Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 8 August 2022

Case Number: T 1171/18 - 3.3.08

Application Number: 13734999.9

Publication Number: 2870180

IPC: C07K16/44

Language of the proceedings: EN

Title of invention:
Anti-biotin antibodies and methods of use

Applicant:
F. Hoffmann-La Roche AG

Headword:
Humanised biotin-antibodies/HOFFMANN-LA ROCHE

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - main request (yes)

Decisions cited:
T 0067/11

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1171/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 8 August 2022

Appellant: (Applicant)	F. Hoffmann-La Roche AG Grenzacher Strasse 124 4070 Basel (CH)
Representative:	Vossius & Partner Patentanwälte Rechtsanwälte mbB Siebertstraße 4 81675 München (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 13 November 2017 refusing European patent application No. 13734999.9 pursuant to Article 97(2) EPC**

Composition of the Board:

Chair	B. Claes
Members:	A. Schmitt
	L. Bühler

Summary of Facts and Submissions

I. The appeal of the applicant (appellant) lies from the decision of the examining division to refuse European patent application No. 13 734 999.9 (application), entitled "*Anti-biotin antibodies and methods of use*".

II. Claims 1 to 5 of the main request considered by the examining division read as follows:

"1. An antibody comprising a VH sequence of SEQ ID NO: 12 and a VL sequence of SEQ ID NO: 16.

2. The antibody according to claim 1, which is a monoclonal antibody.

3. The antibody according to any one of the preceding claims, which is an antibody fragment that binds biotin.

4. A pharmaceutical formulation comprising the antibody according to any one of the preceding claims and a pharmaceutically acceptable carrier.

5. The antibody of any one of claims 1 to 3 for use as a medicament."

III. The examining division decided, *inter alia*, that the subject-matter of claim 1 of the main request did not involve an inventive step (Article 56 EPC) and held, by way of *obiter dictum*, that claims 2 and 3 of the main request lacked clarity (Article 84 EPC).

IV. With the statement of grounds of appeal, the appellant submitted sets of claims of a new main request and

auxiliary requests I and II. The set of claims of the main request is identical to claims 1 to 5 of the main request on which the decision under appeal was based (see section II.).

V. The board summoned the appellant to oral proceedings, in accordance with its request, and issued a communication pursuant to Article 15(1) RPBA, in which it, *inter alia*, noted that no comparison of the affinity of the claimed anti-biotin antibody to that of the murine anti-biotin antibody disclosed in document D17 was available.

VI. By letter dated 17 May 2022, the appellant submitted an experimental report (document D20) containing comparative data on the affinities of the claimed antibody and the murine antibody disclosed in document D17 and arguments supporting its view that the claimed subject-matter involved an inventive step.

VII. The board cancelled the oral proceedings.

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

D1 WO 00/50088 A2

D9 K. L. Wark et al., Advanced Drug Delivery Reviews, 58, 2006, 657-670

D10 H. Wu, "Simultaneous Humanization and Affinity Optimization of Monoclonal Antibodies" in Recombinant Antibodies for Cancer Therapy: Methods and Protocols edited by M. Welschof and J. Krauss, Methods in Molecular Biology, 207, 2003, 197-212

D11 P. S. Chowdhury, "Targeting Random Mutations to Hotspots in Antibody Variable Domains for Affinity Improvement" in Antibody Phage Display: Methods and Protocols edited by P. O'Brien and R. Aitken, Methods in Molecular Biology, 178, 2001, 269-285

D12 WO 2010/056893 A1

D13 "AvantGen's Antibody Humanization and Discovery Technologies", AvantGen, 2009

D14 W. F. Dall'Acqua et al., Methods, 36(1), 2005, 43-60

D15 W. Y. Khee Hwang et al., Methods, 36(1), 2005, 35-42

D16 O. Leger et al., "Humanization of Antibodies" in Chapter 1 of Antibody Drug Discovery, Molecular Medicine and Medicinal Chemistry, vol. 4, 2011, 1-23

D17 WO2011/003557

D19 Experimental report "Determination of the binding of murine Mu33 (muM33) to biotinylated payload" submitted by the appellant on 13 April 2016

D20 Experimental report "Determination of the binding of murine Mu33 (muM33) and humanized antibody (huM33) binding site to biotinylated payload" submitted by the appellant on 17 May 2022

IX. The appellant's arguments relevant to the present decision are summarised as follows.

Main request

Clarity (Article 84 EPC) - claims 2 and 3

Claim 1 only defined the amino acid sequences of the antibody's variable regions but not that of the constant region and was therefore not limited to a monoclonal antibody. Consequently, the feature "monoclonal" expressed in claim 2 further limited the subject-matter encompassed by claim 1 and was thus clear.

No discrepancy existed between claim 1 and claim 3 because, according to the definition provided on page 5, lines 23 to 26 of the application, the term "antibody" encompassed various antibody structures, including antibody fragments.

Inventive step (Article 56 EPC) - claim 1

The disclosure in document D17 constituted the most suitable starting point for the assessment of inventive step because it disclosed the murine monoclonal anti-biotin antibody "muM33" ("parental murine antibody") from which the claimed humanised antibody had been derived (see page 134 of document D17). It further disclosed methods for preparing humanised antibodies by grafting the complementarity-determining regions (CDRs) of a murine antibody into the framework regions (FRs) of a human antibody (see the paragraph bridging pages 22 and 23 of document D17). The claimed antibody differed from that disclosed in document D17 in that it comprised a VH sequence of SEQ ID NO:12 and a VL

sequence of SEQ ID NO:16, which were humanised VH and VL sequences.

The technical effect of the difference was that the affinity of the claimed humanised antibody was about 2.5-fold reduced compared to that of the parental murine antibody of document D17 (see Example 5 of the application and documents D19 and D20), which was an insignificant affinity loss. The objective technical problem was thus the provision of a humanised anti-biotin antibody which exhibited its biotin binding without a significant affinity loss.

The absence of a significant affinity loss in a humanised antibody was surprising since it was unpredictable whether a modification in the amino acid sequence of an antibody resulted in a modified antibody having the desired affinity.

Documents D10 to D16 did not disclose methods of obtaining humanised antibodies without significant affinity loss, nor did their disclosure reflect an expectation of the skilled person that such methods existed. In fact, these documents disclosed that humanisation of (murine) antibodies usually resulted in antibodies exhibiting significant affinity loss higher than about 2.5-fold and that, irrespective of the humanisation approach taken, no general rule or strategy could be derived from the prior art which guaranteed that a humanised antibody would be obtained which retained the binding affinity of the parental murine antibody.

The skilled person knew that grafting the CDRs from a donor antibody onto the FRs of a human acceptor antibody was not sufficient for retaining an antibody's

binding affinity. However, the additional alterations necessary for restoring the binding affinity, if possible at all, were unique for any given antibody and could therefore not be predicted. Moreover, no humanised anti-biotin antibodies were known to the skilled person. There was therefore no pointer in the state of the art towards the point mutations which had to be introduced into the humanised anti-biotin antibody after CDR grafting to improve its affinity.

Consequently, the skilled person would not have introduced the mutations at positions 60 and 61 in the H-CDR2 region of the murine anti-biotin antibody of document D17 to improve the affinity of a humanised version of this antibody because the prior art only highlighted other amino acid positions as relevant for humanisation or antigen contact (see e.g. document D12, paragraphs [00290] and [00305]; document D10, Figure 4 and first full sentence on page 199; document D15, page 38, left-hand column, first full sentence; and document D16, section 1.4.2). The skilled person could therefore not have expected that the mutations in positions 60 and 61 would have a positive effect on the antibody's affinity.

In view of these considerations, the skilled person starting from the disclosure in document D17 could not have reasonably expected to provide a humanised antibody with an affinity only about 2.5-fold reduced compared to that of the parental murine antibody of document D17.

X. The appellant requested that the decision under appeal be set aside and that a patent be granted based on the set of claims of the main request or, alternatively, one of the sets of claims of auxiliary requests I

or II, all claim requests submitted with the statement of grounds of appeal.

Reasons for the Decision

1. The appeal complies with Articles 106 to 108 and Rule 99 EPC and is admissible.

Main request

Amendments (Article 123(2) EPC)

2. The subject-matter of claims 1 to 5 finds a basis in claims 7 and 8, 11, 12, 13, and 14 of the application as filed, respectively. Claims 1 to 5 of the main request therefore meet the requirements of Article 123(2) EPC.

Clarity (Article 84 EPC)

3. In an *obiter dictum* to the decision under appeal, the examining division considered that claim 2, which defined that the antibody of claim 1 was a monoclonal antibody, lacked clarity. The skilled person understood the antibody of claim 1 to be a monoclonal antibody, and thus claim 2 raised doubts as to which other types of antibody claim 1 encompassed. Moreover, claim 3 was not clear because claim 1 related to an antibody and therefore could not encompass antibody fragments as recited in claim 3.

4. However, claim 1 refers to an antibody only defined by the amino acid sequences of its variable heavy (VH) and variable light (VL) amino acid sequences (see section IV.). As defined on page 5, lines 23 to 26 of

the application, the term "antibody" is used "*in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity*". Thus, for example, a multispecific antibody could comprise a VH sequence of SEQ ID NO:12 and a VL sequence of SEQ ID NO:16 but would not be considered a monoclonal antibody. Consequently, the antibody of claim 1 is not necessarily a "monoclonal" antibody. The subject-matter of claim 2 is therefore more limited than that of claim 1.

5. Furthermore, in view of the definition of the term "antibody" in the application (see section 4. above), claim 1 does not only encompass intact antibodies but also antibody fragments. Consequently, the board is not persuaded by the examining division's reasoning why claims 2 and 3 lacked clarity.

6. The board accordingly holds that the claims comply with the requirements of Article 84 EPC.

Novelty (Article 54 EPC) and sufficiency of disclosure (Article 83 EPC)

7. The examining division did not formulate any objections to novelty or sufficiency of disclosure of the claimed invention. The board does not have any objection under these provisions, either. An antibody comprising a VH sequence of SEQ ID NO:12 and a VL sequence of SEQ ID NO:16 is not disclosed in the cited art. The requirements of Articles 54 and 83 EPC are thus met.

Inventive step (Article 56 EPC)

Closest prior art, difference and objective technical problem

8. Claim 1 is directed to an antibody comprising a VH sequence of SEQ ID NO:12 and a VL sequence of SEQ ID NO:16. These sequences are derived from a mouse monoclonal anti-biotin antibody whose VH and VL sequences have been humanised by grafting the murine complementarity-determining regions (CDRs) into a human framework (FR) and introducing two backmutations in the FRs and two "forward mutations" in the CDR2 of the VH sequence (H-CDR2).

9. In appeal, both the disclosure in documents D1 and D17 have been considered suitable starting points for the assessment of inventive step.

10. Document D1 mentions anti-biotin antibodies, which are preferably "*human, humanized, or primatized*" (page 5, lines 19 to 23), and refers to citations disclosing publicly available anti-biotin antibodies (page 18, lines 11 to 17). It furthermore discloses that for the preparation of humanised antibodies, "*non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody*" (see page 21, lines 6 to 9).

11. Document D17 discloses the murine anti-biotin antibody from which the humanised VH and VL sequences recited in the claim were derived (see page 134, SEQ ID NO:61, "parental murine antibody"). Document D17 describes the preparation of humanised antibodies by grafting murine CDRs into the framework of a human antibody, including additional modifications in the constant region for modifying the effector function (see the paragraph

bridging pages 22 and 23). The preparation of chimeric antibodies (see page 22, second paragraph) and human antibodies is also described (see page 23, second paragraph).

12. Both document D1 and D17 therefore disclose murine anti-biotin antibodies and methods for preparing humanised versions of these antibodies by CDR grafting, but neither of them discloses amino acid sequence(s) of a humanised anti-biotin antibody. As document D17 in addition discloses the amino acid sequences of the VH and the VL regions of the parental murine antibody, the board considers the disclosure in this document to represent the closest prior art for the assessment of inventive step.

13. The subject-matter of claim 1 differs from the parental murine antibody disclosed in document D17 in that it comprises a defined VH sequence of SEQ ID NO:12 and a defined VL sequence of SEQ ID NO:16.

14. The technical effect of this difference is that the antibody is humanised and has a dissociation constant (K_D) of 0.63 to 0.97 nM, depending on the experimental conditions (see Example 5 of the application and Figure 3 of document D20). The parental murine antibody has a K_D value of 0.37 nM when directly compared to that of the claimed antibody (see Figure 3 of document D20), which is about 2.5-fold lower than that of the humanised antibody. Since the avidity of a humanised antibody is described in the cited prior art as "*only*" 5- and 8-fold lower than that of the parental antibody in the art (see document D14, page 51, right-hand column, first paragraph), the board agrees with the appellant that this affinity loss is not particularly significant.

15. The objective technical problem may therefore be formulated as the provision of a humanised anti-biotin antibody without a significant affinity loss compared to the murine antibody from which it was derived.

Obviousness

16. The examining division did not consider a loss of affinity in a humanised antibody of less than 2-fold surprising because "*methods to produce humanized antibodies without significant affinity loss were not only available in the art at the effective date, for instance D12-D15, but were in fact textbook knowledge, for instance D10 (see summary of method in pages 198-199), D11 (see summary of method in pages 269-270) and D16 (section 1 .4, in particular 1.4.1-1.4.2 and 1.4.4-1.4.6)*" (see third paragraph on page 9 of the decision). Therefore, "*the skilled person seeking to provide humanized anti-biotin antibodies would apply any of these known methods and in doing so, would expect to obtain humanized antibodies without significant affinity loss*" (see fourth paragraph on page 9 of the decision).

17. The board was unable, however, to identify passages in documents D10 to D16 which supported the examining division's allegation that the skilled person would not have expected a significant affinity loss when humanising a mouse antibody.

18. Document D10 is a chapter from a book published in 2003 entitled "Antibodies for Cancer Therapy: Methods and Protocols" which presents a collection of protocols for the design, construction and characterisation of anticancer antibodies. It discloses that "*CDR-grafted*

antibodies often do not retain full antigen-binding ability" (page 198, lines 3 to 4) and that "*in order to maintain binding affinity, ... key mouse residues need to be maintained*" (page 198, lines 6 to 7). However, "*the complexity arising from the large number of framework residues that are potentially involved in binding ability has slowed the rate of success*" (page 198, lines 9 to 11).

19. Document D10 therefore proposes a method for combining humanisation and affinity optimisation based on the construction of a combinatorial library that contains human/murine wobble residues in key framework residues and single mutations in the H-CRD3 and the L-CDR3 regions (see page 198, second paragraph and page 199, lines 4 to 7). Document D10 claims that this approach "*results in the identification of humanized antibodies that have higher affinity than the parent MAb*" (see page 198, lines 2 to 4 of the second paragraph). This method, however, involves the screening of a large number of clones and was only applied to a single murine monoclonal antibody in 1999 (see page 198, lines 4 to 6 of the second paragraph and the first full paragraph on page 199 and reference (9) cited there).

20. Document D11 is a chapter from a book published in 2001 which presents a compilation of antibody phage display protocols. It discloses a protocol for affinity improvement in antibody variable domains based on the generation of phage-display libraries generated by random mutagenesis at pre-selected mutation hotspots in specific CDRs and FRs (see last paragraph on page 270). Document D11 discloses that "*the method described here has been found to work in several systems*" and refers to two citations from 1999 and 2000 (see the last sentence on page 270 and references (4 and 5) cited

there) but does not appear to disclose any values on the affinity of the humanised antibodies obtained by this method.

21. Consequently, documents D10 and D11 confirm that a significant loss in affinity is to be expected when humanising murine antibodies. They describe two protocols for improving the affinity of humanised antibodies based on the large-scale screening of site-directed mutation libraries that have only been applied in few cases in 1999 and 2000 prior to publication of the protocols in 2002 and 2003. In the board's opinion, these few examples are not sufficient evidence that the laborious screening methods they describe would necessarily result in the identification of humanised antibodies with the same or even higher affinity than the parental antibody.

22. This view is furthermore supported by the fact that the review document D9 published years later than documents D10 and D11 still characterises the humanisation process of murine antibodies as a "*laborious and costly procedure*" that "*often results in a 10-fold decrease in K_D*" (see the last sentence on page 659 of document D9) and that document D16 published in 2011 only mentions the same single example as document D10 for the method recited there (see the paragraph bridging pages 15 to 16 of document D16 and also points 27. and 28. below). The board is therefore not persuaded that the skilled person would have reasonably expected that a humanised antibody without significant affinity loss would necessarily be obtained when carrying out the protocols disclosed in documents D10 and D11 for each parental non-human antibody.

23. Document D12 discloses methods for the preparation of affinity-optimised antibody templates but does not contain any teaching that the described methods normally, let alone inevitably, result in the production of a humanised antibody with a particular affinity, irrespective of the parental antibody. Moreover, document D12 discloses that the commonly used technique for humanisation of monoclonal antibodies is CDR grafting of the non-human donor antibody onto the most similar human acceptor antibody framework, i.e. the same method as proposed in documents D17 and D1 for the humanisation of murine anti-biotin antibodies. Document D12 confirms the teaching in document D9 (see point 22. above) that this method usually results in a significant loss of affinity of the humanised antibodies (see paragraph [0003] of document D12).

24. Document D13 discloses that humanised antibodies produced by the CDR grafting approach including backmutations of critical mouse framework residues "*often exhibit lower (3-5x) affinity than the parental mouse antibodies*" (see last sentence of the second paragraph on page 1) and therefore also confirms the teaching in documents D9, D10 and D12 that the skilled person had to expect a substantial loss of affinity in a humanised antibody produced by this technique. The proprietary technology presented in this publication results in humanised antibodies that "*often exhibit higher affinity than the parental mouse antibodies*" (see last sentence of the third paragraph on page 1). Document D13 therefore contains no teaching that the skilled person could expect to normally obtain a humanised antibody with retained or increased affinity when applying document D13's technology.

25. Document D14 describes a method for antibody humanisation by framework shuffling and discloses that the humanised antibody obtained by this method exhibited "*only a 5- and 8-fold avidity loss when compared with the parental mAb*" (page 51, right-hand column, first paragraph). Document D14 therefore does not support the examining division's conclusion either that the skilled person "*would expect to obtain humanized antibodies without significant affinity loss*" (see point 16. above) when carrying out this method.

26. Document D15 describes an approach for the selection of human framework sequences for CDR-grafting constructions for which "*avidity loss is low*", namely 30-fold and 6-fold compared to the parental mouse antibody (see page 41, left-hand column, last paragraph and Table 4). In the context of clinical products, document D15 concludes that "*[a]ll humanized antibodies end their development with an affinity on par with the original mouse antibody, usually after an in vitro affinity maturation process*" (see the sentence bridging the left- and right-hand columns on page 41). This conclusion, however, formulates a goal that should be achieved for humanised antibodies for clinical purposes but does not express a guarantee that it could be achieved for each and every humanised antibody by affinity maturation.

27. This view is confirmed in document D16, which is a chapter from a book published in 2011 that describes a variety of different techniques for the humanisation of antibodies. CDR grafting is described as the standard technology (see chapter 1.2 on pages 3 to 8), in which, however, reduced affinity is often encountered, requiring the introduction of backmutations (see

page 6, first full paragraph), a procedure which is described as "unpredictable" (see page 7, lines 4 to 7 from the bottom). In chapter 1.4 of document D16, "alternative" approaches are described (see sub-chapters 1.4.2 to 1.4.7). These approaches are exemplified with reference to single monoclonal antibodies to which they had been applied and where varying degrees of changes in affinity were observed depending on the antibody, ranging from an improvement in or retaining of the source antibody's affinity to a 2-, 5-, 6-, 8- and even 30-fold loss in affinity (see first full paragraph and lines 2 to 5 from the bottom of page 10; lines 12 to 21 of section 1.4.3 on page 11; last three lines of the first paragraph on page 12 and the paragraph bridging pages 12 to 13 starting on line 8 from the bottom on page 12; last six lines of the first full paragraph on page 13; lines 1 to 8 of page 14; lines 1 to 2, 10 to 11 and 16 to 17 of page 15, last sentence of the second paragraph on page 15; and the paragraph bridging pages 15 to 16 describing the method of document D10).

28. Thus, document D16 does not point to a humanisation method either which would normally or necessarily result in no or minimal loss of the parental antibody's affinity but instead confirms the teaching of documents D9, D13, D14 and D15 that, depending on the parental antibody and the antigen it recognises, a loss of affinity higher than 2.5-fold has to be expected.

29. Therefore, the examining division's assertion that the skilled person, when applying any of the known humanisation methods *"would expect to obtain humanized antibodies without significant affinity loss*" is not supported by the teaching in any of the documents cited by the examining division. Moreover, since no humanised

anti-biotin antibodies were known at the priority date of the application, the skilled person did not know whether a humanised anti-biotin antibody with a 2.5-fold affinity loss could be provided at all and did not have any guidance on the humanisation protocols or necessary mutations in humanised CDR-grafted antibodies suggested in document D17 itself.

30. The board furthermore finds support for its view in decision T 67/11, which considered that in a situation where "*no particular 'recipe' for reducing the immunogenicity of a specific antibody while not affecting or improving the affinity and specificity of this antibody has been disclosed*" (Reasons, point 21.2) and where "*the prior art failed to provide the skilled person with a clear pointer towards any specific set of mutations which would do the job*" (Reasons, point 22), the impact of each mutation in the antibody on the final properties of the antibody was unpredictable, and thus it was "*the attainment of an antibody ... having indeed the desired characteristics which* [was] *considered surprising, not the theoretical possibility of achieving one*" (Reasons, point 24).

31. In the case at hand, the board could similarly not identify any teaching in documents D9 to D16 which demonstrated that this analysis of the humanisation protocols available to the skilled person had changed before the priority date. The board is therefore of the opinion that the considerations in points 21.2 and 22 of T 67/11 are still applicable to the case at hand.

32. The examining division considered that decision T 67/11 was not relevant to the case at hand because it concerned a humanised antibody which retained or improved the affinity of the parental antibody and not

one which had a lower affinity as in the case at hand. However, since documents D9 to D16 confirm that the skilled person had to expect, in general, a higher loss in affinity than that observed for the claimed antibody (see points 17. to 29. above), this argument does not convince the board.

33. Consequently, taking into account the disclosure in documents D10 to D16 and the fact that no humanised anti-biotin antibody was known in the art, it was not evident to the skilled person which of the known humanisation techniques should be followed and, when following the standard method of CDR grafting, which back- or forward mutations were necessary to obtain a humanised anti-biotin antibody exhibiting about a 2.5-loss in affinity compared to the parental murine antibody, nor could the skilled person reasonably expect that such an antibody could be obtained at all with any of the known humanisation methods.

34. In view of the above considerations on the evidence and arguments of the examining division, the board is of the opinion that the subject-matter of claim 1 of the main request was not obvious to the skilled person and involves an inventive step (Article 56 EPC).

35. Dependent claims 2 and 3 refer to the antibody of claim 1. The subject-matter of claim 4 relates to a pharmaceutical composition comprising the antibody of claim 1. The subject-matter of claim 5 relates to the antibody of claim 1 for use as a medicament (see section IV.). The subject-matter of claims 2 to 5 therefore involves an inventive step for the same reasons as that of claim 1 (Article 56 EPC).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent with the following claims and a description and figures possibly to be adapted:

   Claims 1 to 5 of the main request filed with the statement of grounds of appeal.

The Registrar: The Chair:

L. Malécot-Grob B. Claes

Decision electronically authenticated