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Datasheet for the decision of 6 September 2018

Case Number: T 2409/13 - 3.3.04

Application Number: 05723312.4

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Title of invention:
CDR-Repaired Antibodies

Patent Proprietor:
Genentech, Inc.

Opponent:
Glaxo Group Limited

Headword:
CDR-Repaired Antibodies/GENENTECH

Relevant legal provisions:
EPC Art. 54, 56, 84, 123(2)

Keyword:
Novelty - main and auxiliary requests 1 and 3 (no)
Inventive step - auxiliary requests 1 and 2 (no)
Amendments - auxiliary request 4 - added subject-matter (yes)

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T 0681/01

Catchword:

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Beschwerdekammern

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Case Number: T 2409/13 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 6 September 2018

Appellant: (Patent Proprietor)	Genentech, Inc. 1 DNA Way South San Francisco, CA 94080-4990 (US)
Representative:	Vossius & Partner Patentanwälte Rechtsanwälte mbB Siebertstrasse 3 81675 München (DE)
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Representative:	Hitchcock, Lucy Rose GlaxoSmithKline Global Patents (CN925.1) 980 Great West Road Brentford, Middlesex TW8 9GS (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 7 October 2013 concerning maintenance of the European Patent No. 1716181 in amended form**

Composition of the Board:

Chairwoman	M. Blasi
Members:	A. Chakravarty
	B. Claes

Summary of Facts and Submissions

I. European patent EP 1 716 181, entitled "*CDR-Repaired Antibodies*" derives from European application 05 723 312.4 and was published as international application WO 2005/080432.

II. In an interlocutory decision, the opposition division decided that, account being taken of the amendments in the form of auxiliary request 1, the patent and the invention to which it related met the requirements of the EPC (Article 101(3)(a) EPC). In the decision under appeal, the opposition division further considered that the subject-matter of claim 1 of the main request did not meet the requirements of Article 54 EPC.

III. Appeals were filed by the patent proprietor (appellant I) and the opponent (appellant II) against the interlocutory decision of the opposition division.

IV. The main request of appellant I was that the patent be maintained as granted. Alternatively, the patent should be maintained on the basis of the claims of auxiliary requests 1 to 4, filed in the proceedings before the opposition division and re-filed with the statement of grounds of appeal.

V. With their reply to the statement of grounds of appeal of appellant I, appellant II submitted a declaration of Dr P Hamblin.

VI. Oral proceedings before the board were held on 6 September 2018. At these oral proceedings the patent proprietor withdrew their appeal (becoming respondent) and designated the set of claims filed as auxiliary request 1 with the statement of grounds of appeal as

the main request. They furthermore filed sets of claims of auxiliary requests 2 and 4. At the end of the oral proceedings, the chairwoman announced the decision of the board.

VII. Claims 1 and 6 of the main request read:

"1. A method of making an altered antibody comprising (a) incorporating non-human hypervariable region residues into an acceptor human framework,

(b) further comprising introducing one or more amino acid substitutions in one or more hypervariable regions, without modifying the acceptor human framework sequence, wherein a library of altered antibodies is created and whereby substitutions in the hypervariable regions are made under conditions which maintain a bias towards the non-human hypervariable region sequence, and

(c) selecting an antibody with a binding affinity (Kd) value of no more than about 5 X 10^{-7}M."

"6. A method of selecting an altered antibody comprising:

(a) preparing nucleic acid encoding at least the variable heavy (VH) and variable light (VL) domains of an antibody, each comprising an acceptor human framework and hypervariable regions of a non-human antibody;

(b) substituting hypervariable region residues by introducing an approximately 10-50 percent mutation rate into the nucleic acid so as to maintain a bias

towards the non-human hypervariable region sequences; and

(c) selecting one or more altered antibodies that bind antigen".

VIII. Claim 1 of auxiliary request 1 reads:

"1. A method of making an altered antibody comprising

(a) incorporating non-human hypervariable region residues into an acceptor human framework,

(b) further comprising introducing one or more amino acid substitutions in one or more hypervariable regions, without modifying the acceptor human framework sequence, wherein a library of altered antibodies is created, whereby substitutions in the hypervariable regions are made under conditions which maintain a bias towards the non-human hypervariable region sequence, which conditions comprise introducing an approximately 10-50 percent mutation rate into a nucleic acid encoding each hypervariable region position to be substituted, and

(c) selecting an antibody with a binding affinity (Kd) value of no more than about 5 x 10^{-7}M".

Claim 5 of auxiliary request 1 and claim 4 of auxiliary request 3 are identical to claim 6 of the main request.

The set of claims of auxiliary request 2 is identical to the one of auxiliary request 1 except that claims 5 and 6 are deleted.

Claim 1 of auxiliary request 4 reads:

"1. A method of making an altered antibody comprising

(a) incorporating hypervariable region residues of a non-human antibody corresponding to the Kabat CDR residues, the Chothia hypervariable loop residues, the Abm residues, or the contact residues into an acceptor human framework to generate a hypervariable region-grafted antibody, wherein said incorporation results in a perturbation of the hypervariable regions and a loss of antigen binding affinity,

(b) subsequent to step (a), remodeling the hypervariable region-framework interface by introducing one or more amino acid substitutions in one or more hypervariable regions, without modifying the acceptor human framework sequence, wherein a library of altered antibodies is created and whereby substitutions in the hypervariable regions are made under conditions which maintain bias towards the non-human hypervariable region sequence, and

(c) selecting a remodelled antibody with
(i) a binding affinity (Kd) value of no less than 100 fold or no less than 10 fold the affinity of the non-human antibody; or
(ii) an improved binding affinity (Kd) relative to the non-human antibody; and
wherein said Kd value is no more than about 5 X 10^{-7}M."

IX. The final requests as noted by the Chairwoman and confirmed by the parties were as follows:

Appellant I requested that the appeal of appellant II be dismissed (main request), or alternatively, that the

decision under appeal be set aside and the patent be maintained in amended form on the basis of one of the sets of claims of auxiliary request 1, filed as auxiliary request 2 with the statement of grounds of appeal, auxiliary request 2, filed during the oral proceedings, or auxiliary request 3, filed with the statement of grounds of appeal, or auxiliary request 4, filed during the oral proceedings.

Appellant II requested that the decision under appeal be set aside and the patent be revoked in its entirety.

X. The following documents are referred to in this decision:

D1: Vasserot et al., Drug Discovery Today, 3 February 2003, 8, 118-126.

D5: Tan et al., Journal Of Immunology, 15 July 2002, 169, 1119-1125.

D6: Wu et al., Proceedings of the National Academy of Sciences of the United States of America, 26 May 1998, 95(11), 6037-6042.

D26: Huse et al., International Reviews Of Immunology, 1 January 1993, 10(2/03), 129-137.

D27: Glaser et al., Journal Of Immunology, 15 December 1992, 149(12), 3903-3913.

D31: Declaration of Dr P Hamblin, dated 19 June 2014.

XI. The respondent's arguments are summarised as follows:

Main request - claim 1
Claim construction

The claimed subject-matter was novel for the following reasons. The claim was for a method of humanising a non-human antibody by complementary determining region (CDR) grafting. The prior art comprised methods of humanising antibodies by CDR grafting followed by making amino acid substitutions in the framework region. It was explained, for example, in document D5 that "*avoidance of any perturbation of the CDRs has been an overriding principle in the design of humanized Abs*" (see page 1120, left column, penultimate paragraph).

The claimed method comprised three steps (a) to (c), where step (a) was the CDR grafting step, as explained on page 29, paragraph 2 of the application and was followed by step (b), the introduction of one or more amino acid substitutions in the CDRs using a "*soft randomisation*" approach, as explained on page 75, penultimate paragraph 2 of the application. Given that it was apparent from the description that the claimed invention related to CDR grafting, the skilled person reading the claim would have understood that steps (a) to (c) of the claimed method were to be carried out in the particular order presented in the claim.

Novelty (Article 54 EPC)

Document D1 did not disclose a method of CDR grafting, since its first step was the creation of a library of nucleic acids encoding CDR variants by codon-based mutagenesis and only afterwards introducing these

variants into nucleic acids encoding human framework regions.

Document D1 did disclose a method for making humanised antibodies involving the substitution of the hypervariable regions under conditions that did not maintain a bias towards the non-human hypervariable domain sequence.

The frAMEworks™ process disclosed in document Dl used codon based mutagenesis to, "*explore all possible amino acid changes at each position of the CDRs*" (document D1, legend to Figure 1, lines 3 to 4) and this represented a completely unbiased exploration of all possible substitutions in each CDR.

The disclosure in document D1 that the method used was based on CDR variants that were "*closely related*" to the parent sequence (see page 121, right-hand column, lines 6-8) did not alter the frAMEworks™ process described in Figure 1, which explored all possible amino acid changes at each CDR residue. In particular, the skilled person would not have taken a teaching from document D1 that a bias toward the parent sequence should be maintained.

In addition, the method disclosed in document D1 required that the modification and screening steps were only the first step of the entire process. In particular, Figure 1 disclosed that, following the initial functional assay, the process was reiterated until the desired improvement was obtained (see Figure 1 caption, lines 4 to 7). Thus, regardless of the starting CDR sequence, the frAMEworks™ process differed from that claimed in that it involved the

repeated mutation of CDR residues without any bias. It followed that the subject-matter of claim 1 was novel.

Auxiliary requests 1 and 2 - claim 1
Inventive step (Article 56 EPC)

The claimed method was not obvious to the skilled person for the following reasons: It differed from that of claim 1 of the main request in that it further defined the conditions of how the bias was introduced - by stating "which conditions comprise introducing an approximately 10-50 percent mutation rate into a nucleic acid encoding each hypervariable region position to be substituted".

Document D1 disclosed a method of codon based mutagenesis in which unbiased mutations were made in the CDRs. Even if the disclosed method introduced mutations so as to retain a bias towards to parent sequence (which was disputed), no mutation rate for each hypervariable region position to be substituted was disclosed and thus the method of document D1 could not anticipate the claimed subject-matter. It could, for the sake of argument be taken to represent the closest prior art for the claimed invention.

Given the above difference between the closest prior art and the claimed subject-matter, the problem to be solved was that formulated in the decision under appeal, i.e. [providing a method for] "*the humanisation of therapeutically interesting antibodies without the problem of high immunogenicity caused by the murine sequences present in the antibody*".

Combining the disclosure in document D1 with that in documents D26 or D27 did not render the claimed

invention obvious to the skilled person either. As recognised in the decision under appeal, the skilled person would not have considered combining the disclosure in documents D26 or D27 with that in document Dl (see point 10.3.1 of the decision under appeal). Even if the teaching of these documents was taken into account by the skilled person, there was nothing in either document that would have led them to the conclusion that the mutation method disclosed therein was inherently associated with a bias towards the parental amino acid sequence. In fact, both documents disclosed that any desired mutation rate could be achieved. Thus the skilled person was left with the teaching in document D1 as discussed above.

Auxiliary request 3 - claim 4 (identical to claim 6 of the main request and claim 5 of auxiliary request 1) Novelty (Article 54 EPC)

The claimed subject-matter was novel for the following reasons: It was a method of selecting a CDR grafted antibody after modification in the CDR region and screening for antibodies with an improved binding to the antigen. The modifications in the CDR region residues were effected by introducing an approximately 10-50% mutation rate into the nucleic acid encoding each position to be substituted so as to maintain a bias to the donor/parental region sequences.

Document D6 did not directly and unambiguously disclose a method in which the substitution of the hypervariable region residues was effected by introducing an approximately 10-50% mutation rate into the encoding nucleic acid so as to maintain a bias to the donor/ parental region sequences.

The disclosure in document D6 did not include the disclosure in documents D26 or D27, incorporated by reference. As set out above, the skilled person would not have considered the disclosure in documents D26 or D27 as forming part of that of document D6. Even if they had done so, neither of these documents disclosed or suggested antibody selection methods comprising the introduction of a 10-50% mutation rate in the encoding nucleic acid sequences as claimed.

Auxiliary request 4 - claim 1
Clarity (Article 84 EPC)

The claim was clear for the following reasons: The skilled reader would immediately realise that the "binding affinity value" referred in step (c) as being "no less than 100 fold or no less than 10 fold the affinity of the non-human antibody" related to how strongly the antibody bound to the antigen, with "no less than 100 fold [...] the affinity of the non-human antibody" being the minimum binding affinity.

Amendments (Article 123(2) EPC)

The claims of auxiliary request 4 met the formal requirements of the EPC. The subject-matter of the amended claims found support in the application as filed, at least at page 3, lines 10 to 12; page 29, lines 8 to 15, 26 to 27 and 36 to 37; page 49, lines 32 to 34 and page 50, lines 6 to 7.

XII. Appellant II's arguments are summarised as follows.

Main request - claim 1
Claim construction

The claimed subject-matter was a method for producing an altered humanised antibody, not limited to methods of CDR grafting. The method 'comprised' steps (a) to (c) and therefore could also include additional steps. Moreover the claim did not require the steps to be performed in the order (a) to (c). Thus, the claimed subject-matter included methods for making an altered antibody in which step (b) preceded step (a). The bias mentioned in step (b) of the claim should be understood to mean that the antibody produced after carrying out the step comprised a CDR which was similar in sequence over its length to the sequence of the CDR found in the unmodified parent antibody.

Novelty (Article 54 EPC)

The claimed subject-matter lacked novelty for the following reasons: the method was not limited to "*CDR-grafting*" because there was no required sequence to two steps (a) and (b). Document D1 disclosed a method of making an altered antibody, having all the features of the claimed method, which aimed at improving the affinity of humanised antibodies to their antigen by using codon-based mutagenesis of the CDRs under the so-called "frAMEworks™" approach. The CDR sequences produced by this method maintained a bias towards the parent sequence, as evident from on page 121, right-hand column, lines 6 to 8, which stated that the CDR variants of the frAMEworks™ method were prepared so that they are "*closely related to those of the parent molecule*". The skilled person would have understood

this to mean that the technique described included the feature "whereby substitutions in the hypervariable regions are made under conditions which maintain a bias towards the non human hypervariable region sequence" (see document D31).

The sentence in the legend of Figure 1 of document D1, stating that codon-based mutagenesis was used to create the variant libraries and to "explore all possible amino acid changes at each position of the CDRs", did not mean that the library was generated in an unbiased fashion. In fact, it said nothing about the degree of bias in the method but merely referred to the fact that the codon-based mutagenesis procedure enabled the user to investigate all 20 natural amino acids at each position should they so wish. Crucially, the method allowed exploration whilst maintaining a desired degree of bias towards the parental sequence by varying the conditions. This view was confirmed in document D31.

It was self-evident that the mutations made by the method disclosed in document D1 were done so as to maintain a bias towards the non-human donor sequence since the alternative was completely illogical. If no bias were maintained towards the non-human donor CDR sequences, then would simply be no reason for the skilled person to start with a non-human donor antibody in the first place.

Auxiliary requests 1 and 2 - claim 1
Inventive step (Article 56 EPC)

The claimed method was obvious to the skilled person for the following reasons:

The closest prior art

The disclosure of document D1 represented the closest prior art for the claimed method. It was directed to the same purpose as the patent, which was the provision of altered antibodies that possessed reduced immunogenicity in humans (see, for example, paragraphs [0071] and [0247] of the patent). It highlighted that immunogenicity was a primary concern which had curtailed the development of certain therapeutics (see page 120, end of right-hand column).

The problem and its solution

Document D1 disclosed a method (frAMEworks™) in which fully human frameworks were combined with synthetic CDRs, with the resultant antibodies being devoid of any potentially immunogenic murine residues in the framework regions (see page 120, left-hand column, first full paragraph). The only difference between the claimed method and that disclosed in document D1 was that, in the step of introducing one or more amino acid substitutions in one or more hypervariable regions, without modifying the acceptor human framework, the "substitutions in the hypervariable regions are made under conditions which maintain a bias towards the non-human hypervariable region sequence, which conditions comprise introducing an approximately 10-50 percent mutation rate into a nucleic acid encoding each hypervariable region position to be substituted".

The technical effect of this difference was that the resultant hypervariable region was biased towards the non-human sequence. A higher mutation rate led to a lower bias towards the parent sequence and *vice-versa*.

The objective technical problem was therefore the provision an alternative method of making a humanised antibody.

Obviousness

The claimed solution was obvious in light of the teaching of document D1 in combination with document D26 and/or D27. Document D27 was the seminal paper on codon-based mutagenesis. Its authors described a specific example of an application of the technique which "*results in a mixture of oligonucleotides coding for randomized amino acids within a predefined region while maintaining a 50% bias toward the parental sequence at any position*" (page 3904, right-hand column, end of second full paragraph, emphasis added). That the technique disclosed in document D27 led to CDRs having a 50% bias to the parent (equivalent to 50% mutation rate) was illustrated in Figure 1 on page 3905 and re-confirmed on page 3904, right-hand column, penultimate paragraph "*In some mutants, only one of the five CDR codons was altered, whereas in others multiple codon changes were effected. Both the observed codon changes and resulting amino acid substitutions shown in Table II appeared to be random, with no discernible pattern of clustering and the expected 50% substitution per position (31 substitutions in 60 positions)*".

The skilled person consulting document D27 would have been motivated to apply this same procedure to the frAMEworks™ method disclosed in document D1, thus arriving at the subject-matter of claim 1. Document D1 provided an incentive to introduce a bias towards the non-human sequence because the method required libraries of CDR variants that were closely related to those of the parent molecule.

Document D1 was a short 9-page review with various small sections, each of which were by the same authors and connected. It was therefore completely illogical to suggest that a skilled person would not have linked information given in neighbouring sections.

Auxiliary request 3 - claim 4 (identical to claim 6 of the main request and claim 5 of auxiliary request 1) Novelty (Article 54 EPC)

The claimed subject-matter was a method of selecting an altered antibody and merely required that the nucleic acid mentioned in step (a) encoded a variable heavy domain and variable light domain of an antibody having an acceptor human framework and hypervariable regions of a non-human antibody and that the hypervariable regions were mutated as described in step (b). The "comprising" language meant that, whilst the claimed method included a step of mutating the hypervariable regions, the addition of further steps was not precluded and it was not required that the acceptor human framework remain unmodified. Accordingly methods where the acceptor human framework was modified, as well as those where the acceptor human framework was not modified were claimed.

Document D6 disclosed the step-wise, *in vitro*, affinity maturation of Vitaxin, a humanised antibody recognising $\alpha_v\beta_3$ integrin. Vitaxin was a CDR-grafted antibody which also included some framework mutations. Detailed methodology as to how to carry out CDR affinity maturation was provided in the "*Materials and Methods*" section on page 6038. According to that method, the CDRs were identified, oligonucleotides prepared and M13 phage libraries made for each CDR, with the oligonucleotides designed to mutate a single CDR

residue in each clone, i.e. mutating one amino acid in each CDR at a time.

The subsection "*Screening of Expression Libraries*" (page 6038, left-hand column) made it clear that Fab libraries were used to screen for $\alpha_v\beta_3$-specific binders. It was therefore clear that nucleic acids were prepared encoding V_H and V_L domains in which a non-human hypervariable region (mutated CDR from the M13 library) had been incorporated into a human acceptor framework, as required by step (a) of claim 6.

Furthermore, the "*Construction of CDR Libraries*" subsection disclosed the "*NN(G/T)*" method of incorporating and it would be clear to a person skilled in the art that this approach inevitably resulted in a 50% bias towards the parental sequence at each position.

The claimed method did not require the construction of libraries of altered antibodies and step (c) did not refer to a mutation rate at each hypervariable region position to be substituted (cf. claim 1 of auxiliary request 1, see Section VIII., above). The mutation rate could therefore mean the percentage of mutation introduced over the length of the CDR. Table 2 of document D6 disclosed the different CDRs and the single mutations introduced therein. It was a simple matter to calculate which of the mutated CDRs had a mutation rate of 10-50%. The bias towards the non-human (parent) sequence was given, since only a single amino acid in each CDR was mutated.

Accordingly, document D6 disclosed a method having all the features required by claim 6 of the main request

and of the identical claims of the first and third auxiliary requests, which all lacked novelty.

Auxiliary request 4 - claim 1
Clarity (Article 84 EPC)

The wording in step (c) (ii) was unclear. It mentioned "a binding affinity value of no less than 100 fold or no less than 10 fold the affinity of the non-human antibody". However the expression "binding affinity" was followed by the term "Kd", referring to the dissociation constant. The concept of binding affinity of "no less than" a certain value and dissociation constant of "no less than" a certain value, were contradictory, since a lower Kd indicated a higher binding affinity. Hence the claim lacked clarity.

Amendments (Article 123(2) EPC

The combination of the phrase "binding affinity value of no less than 100 fold or no less than 10 fold the affinity of the non-human antibody" with the term "Kd" in part (c)(i), had no basis in the application as filed. While the application as filed provided a basis for screening antibodies for binding affinity where the affinities achieved are "no less than 100 fold or no less than 10 fold the affinity of the non-human parent antibody" on page 49, lines 32 to 35, this passage did not mention the dissociation constant, "Kd". Thus, claim 1 did not meet the requirements of Article 123(2) EPC.

Reasons for the Decision

1. The patent proprietor withdrew their appeal during the oral proceedings. Thus the patent proprietor (former appellant I) has the status of respondent.

2. The appeal of appellant II is admissible.

Background to the invention

3. The invention concerns a method of making a humanised antibody starting from a non-human antibody in which non-human variable regions (CDRs) are incorporated into a human framework and the humanised antibody is modified to compensate for the loss of binding affinity caused by the humanisation. According to the description of the patent, a problem with murine antibodies in a clinical setting is that their use "*can result in a human anti-mouse antibody response (HAMA) thus negating their utility*" (see paragraph [0002]). To mitigate this a "*method to transfer the murine antigen binding information to a non-immunogenic human antibody acceptor, a process known as humanization*" was developed (*ibid.*). However, "[*f]ollowing transfer of CDR residues into an acceptor chosen by either of these methods, it has been necessary to alter framework residues in the acceptor in order to restore and enhance antigen binding affinity*" (see paragraph [0003]).

4. Furthermore, the description of the patent states that the invention concerns an approach to restoring antigen binding affinity of humanised antibodies in which "*rather than transferring murine residues that interact with the hypervariable region(s) to the new framework, [...] the molecular fit between the new framework and*

the grafted hypervariable region can be restored by changing residues residing within the hypervariable region(s)" (see paragraph [0007] of the patent). This rationale finds expression in step (b) of claim 1 of all pending claim requests, which comprises "introducing one or more amino acid substitutions in one or more hypervariable regions, without modifying the acceptor human framework sequence".

5. Note on nomenclature: the expression "hypervariable region", used in the patent is equivalent to "*complementary determining region*" or CDR and the two expressions are used interchangeably in this decision.

Main request - claim 1
Claim construction

6. It is established case law of the boards of appeal that claims should be construed to arrive at an interpretation which is technically sensible and takes into account the whole disclosure of the patent but also bearing in mind that only that technically illogical interpretations should be excluded (see also Case Law of the Boards of Appeal of the European Patent Office, 8th edition 2016, II.A.6.1).

7. There was disagreement between the parties as to whether or not the claim prescribed a temporal order on the steps, in particular on steps (a) and (b). In the absence of explicit language in the claim specifying an order to steps (a) and (b) and given that the board has seen no evidence of a technical reason for a particular order of steps (a) and (b), the claim is construed as including methods comprising steps (a) and (b) in either order. The fact the the steps are presented in alphabetical order is not considered by the board to

impart an compulsory order on the steps. Thus, contrary to the view of the respondent, the claimed method is not limited to methods of CDR grafting.

8. Furthermore, the respondent took the view that the claimed subject-matter did not include methods having additional mutation, screening and selection steps beyond steps (b) and (c), while appellant II considered that the claimed method could include additional steps. The board considers that in view of the "comprising" language used, the claimed method may also include further steps in addition to steps (a) to (c).

9. There was also disagreement between the parties about the meaning of the phrase "a bias towards the non-human hypervariable region sequence" in the context of step (b) of the claim. The parties put forward essentially two alternative constructions of this feature. The construction put forward by appellant II was that the bias may be calculated over the entire length of the hypervariable region. The respondent on the other hand, considered that the bias should be interpreted in line with claim 4, i.e. that it refers to the mutation rate into the nucleic acid encoding each hypervariable region position to be substituted.

10. Applying the principles set out in point 6. to the case at hand, the board considers that the skilled person would have recognised that the claimed method involves making modifications to a humanised antibody in the non-human CDR sequence with aim of restoring the binding activity to that of the non-human "parent" antibody. The skilled person would have further recognised that a technically sensible understanding of the phrase "whereby substitutions in the hypervariable regions are made under conditions which maintain a bias

towards the non-human hypervariable region sequence" is that the final, modified sequence maintains a bias (i.e. a close structural relationship) towards the parent sequence. This interpretation finds support in document D31 which states that "*[i]f no bias is maintained towards the non-human donor CDR sequences, then there is simply no reason for the skilled person to start with a non-human donor antibody in the first place*" (see paragraph 12). The board is also persuaded by appellant II's submission that "*[i]f the skilled person did not make substitutions under a method that maintained bias, then the output would be entirely random and resultant antibodies would have CDRs the overwhelming majority of which* [*bore*] *no resemblance to the donor CDRs, the binding properties of which the skilled person is trying to retain*" (see statement of grounds of appeal of appellant II, paragraph bridging pages 18 and 19). The board also considers that the interpretation suggestion by the respondent represents a second technically sensible interpretation of the above phrase and therefore accepts the interpretations put forward by both parties.

11. In view of the above considerations, the board construes the claim as including those methods in which the modified hypervariable region sequence "maintains a bias towards the non-human hypervariable region sequence" over its length and also those in which the bias refers to the mutation rate into the nucleic acid encoding each hypervariable region position to be substituted.

Novelty (Article 54 EPC)

12. Document D1, a review article about optimisation of protein therapeutics by directed evolution (see title),

contains a section entitled "*Engineering antibodies with fully human frameworks*" (see page 120). Here, a so-called "frAMEworks™" humanisation process is described which addresses the problem of "*loss of activity*" inherent in "*traditional antibody humanization* [i.e.] *the direct transfer, or grafting, of murine complementarity-determining regions (CDRs) into a human framework*" (see page 121, left-hand column).

13. The humanisation method disclosed involves creating "[l]*ibraries of CDR variants derived from the original murine or chimeric antibodies* [...] *inserted into a fully human, germline framework. Codon-based mutagenesis is* [then] *used to create the variant libraries and to explore all possible amino acid changes at each position of the CDRs. A functional screen is then used to identify humanized variants that maintain or improve antigen binding. Subsequently, additional amino acid substitutions are introduced into the CDRs of the antibody and the process is reiterated until the desired improvements are reached. This approach generates antibodies with fully human frameworks and significantly enhanced affinity and/or activity*" (see page 121, legend to Figure 1).

14. The board is of the view that the method disclosed in document D1 falls within the ambit of the claim for the following reasons:

i) it is a method for making an altered antibody,

ii) it involves generating "*Libraries of CDR variants derived from the original murine or chimeric antibodies*" (see document D1, legend to Figure 1), also described as generating "*libraries of synthetic CDR*

variants that are closely related to those of the parent [murine] molecule" by codon based mutagenesis (see page 121, right-hand column, paragraph 1), which is considered to correspond to "comprising introducing one or more amino acid substitutions in one or more hypervariable regions" in step (b) of claim 1 because of the reference to CDR **variants**.

iii) it comprises the insertion of these CDR variants "*into the selected frameworks*" (*ibid.*), which is considered to correspond to the feature in step (a) of claim 1 of "incorporating non-human hypervariable region residues into an acceptor human framework". The reference to the insertion of the CDR variants "*into a fully human, germline framework*" (see paragraph 13., above) also inherently discloses that the human acceptor framework is not modified (cf. claim 1 step (b)) and finally,

iv) the method disclosed in document D1 involves employing "*a functional assay [...] to identify amino acid changes that accommodate the new framework and simultaneously improve affinity or any other crucial property of the antibody*" and it is disclosed that "*the frAMEworks™ approach has generated several antibodies with femtomolar affinities*" (see page 121, right-hand column, paragraph 1), which is considered to correspond to the features in step (c) of claim 1.

15. The respondent provided several lines of argument as to why the subject-matter of claim 1 differed from that disclosed in document D1 as follows.

16. The first was that the claim was for a method of CDR grafting, which entailed as a first step, incorporating non-human CDRs into a human framework. The methods

disclosed in document D1 were not CDR grafting methods, instead entailing the creation of a library of CDR variants and the subsequent insertion of these into human frameworks. The skilled person would have immediately understood that CDR grafting was an entirely different procedure to the frAMEworks™ process disclosed in document D1.

17. The board notes that the first line of argument depends on the claim prescribing a temporal order to the steps recited in the claim, i.e. requiring that step (a) is performed before step (b). However, as set out in point 7. above, the claim prescribes no such order. Hence the claimed subject-matter includes both methods in which non-human CDRs are first grafted into a human framework and then modified and also methods in which the non-human CDRs are first modified and then inserted into a human framework. Thus, the first line of argument must fail.

18. The second line of argument was that the subject-matter of claim 1 differed from the method disclosed in document D1 at least in that the latter did not include the feature of substitution of the hypervariable regions under conditions that maintain a bias towards the non-human hypervariable domain sequence. The disclosure in document D1 (see legend to Figure 1) to that codon based mutagenesis was done to "*explore all possible amino acid changes at each position of the CDRs*" could only mean that a completely unbiased exploration of all possible substitutions in each CDR was disclosed.

19. The second line of argument relates to whether or not the method disclosed in document D1 involves making "substitutions in the hypervariable regions" "under

conditions which maintain a bias towards the non-human hypervariable region sequence". This issue is dealt with in point 14., above.

20. A third line of argument was that in the method disclosed in document D1, the functional assay to determine resulting antibody properties was only a first step. Figure 1 disclosed that following this initial functional assay, the frAMEworks™ process involved the introduction of further mutations into the CDRs and reiterating this process until the desired improvement was reached.

21. The respondent's third line of argument is not persuasive either because, as set out in point 7., the claim is for methods "comprising" steps (a) to (c) and therefore includes methods having additional steps. Therefore, the fact that document D1 discloses a method in which the functional assay to determine resulting antibody properties is followed by reiterating the mutation and selection process until the desired improvement is reached does not differentiate the disclosure in document D1 from the claimed subject-matter.

22. The above considerations lead to the finding that the subject-matter of claim 1 of the main request lacks novelty with respect to the disclosure in document D1. The main request therefore does not meet the requirements of Article 54 EPC.

Auxiliary request 1
Novelty (Article 54 EPC) - claim 1
Amendments (Article 123(2) EPC) - claims 1 to 6

23. In the course of the oral proceedings, the board informed the parties that it was of the opinion that the claims of auxiliary request 1 met the requirements of Article 123(2) EPC and that the subject-matter of claim 1 was novel. In view of the decision on inventive step relating to the inventive step of this request, set out below, reasoning for the above mentioned opinions need not be given in this decision.

Auxiliary requests 1 and 2 - claim 1
Inventive step (Article 56 EPC)

The closest prior art

24. As set out in point 12. above, document D1 discloses a method for making humanised antibodies in which the CDR sequences have been modified by substitution by codon mutagenesis followed by screening the resulting antibodies for the binding to a selected antigen. It therefore has the same aim as the claimed method. The parties were in agreement that this disclosure in document D1 can be taken as the closest prior art for the invention claimed. The board agrees.

The problem and its solution

25. The method disclosed in document D1 differs from the claimed one in that the latter specifies that the substitutions in the hypervariable regions are made under conditions which maintain a bias towards the non-human hypervariable region sequence, which conditions comprise introducing an approximately 10-50 percent

mutation rate into a nucleic acid encoding each hypervariable region position to be substituted (emphasis added by the board). Document D1 on the other hand states that the codon based mutagenesis is done such that the produced variants are "*closely related to those of the parent molecule*" (see document D1, page 121, right-hand column, paragraph 1). Document D1 therefore does not specify a particular mutation rate in the nucleic acid encoding each hypervariable region position to be substituted.

26. The mutation rate of approximately 10-50 percent mutation rate into a nucleic acid encoding each hypervariable region position to be substituted has the technical effect of determining the ratio of mutant to parent amino acids at each position of the hypervariable region. A higher rate of mutation leads to a higher proportion of molecules in the variant "*library*" having an amino acid differing from that of the parent at each position leading to an increase in the average number of mutations in each CDR.

27. In view of the above difference and the technical effect thereof, the problem to be solved by the claimed subject-matter is to put into practice the frAMEworks™ antibody humanisation method suggested in document D1. This differs, at least in formulation, from the problem suggested in the decision under appeal of [providing a method for] "*the humanisation of therapeutically interesting antibodies without the problem of high immunogenicity caused by the murine sequences present in the antibody*". However, in essence both problems aim at providing humanised therapeutically interesting antibodies without the problem of high immunogenicity caused by the murine sequences present in the antibody. They differ in that the problem adopted by the board

takes more account of the disclosure in document D1. The problem formulated in the statement of grounds of appeal of former appellant I (respondent) was "*provision of a method for producing an altered antibody that maintains/restores the affinity/avidity lost during a CDR-grafting process*" (see respondent's statement of grounds of appeal, D.3.2). The board considers that this cannot be the problem to be solved because it has construed the claimed subject-matter as not being limited to CDR grafting methods (see point 7. above).

Obviousness

28. The question to be answered is whether it was obvious to the skilled person, faced with the above formulated technical problem and starting from the method disclosed in document D1, to carry out that method under conditions resulting in the introduction of an approximately 10-50 percent mutation rate into a nucleic acid encoding each hypervariable region position to be substituted.

29. Document D1 contains no details on the conditions to be used when carrying out codon mutagenesis, but in the context of explaining the "*Benefits of codon based mutagenesis and focused libraries*" (see heading on page 120, left column), states - "*We have pioneered a mutagenesis procedure that introduces diversity into nucleic acids encoding the protein of interest through the targeted insertion of synthetic oligonucleotide pools generating changes at the codon level. This codon-based strategy enables full control over the location and extent of changes and permits the evaluation of the entire repertoire of natural amino*

acids at every position [13,20]" (see heading on page 120, right column, first paragraph).

30. The referenced documents are documents D26 and D27 in the appeal procedure. The board considers that the skilled person starting from document D1 and seeking to solve the technical problem and therefore seeking further details on how to carry out codon based mutagenesis, would have turned to either or both of these documents, since these are referenced in exactly the context of providing background information on this method.

31. Document D27, entitled "*Antibody engineering by codon-based mutagenesis in a filamentous phage vector system*", concerns the use of codon based mutagenesis in conjunction with the M13 antibody expression and screening system to provide an efficient and general approach for redirecting the specificity and potentially improving the affinity of antibodies *in vitro* (see abstract) and is therefore not concerned directly with restoring antibody binding affinity after humanisation. It does however contain a detailed description of codon based mutagenesis. On the topic of the level of mutations to be introduced, the following disclosure is made "*Several options are available in the choices of what CDR to mutate and the level of mutations introduced. At low levels of mutations the libraries become very large increasing the screening task, but at high levels of mutation there is a risk that the preponderance of multiple mutations per antibody will create a large number of functionally useless antibodies*" (see page 3904, left-hand column, "*Results*", lines 5 to 11 of the paragraph). In the particular method disclosed in the document "*The choices of L2 and L2 were essentially arbitrary* ***as was***

the choice to insert a level of 50% substitution at each codon in the CDR" (*ibid.*, lines 14 to 16 of the paragraph; emphasis added by the board). This is further explained as follows "*This particular application of codon-based synthesis results in a mixture of oligonucleotides coding for randomized amino acids within a predefined region while maintaining a 50% bias toward the parental sequence at any position*" (*ibid.*, right-hand column, second full paragraph). The mutagenesis successfully produced CDR variants with the desired altered binding affinity (see page 3908, right-hand column, "*Discussion*", first paragraph).

32. The respondent argued that documents D26 and D27 both disclosed that in codon based mutagenesis the mutation rate was fully selectable and that in fact the skilled person reading document D1 in combination with documents D26 and D27 would consider that "*the completely unbiased mutation achievable by codon based mutagenesis [...] forms the core of the frAMEworks™ process*" and allowed the exploration of all possible amino acid changes at each position of the CDR (see statement of grounds of appeal, E.2.1.2).

33. It is correct that document D27 teaches that codon based mutagenesis has the potential to any mutation rate at each position to be mutated. However, the document then exemplifies a "*particular application of codon based mutagenesis*" which "*results in a mixture of oligonucleotides coding for randomized amino acids within a predefined region while maintaining a 50% bias toward the parental sequence at any position*" (see document D27, page 3904, right-hand column) and finally concludes that "*we have successfully mutagenized antibody hypervariable regions resulting in 50%*

saturation at the level of each codon" (see paragraph bridging pages 3909 and 3910). The board therefore agrees with appellant II that the skilled person seeking guidance on a suitable mutation level for each position would have learned that maintaining a 50% bias toward the parental sequence at any position was a successful strategy. They would therefore have applied this level of bias to the method disclosed in general terms in document D1. Thus, the claimed subject-matter of claim 1 of auxiliary requests 1 and 2 was obvious to the skilled person. Auxiliary requests 1 and 2 do not meet the requirements of Article 56 EPC.

Auxiliary request 3 - claim 4 (identical to claim 6 of the main request and claim 5 of auxiliary request 1)Novelty (Article 54 EPC)

34. The claim is for a method of selecting an altered antibody comprising steps (a) to (c). In contrast to the method of claim 1 of auxiliary request 3, it does not require that the human acceptor framework sequence is not modified. The board construes step (b) of this claim analogously to step (b) of claim 1 in that the phrase "substituting hypervariable region residues by introducing an approximately 10-50 percent mutation rate into the nucleic acid so as to maintain a bias towards the non-human hypervariable region sequences" may refer to both methods in which the mutation rate and the bias are calculated with respect to the length of the CDR, i.e. such that 10-50 percent of the amino acids in the final sequence are mutated, and to methods in which the mutation rate refers to the rate of mutation at a single position and hence 10-50 percent of molecules in a library will have a mutated amino acid at said position (see point 11. above).

35. Document D6 discloses a method for improving the binding characteristics of Vitaxin (a humanised antibody specific for a conformational epitope of the $\alpha_v\beta_3$ integrin complex). To do this, phage-expressed libraries of Vitaxin Fab variants were constructed and subjected to "*a limited initial mutagenesis strategy in which every position of all six CDRs was methodically and efficiently mutated ... followed by the expression and screening of a combinatorial library consisting of the best mutations*" (see page 6037, right-hand column, final paragraph). Beneficial mutations obtained by this method are listed in Table 2. Four mutants having a single substitution in the CDR3 sequence of the L chain are shown to have good binding affinities. The parent CDR is the nine amino acid sequence "QQSGSWPHT". The mutant sequence have the amino acids N or T in place of the parent G, or L or Q in place of the parent H. A single amino acid represents 11.11% of the total sequence. Thus, the method disclosed in document D6 has all the feature of the claimed method and anticipates it.

36. The respondent argued that the method disclosed in document D6 was not relevant to the novelty of the claimed subject-matter by relying on a construction of the claim that interpreted the phrase "substituting hypervariable region residues by introducing an approximately 10-50 percent mutation rate into the nucleic acid so as to maintain a bias towards the non-human hypervariable region sequences" as used in the claim to refer only to methods in which the mutation rate refers to the rate of mutation at a single position and leading 10-50 percent of molecules in a library to have a mutated amino acid at said position. However, in view of the board's claim construction (see point 11. above), this argument is not successful.

37. It follows that the subject-matter of claims 6, 5 and 4 of the main and auxiliary requests 1 and 3, respectively, does not meet the requirements of Article 54 EPC.

Auxiliary request 4 - claim 1
Clarity (Article 84 EPC)

38. During the oral proceedings, the board gave an negative opinion with regard to the clarity of this claim. However, in view of the decision on added subject-matter below, a detailed reasoning for this opinion is not required here.

Amendments (Article 123(2) EPC)

39. Article 123(2) EPC provides that a European patent may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed. It is an accepted principle in the established case law of the Boards of Appeal that, to comply with Article 123(2) EPC, the amendment should be made within the limits of what a skilled person would derive directly and unambiguously, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the application as filed (see Case Law of the Boards of Appeal of the EPO, 8th edition 2016, II.E.1).

40. Claim 1 of auxiliary request 4 has no direct equivalent in the application as filed.

41. The respondent stated that the basis for the subject-matter of step (c)(ii) of claim 1, i.e. "selecting a remodelled antibody with an improved binding affinity

(Kd) value no less that 100 fold or no less than 10 fold the affinity of the non-human antibody" was to be found in the final paragraph of page 49 of the application as filed.

42. The relevant passage of page 49 reads as follows: "*In a preferred embodiment of the invention, a panel of antibody variants are prepared and are screened for binding affinity for the antigen and/or potency in one or more biological activity assays. The affinities achieved are preferably similar to, e. g. no less than 100 fold, or no less than 10 fold the affinity of the non-human parent antibody*".

43. The board considers that the above cited passage in the application provides a basis for a method having a step of selecting a remodelled antibody with a binding affinity *"no less than 100 fold, or no less than 10 fold the affinity of the non-human parent antibody*" but not for selecting antibodies with a lower dissociation constant (Kd) than the non-human parent antibody.

44. The respondent further considered that the basis for the subject-matter of claim in relation to the features of step (c)(ii), i.e. "selecting a remodelled antibody with an improved binding affinity (Kd) relative to the non-human antibody" was to be found in the application as filed on page 50, lines 4 to 7 which reads "*One or more of the antibody variants selected from an initial screen are optionally subjected to one or more further biological activity assays to confirm that the antibody variant(s) have improved activity in more than one assay*".

45. The board however holds that this passage does not directly and unambiguously disclose that improved

activity referred to is "relative to the non-human antibody", since the improvement could also be with relative to the humanised antibody obtained in step (a) of the claimed method. Moreover, the cited passage in fact concerns "improved activity in more than one assay" and not "improved binding affinity (Kd) relative to the non-human antibody".

46. In view of the above considerations, the claimed subject-matter extends beyond the content of the application as filed, contrary to Article 123(2) EPC.

47. Since none of the claim requests is allowable, the patent has to be revoked.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairwoman:

S. Lichtenvort M. Blasi

Decision electronically authenticated