only reduced, rather than the mice being rendered infertile.

72. Summarizing the above, the board has no reason to doubt that the invention as claimed in claims 1, 5 and 6 is sufficiently disclosed as required by Article 83 EPC.

Article 54 EPC - Novelty

73. In the decision under appeal, the opposition division acknowledged novelty of the subject-matter of the claims of the seventh auxiliary request (see section 1 on page 23 in connection with the reasons given in the passage from page 13, line 5 to page 16, line 11). In appeal proceedings, the respondents have contested the opposition division's findings on novelty relying on documents (1), (4), (8) and (9).

Document (1)

74. Document (1) relates to the production of xenogeneic, in particular human antibodies in transgenic mice. The transgenic mice, which are characterised by being incapable of producing endogenous immunoglobulin heavy and light chains, but capable of producing xenogeneic immunoglobulin light and heavy chains to produce a

xenogeneic immunoglobulin or immunoglobulin analogue, are obtained by a method which comprises introducing a xenogeneic, particularly human DNA segment of at least 100 kb, into an embryonic stem cell (ES cell), selecting the ES cells having a DNA segment integrated into the genome, introducing these cells into embryos and generating chimeric mice from the embryos (see passage from column 3, line 59 to column 4, line 5).

75. The transgenic mice may have the xenogeneic immunoglobulin locus inserted into a chromosome and an inactivated endogenous immunoglobulin region, or an entire endogenous immunoglobulin locus substituted by a portion of, or an entire, xenogeneic immunoglobulin locus (see column 3, lines 50 to 55). In the second embodiment, at least portions of the human heavy and light chain immunoglobulin locus are used to directly replace the corresponding endogenous immunoglobulin loci by homologous recombination in embryonic stem cells (see column 6, lines 30 to 34). It is apparent from the passage on column 10, lines 11 to 16 that, in this case, where the xenogeneic DNA insert is large (up to about 1000 kb), a yeast artificial chromosome (YAC) is used for cloning of the targeting construct (see also Examples V and VI).

76. In support of their objection of lack of novelty, the respondents relied on the passages in column 5, lines 48 to 57; and column 10, line 50 to column 11, line 2, as well as on Example VIII and Figure 16c. The passages in columns 5 and 10 read:

"*Novel transgenic non-human hosts, particularly mammalian hosts, usually murine, are provided, where the host is capable of mounting an immune response to an immunogen, where the response produces antibodies*

having xenogeneic, particularly primate, and more particularly human, constant ***and/or variable regions*** *or such other effector peptide sequences of interest.*" (emphasis added by the board)

"*As already indicated, the target locus may be substituted with the analogous xenogeneic locus. In this way, the xenogeneic locus will be placed substantially in the same region as the analogous host locus, so that any regulation associated with the position of the locus will be substantially the same for the xenogeneic immunoglobulin locus. For example, by isolating* ***the variable region of the human IgH locus (including V, D, and J sequences)****, or portion thereof, and flanking the human locus with sequences from the murine locus, preferably sequences separated by at least about 5 kbp, in the host locus, preferably at least about 10 kbp in the host locus, one may insert the human fragment into this region in a recombinational event(s), substituting the human immunoglobulin locus for the endogenous variable region of the host immunoglobulin locus. In this manner, one may disrupt the ability of the host to produce an endogenous immunoglobulin subunit, while allowing for the promoter of the human immunoglobulin locus to be activated by the host enhancer and regulated by the regulatory system of the host.*" (emphasis added by the board)

77. According to the established jurisprudence of the boards of appeal, in order to assess the information content of a prior art document the technical disclosure of the document must be considered as a whole (see T 56 87, OJ EPO 1990, 188). Thus, the information content of these two passages must be assessed in the light of the meaning given in

document (1) to the term "*human variable region*". It is stated in the passage in column 6, lines 45 to 49 that, in the 5'-3' direction of transcription, the human immunoglobulin heavy chain locus "... *comprises a large cluster of variable region genes* (V_H)*, the diversity (D) region genes, followed by the joining* (J_H) *region genes and the constant* (C_H) *gene cluster*". Thus, it appears that what is meant in document (1) by "*human variable region*" does not include the D and J genes, but is restricted to the V genes.

78. In the light of the meaning given to the term "*human variable region*" in document (1), the disclosure content of the passages on which the respondents relied is rather obscure. This is particularly true for the passage in column 10, in which first "*... the variable region of the human IgH locus (including V, D, and J sequences) ...*" is isolated, but then "*... the human locus ...*" is flanked with sequences from the murine locus, and subsequently "*... the human immunoglobulin locus ...*" is substituted for the endogenous variable region of the host immunoglobulin locus.

79. For the boards of appeal to accept that the content of a prior art document destroys novelty, it is a prerequisite that the claimed subject-matter is directly and unambiguously derivable from the prior art. In the present case, the board considers that a person skilled in the art reading the two passages of document (1) quoted above in the light of the technical disclosure of this document as a whole, would not be able to derive, directly and unambiguously, from these passages an ES cell or a mouse comprising a genetically modified immunoglobulin heavy chain locus in which the endogenous V, D, and J gene segments are replaced *in situ* by the orthologous human V, D, and J gene

segments, and which produces hybrid antibodies containing human variable regions and mouse constant regions.

80. As regards Example VII, which relates the production of chimeric mice by introducing human immunoglobulin sequences using homologous recombination, the passage in column 38, lines 24 to 33 describes the replacing of human sequences as including a 100 kb fragment of genomic DNA which encompassed the human VH6-D-J-C_μ-C_δ heavy chain region flanked by mouse sequences from the immunoglobulin heavy chain locus. As apparent from Figure 16C which illustrates the construct of Example VII, the constant region comprises the human C_μ and C_δ genes followed by the *neo* marker gene and the mouse C_ε and C_δ genes.

81. It is undisputed that, upon integration of this construct into the genome of an ES cell and selection of transformants for neomycin resistance, the chimeric locus will include the human C_μ and C_δ genes. Thus, transgenic mice generated from such an ES cell as taught in Example VIII (and Figure 17) are bound to produce hybrid antibodies that contain at least part of the human immunoglobulin heavy chain constant region. Such mice do not destroy the novelty of claims 5 and 6.

82. The board does not accept the respondents' argument relying on documents (4) and (79) that in heterozygous mice the chimeric construct of Figure 16C could undergo recombination (so called "trans-switching") with the endogenous immunoglobulin heavy chain locus to generate a modified locus that produces a hybrid heavy chain (and a hybrid antibody) containing human variable regions and mouse constant regions, as required by the present invention. Not only is trans-switching a rather

rare event which occurs at random, but also the low probability that recombination occurs precisely in the junction between the human variable and constant regions must be considered. Under these circumstances, it is not tenable to argue, as the respondents do, that the subject-matter of present claims 5 and 6 is the inevitable result of repeating Examples VII and VIII of document (1), and the question whether or not the presence of the *neo* marker gene in the chimeric construct described in document (1) interferes with trans-switching is irrelevant. Hence, in accordance with the case law of the boards of appeal since T 12/81 (OJ EPO 1982, 296) the subject-matter of claims 5 and 6 cannot be regarded as disclosed in document (1).

83. The same reasons apply to the subject-matter of claim 1. In addition, the board remarks that, contrary to respondent II's view, even if the generation of targeting vectors by bacterial homologous recombination is considered to be part of the common general knowledge, the specific combination of step b) with the further steps specified in claim 1 is not anticipated by document (1). As stated above, document (1) teaches that, for replacement of large DNA fragments, a YAC vector is used for cloning the targeting construct. Respondent II's objection based on document (1) in combination with the common general knowledge seems to blur the boundary between lack of novelty and lack of inventive step.

84. Furthermore, the board does not share respondent II's view that document (1) describes step d) of the method of claim 1, in which modification of allele (MOA) is detected using a quantitative assay to identify ES cells in which replacement *in situ* has occurred. According to the invention, in the quantitative assay

to detect MOA the probe detects the unmodified allele, not the modified one, as it was known in the art at the priority date (see paragraph [0091] of the patent in suit). In contrast, in the method described in document (1) homologous integration at the right location is detected by DNA analysis by Southern blot hybridization, or using probes for the insert and the sequences at the 5' and 3' regions flanking the region where homologous integration would occur (see column 9, lines 40 to 60).

85. In conclusion, the subject matter of claims 1, 2, 5 and 6 is novel over document (1).

Documents (4), (8) and (9)

86. In the decision under appeal, the opposition division found that none of these documents discloses a cell or mouse containing a modified immunoglobulin heavy chain locus *in situ* in place of the endogenous locus. This has not been disputed in appeal proceedings. Rather, the respondents argued that such cell or mouse would be the product of a trans-switching event as described in document (4) (see chapter under the heading "Trans-Switching" starting on page 85, line 9).

87. The board disagrees with this view. As explained above in connection with document (1), due to the unpredictability and random occurrence of trans-switching, it cannot be accepted that a cell or mouse according to claim 5 or 6 is the inevitable result of the method described in documents (4), (8) or (9). Document (168), on which respondent II relied, shows that isotype switching of an immunoglobulin heavy chain transgene can occur by interchromosomal recombination, in the particular case of Figure 4d recombination

between chromosomes 12 and 5, but does not provide evidence that the trans-switching event occurs reliably and inevitably leads to the modified heavy chain locus being integrated *in situ* in place of the endogenous locus.

88. For these reasons, the objection of lack of novelty in view of documents (4), (8) and (9) must fail.

Article 56 EPC – Inventive step

89. In the decision under appeal, the opposition division held that, starting from either document (1) or (4) as the closest state of the art, the problem to be solved had not been plausibly solved over the whole scope of the claims of the seventh auxiliary request, and that the subject-matter of the claims was obvious over a combination of documents (4) and (7), account being taken of the common general knowledge in the field (see page 24, lines 6 to 10 of the decision under appeal). In appeal proceedings, the opponents relied on documents (4), (1), (8) and (3) as the closest state of the art for the subject-matter of claim 1, and on documents (4) and (1) as the closest state of the art for the subject-matter of claims 5 and 6.

Document (4) as the closest state of the art

90. Document (4) describes transgenic animals, in particular mice capable of producing human antibodies, and methods to generate them. The transgenes inserted into the murine genome should be able to undergo functional V-D-J rearrangement to generate recombinational diversity and junctional diversity (see page 3, lines 3 to 6) and be capable of switching from an isotype needed for B cell development to an isotype

that has a desired characteristic for therapeutic use (see page 4, lines 22 to 24). According to document (4), the transgenic mice can express both fully human heavy chains and chimeric heavy chains comprising a human heavy chain variable region and a murine heavy chain constant region resulting from trans-switching between a human heavy chain transgene and an endogenous murine heavy constant region gene (see page 13, lines 9ff and page 86, lines 25 to 29). As advantages of the presence of a murine constant region over a human constant region, retention of effector functions in the murine host and presence of murine immunological determinants for binding to a secondary antibody are mentioned in the section under the heading "Trans-Switching" starting on page 85, line 9. It is also hypothesized that such chimeric antibodies may also have repertoire advantages (see page 85, lines 20 to 32).

91. The transgenic mice described in document (4) are generated by introducing human immunoglobulin heavy chain segments, either in unrearranged or rearranged form (see passage from page 51, line 31 to page 52, line 14) into the genome of mice and subsequently disrupting the endogenous immunoglobulin locus by homologous recombination (see section under the heading "Functional Disruption of Endogenous Immunoglobulin Loci" starting on page 74, line 18). Candidate hybridomas which produce chimeric antibodies are identified from a pool of hybridoma clones comprising fully human and chimeric heavy chain genes by a method described in Example 24 starting at the bottom of page 211).

92. It is undisputed that document (4) describes random integration of a fully human immunoglobulin heavy chain

transgene, rather than targeted integration of immunoglobulin heavy chain V, D and J gene segments *in situ* to replace endogenous V, D and J gene segments (step c) of the method of present claim 1). Hence, document (4) does not disclose either the use of a targeting vector (LTVEC), or a quantitative assay to detect modification of allele (MOA) as required by the method of claim 1. Moreover, the transgenic mice described in document (4) do not reliably produce hybrid antibodies containing human variable regions and mouse constant regions, chimeric transgenes producing such antibodies being only the result of a random trans-switching event. Hybridomas expressing hybrid antibodies have to be obtained by screening individual hybridomas as described in Example 24 of document (4).

93. The method of modifying an endogenous immunoglobulin heavy chain variable region gene locus according to the present invention is more reliable than the method of document (4) because it does not require a random trans-switching event in order to generate the chimeric locus. As admitted by respondent I, it is also more direct since insertion of the human immunoglobulin sequences and inactivation of the murine locus are effected in one step. Thus, starting from document (4), the problem to be solved is to provide improved methods and means for generating a repertoire of hybrid antibodies having a heavy chain which contains human variable regions and mouse constant regions.

94. The board has no doubts that this problem is solved over the whole scope of the claims 1 to 6. Even if only some human V gene segments are inserted into the mouse genome together with the D and J gene segments, a repertoire of hybrid heavy chains and, consequently, of

hybrid antibodies arises from the rearrangement of the V, D and J gene segments.

95. The solution proposed in the present claims was not obvious to a person skilled in the art at the priority date. As regards the method of claim 1, the lines of argument put forward by the respondents relied on a combination of the teachings of document (4) with methods well known in the art as apparent from documents (10) and (77), or a combination of document (4) with either document (7) or (1).

96. Document (10) describes a restriction endonuclease-independent approach to engineering large DNA fragments in *E. coli* based on (bacterial) homologous recombination and suggests to apply the same strategies for insertion and/or deletion of sequences by homologous recombination in, e.g., mouse embryonic stem cells (see page 127, left-hand column, last two paragraphs of the discussion). In this document only short homology arms (up to 60 bp) flanking the cloned fragment are used, and no information whatsoever is provided as to the length of homologous arms required for targeting a DNA fragment into the genome of ES cells. Moreover, an assay to detect modification of allele as required in present claim 1 cannot be derived from this document.

97. Document (77) describes the generation of a knock-out vector by bacterial homologous recombination and the use of this vector for disrupting the mouse tubby gene in ES cells by homologous recombination. Homology arms ("recombination sequence") of less than 1 kb, more preferably less than 500 bp, and most preferably less than about 100 bp are described (see column 5, lines 43 to 52). However, this document does not describe the

use of a vector having a genomic fragment flanked by large homology arms (LTVEC) for targeting the genome of an ES cell, nor the detection of a modification of allele (MOA) in the ES cell.

98. Hence, starting from document (4) a person skilled in the art could not arrive at the method according to present claim 1 by combining the teachings of this document with the methods described in either document (10) or document (77).

99. Document (7) describes the insertion of a rearranged human V region gene (V_HT15) at a chromosomal position where rearranged V_H genes locate, 5' to the heavy chain enhancer (see Figure 1A) by homologous recombination. It is stated in this document that the proper location of the transgene relative to the constant region genes allows it to participate in isotype switching and undergo somatic hypermutation (see Abstract). The method is said to be not only applicable to "*... any rearranged VH gene, but can be easily extended to light chain genes as well. Transgenic mice of this kind, generated by gene targeting, should provide useful mouse models for the in vivo analysis of antigen-specific B cell activation and tolerance, including models of allergic and autoimmune disease*" (see page 1270, right-hand column, last nine lines).

100. The board is unable to see how a person skilled in the art could arrive at the subject-matter of claims 1, 5 or 6 by combining the teachings of documents (4) and (7). Apart from the fact that document (7) – like document (4) - does not describe a quantitative MOA assay as required in claim 1, but rather a non-quantitative Southern blot analysis of a PCR-positive transfectant and offspring of germline chimeras (see

Figure 1C of document (7)), it is not apparent to the board why a person skilled in the art would have turned to this document when seeking to provide improved methods and means for generating a repertoire of hybrid antibodies. Document (7) is solely concerned with a model system in which the J_H region of the human variable region locus is replaced by a single rearranged V_H gene. Moreover, as is apparent from Figure 1A, the constant region in the construct of document (7) is of human origin (C_H); thus, the heavy chain resulting from the expression of this construct must be a human heavy chain, rather than a hybrid one. Hence, neither the subject-matter of claim 1 nor that of claims 5 and 6 can be regarded as obvious to a skilled person in view of the teachings of document (4) combined with those of document (7).

101. As regards document (1), which describes integration of a xenogeneic immunoglobulin locus or part of it by homologous recombination into the genome of an ES cell, the board observes that the method described in this document uses a yeast artificial chromosome (YAC) to clone and introduce the xenogeneic locus into the ES cell. It is not apparent why a skilled person, who is aware of the advantages of YACs (higher capacity and stability), would not use a YAC to create a targeting vector as described in document (1), but rather resort to bacterial homologous recombination, as is required in claim 1. Moreover, neither document (4) nor document (1) describes a quantitative assay to detect modification of allele (MOA). Hence, contrary to respondent I's view, the skilled person would not arrive at the subject-matter of claim 1 by combining the teachings of these two documents.

102. The same applies to the subject-matter of claims 5 and 6. It should be noted that the purpose of the methods described in document (1) is to generate human antibodies, and that the sole construct described in document (1) that contains murine constant region genes includes also the Cμ and Cδ genes of the human constant region. Thus, in order to arrive at the subject-matter of claims 5 and 6, the skilled person would have to delete the human constant region genes from the construct. However, there is no suggestion to do so in document (1). The statements in column 6, lines 30ff and column 8, lines 37ff of document (1) suggesting that at least portions of the human heavy and light chain immunoglobulin loci can be used to replace the corresponding endogenous immunoglobulin loci are vague as to the extent and nature of the portion. Additionally, a person skilled in the art reading the passage in column 2, lines 21 to 32 of document (1) would be strongly discouraged from trying to delete the human Cμ and Cδ genes from the construct described in Example VII in order to join human variable region gene segments to murine constant region gene segments. This passage reads:

"*While the resulting chimeric partly xenogeneic antibody is substantially more useful than using a fully xenogeneic antibody, it still has a number of disadvantages. The identification, isolation and joining of the variable and constant regions requires substantial work. In addition, the joining of a constant region from one species to a variable region from another species may change the specificity and affinity of the variable regions, so as to lose the desired properties of the variable region. Also, there are framework and hypervariable sequences specific for a species in the variable region. These framework and*

hypervariable sequences may result in undesirable antigenic responses."

103. In view of the warnings in this passage that appear to contradict the statements in document (4), the skilled person would be uncertain whether or not success in providing ES cells or transgenic mice that produce hybrid antibodies with at least the same affinity and specificity than fully human antibodies was to be expected if the Cμ and Cδ genes were eliminated from the construct of document (1). Since document (1) makes him/her aware of the fact that substantial work is required, the risk of not succeeding would discourage the notional skilled person – conservative by definition - from even trying to modify the chimeric immunoglobulin locus described therein. Thus, the subject-matter of claims 5 and 6 cannot be considered to be obvious to a skilled person in view of a combination of documents (4) and (1).

Document (1) as the closest state of the art

104. The technical content of document (1) is outlined in sections 74 to 76 above. Essentially, this document describes methods aiming at the generation of transgenic mice that produce human antibodies as well as the transgenic mice generated by these methods.

105. In the decision under appeal, the opposition division formulated the technical problem to be solved starting from document (1) as "*... the provision of an alternative method of making antibodies comprising at least a human variable region and a mouse constant region*" (see page 21, lines 3 to 5 under the heading "Less ambitious problem and its solution"). The board

shares the appellant's view that the problem formulated in the decision under appeal is tainted with hindsight.

106. As stated above, document (1) strongly discourages the skilled person from trying to apply the methods disclosed therein to replace only the murine endogenous variable region by the human variable region, while keeping the murine constant region, so that hybrid antibodies are produced. Having this in mind, the board regards it as doubtful whether the skilled person would be motivated by the suggestions in document (4) to try to modify the chimeric locus described in Example VII of document (1) by deleting the human constant region gene segments.

107. The respondents pointed to documents (83) and (156) allegedly supporting the well-recognised value of the insertion at the endogenous locus. The passages of these documents on which the respondents rely, relate to the experiments and results reported in document (7) in which the board has been unable to find a hint at the solution proposed in the claims (see section 100 above).

108. Nor is there a hint in the passage of document (83) on which the respondents relied (see page 139, left-hand column, seven lines from the bottom of the second full paragraph). It is stated in this passage that transgenic animals, such as those described in document (7) "*... will greatly facilitate research on basic mechanisms of the mammalian immune response, including autoimmune diseases. They create a system for producing "human antisera" to antigens that could not safely be administered to human subjects. These mice are also a source of cells for making hybridomas that*

stably secrete ***human antibodies.***" (emphasis added by the board).

109. Document (156) (see page 400, first paragraph of the left-hand column), which is a chapter of a book dealing with the preparation of **human** antibodies from mice harbouring **human** immunoglobulin loci, mentions the use of endogenous regulatory sequences in combination with transgenic exons for securing controlled expression rates of the immunoglobulin locus. However, it does not teach or suggest creating a chimeric immunoglobulin heavy chain locus that produces hybrid antibodies containing human variable regions and mouse constant regions.

110. In view of the above, the board holds that the objection of lack of inventive step based on document (1) as the closest state of the art is not justified.

Document (8) as the closest state of the art

111. Document (8) relates to a transgenic mouse having incorporated into its germline unrearranged DNA fragments bearing exogenous, in particular human immunoglobulin gene segments which can be rearranged to produce immunoglobulins characterised by the presence of human heavy chains (see page 1, lines 6 to 9 and claims 1 and 2). The purpose of the methods and transgenic animals described in document (8) is to enhance the diversity of B lymphocytes produced by the animal. For this purpose, it is proposed that the unrearranged gene fragment which is introduced into a fertilized egg or a stem cell of the animal includes at least one exogenous (human) variable immunoglobulin gene segment, at least one D gene segment - which does

not need to be of human origin -, at least one exogenous (human) J segment, and at least one μ constant immunoglobulin region (see page 9, lines 19 to 25). The μ constant region may be of exogenous origin or from the animal itself, but it is preferably human (see page 10, lines 3 and 4). Desirably the fragment further contains an exogenous (human) γ constant immunoglobulin region (see page 10, lines 1 and 2). Human δ, ε and α constant regions may be included in the fragment (see page 12, lines 15 to 17).

112. In Example 2 of document (8), an unrearranged human immunoglobulin gene is constructed which comprises an unrearranged human V_H gene segment, the human J_H locus with a single upstream, unrearranged D segment, the murine μ gene including its upstream μ switch region, the murine γ2b switch region, and the human γ1 coding region. It is stated that the murine μ "*... may be changed for the human μ region, since both regions have been found to signal allelic exclusion in transgenic mouse models. Human switch regions may be substituted for switch regions of murine origin*" (see page 32, lines 12 to 21).

113. It has not been disputed that document (8) does not describe LTVEC prepared by homologous recombination. However, it is subject of dispute between the parties whether or not document (8) describes *in situ* replacement of the endogenous (murine) V, D and J gene segments with the orthologous human V, D and J gene segments. Respondent II's argument that, by reference to document (112), which describes methods for site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells, document (8) describes *in situ* replacement, fails, however, to convince the board. The passage on page 20, lines 4 to 6 on which respondent II

relied must be read in the context of the previous statements. The relevant passage reads:

"*Alternatively, a naturally unrearranged human DNA fragment, as described above, can be introduced into isolated embryonal stem cells of the animal to create chimeric animals. Cells which have integrated the human DNA are grown in culture, and can be used to repopulate animal embryos. Such cells could be selected for those bearing the human DNA by the polymerase chain reaction, with appropriate primers [See, e.g., R. K. Saiki, et al, Science, 239:487 (1988). Alternatively a drug resistance, e.g., neo, may be linked to the introduced DNA and the cells selected for growth in the presence of the drug. The resulting chimeric animal contains a population of cells, some of which descend from the altered pluripotent stem cell which carry the human DNA.*

Other cells of the animal descending from unaltered stem cells do not carry the human DNA. A transgenic animal can then be derived from such a chimeric animal by breeding. Such techniques for obtaining chimeric and transgenic animals are described in K. R. Thomas et al, Cell, 51:503 (1987)[document (112); note by the board)*; E. Robertson et al, Nature, 323:445 (1986); M. Hooper et al, Nature, 326:292 (1987) and M. R. Kuehn et al, Nature, 326:295 (1987).*"

114. In the board's view, the reference to document (112) would be understood by the person skilled in the art reading document (8) as a reference to methods for isolating, culturing and transfecting ES cells as described in page 511 of document (112). Since the skilled person cannot derive either from the passage quoted above or from any other part of document (8) any

indication that homologous sequences for driving targeted insertion are present in the constructs described in document (8), he/she would assume that the transgenes are integrated randomly in the genome of ES cells.

115. Starting from document (8), the problem to be solved can be formulated as the provision of improved methods and means for generating a repertoire of hybrid antibodies. In view of the advantages of *in situ* replacement of the endogenous immunoglobulin heavy chain V, D, and J gene segments of the murine ES cell by human V, D, and J gene segments, the board is convinced that this problem is solved by the subject-matter of the present claims.

116. Contrary to respondent II's objection, the solution proposed in claim 1 is not obvious in view of a combination of document (8) with document (94), in particular the section entitled "Generating larger deletions along with marker gene removal" bridging pages 53 and 54, which may be considered as being part of the common general knowledge at the priority date. In this section, gene targeting techniques to delete and replace up to approximately 20 kb of genomic sequence are described. It is stated that, by adding a site specific recombination step, the deletion can be extended by approximately 10 kb, allowing for gene deletions of up to approximately 30 kb. It is further stated that:

"*PCR and Southern blot strategies to identify proper gene replacement events are essentially as for conventional gene targeting (see Chapters 1 and 3), the major differences are that the presence of all recombinase target sites must be confirmed by Southern*

blot analyses or PCR, and deleted alleles must be distinguished from non-deleted. This involves using probes capable of detecting incorporation of the second loxP or FRT site, and restriction enzyme digests that differentiate the deleted product of site-specific recombination from the original floxed or flrted gene."

117. It should be noted that this passage describes only deletion - not replacement – of sequences up to 30 kb. Furthermore, there is no disclosure of the use of LTVECs, nor of the requirement of large homology arms to drive *in situ* homologous recombination. Although the board accepts that bacterial homologous recombination was part of the common general knowledge at the priority date, targeted insertion/replacement of large DNA fragments into the genome of ES cells by homologous recombination was not. Consequently, the board cannot accept that a person skilled in the art would arrive at the method of claim 1 by combining the teachings of documents (8) and (94).

118. The same applies as regards a combination of the teachings of documents (8) and (77). As stated above (see section 97), document (77) does not describe the use of a vector having a genomic fragment flanked by large homology arms (LTVEC) for targeting the genome of an ES cell, nor the detection of a modification of allele (MOA) in the ES cell. Even if, as respondent II argued, the skilled person takes into account also the teachings of document (112), he/she does not arrive at the method of claim 1, because also this document does not describe LTVECs or MOA.

119. The board therefore concludes that the objection of lack of inventive step of the subject-matter of claim 1

based on document (8) as the closest state of the art are not justified.

Document (3) as the closest state of the art

120. Document (3) describes the use of Cre-loxP-mediated gene replacement to generate a transgenic mouse strain that would produce antibodies with a constant (C) region of human rather than mouse origin. In the transgenic mouse strain, the entire murine Cγ1 gene is replaced by its human counterpart, except for the exons encoding the transmembrane and cytoplasmatic portions of the γ chain, with the aim at minimizing the danger of disturbing membrane expression and signalling of the humanized IgG1 in the mouse. According to the authors, these transgenic animals should enable the *ex vivo* production of humanized, chimeric monoclonal antibodies specific for any antigen to which the mouse can respond (see Abstract).

121. The technical content of document (3) has little in common with the present invention, except that it relates to a partly humanized immunoglobulin heavy chain locus engineered using Cre-loxP-gene replacement. Respondent II alleged that, starting from this document, the problem to be solved would be to provide hybrid antibodies with human variable and mouse constant regions. The problem so formulated contains however elements of the claimed solution.

122. Starting from document (3) as the closest state of the art, the problem to be solved must be seen as the provision of alternative methods and means to produce hybrid antibodies. This problem is solved by the subject-matter of claim 1. Contrary to respondent II's view, the solution proposed in this claim is not

obvious to a person skilled in the art from a combination of the teachings of documents (3) and (1). As stated above, the purpose of the methods and transgenic animals described in document (1) is the production of fully human antibodies, chimeric antibodies being described as disadvantageous. In the board's view, the skilled person does not find in document (1) either an incentive to modify the chimeric locus described in document (3) nor any hint towards a chimeric locus that produces hybrid antibodies containing human variable regions and mouse constant regions, as proposed by the invention. Hence, the objection of lack of inventive step based on a combination of documents (3) and (1) fails.

Adaptation of the description

123. In order to meet the requirement of Article 84 EPC that the claims have to be supported by the description, the adaptation of the description to the amended claims must be performed carefully to avoid inconsistencies between the claims and the description which could render the scope of the claims unclear. Any disclosure in the description inconsistent with the amended subject-matter should normally be excised. Reference to embodiments no longer covered by amended claims must be deleted, unless these embodiments can reasonably be considered to be useful for highlighting specific aspects of the amended subject-matter. In such a case the fact that an embodiment is not covered by the claims must be prominently stated (see T 1883/11, point 2 of Reasons).

124. The appellant submitted amendments to the description at a late stage of the oral proceedings. The board has briefly perused these amendments and considers that to

ensure full consistency between the claims of the main request and the description requires careful consideration. The board therefore considers it expedient to remit the case to the department of first instance for the adaptation of the description, in accordance with the discretion given to the board pursuant to Article 111(1) EPC (see T 1149/97, point 7.3 of the Reasons, OJ EPO 2000, 279).

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 claims 1-6 of the main request received during the oral proceedings on 9 November 2015 at 16:00 hours, and a description to be adapted.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated