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Datasheet for the decision of 5 July 2023

Case Number: T 0047/22 - 3.3.04

Application Number: 16703351.3

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IPC: C07K16/00, C07K16/24, C07K16/28

Language of the proceedings: EN

Title of invention:
Methods for producing optimised therapeutic molecules

Patent Proprietor:
Crescendo Biologics Limited

Opponent:
Strawman Limited

Headword:
Immunoglobulin library/CRESCENDO BIOLOGICS

Relevant legal provisions:
EPC Art. 83, 56, 113
RPBA 2020 Art. 12(2), 12(4), 12(6)

Keyword:
Sufficiency of disclosure - main request (no) - auxiliary request (yes)
Late-filed evidence - document could have been filed in first instance proceedings (yes)
Inventive step - auxiliary request (yes)

Decisions cited:
T 1213/19, T 1817/15, T 1380/04, T 0238/92

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Case Number: T 0047/22 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 5 July 2023

Appellant: (Opponent)	Strawman Limited Orchard Lea Horns Lane Combe, Witney Oxfordshire OX29 8NH (GB)
Representative:	Potter Clarkson Chapel Quarter Mount Street Nottingham NG1 6HQ (GB)
Respondent: (Patent Proprietor)	Crescendo Biologics Limited Meditrina Building Babraham Research Campus Babraham Cambridge CB22 3AT (GB)
Representative:	Appleyard Lees IP LLP 15 Clare Road Halifax HX1 2HY (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 10 November 2021 concerning maintenance of the European Patent No. 3245226 in amended form.**

Composition of the Board:

Chairwoman	M. Pregetter
Members:	O. Lechner
	R. Romandini

Summary of Facts and Submissions

I. The appeal lodged by the opponent (appellant) lies from the opposition division's interlocutory decision that the patent as amended in the version of the set of claims of a main request as filed on 13 May 2021 and a description as filed during oral proceedings and the invention to which it relates met the requirements of the EPC.

II. The patent is based on European patent application No. 16 703 351.3, which had been filed as an international application and published as WO 2016/113556 (the "application as filed").

III. In its decision the opposition division held that the main request (as filed on 13 May 2021) complied with Articles 52(2), 83, 54 and 56 EPC.
Auxiliary requests 1 to 18 (as filed on 13 May 2021 were not dealt with in the decision).

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

"1. A method of designing an immunoglobulin library for optimisation of a biological property of a first lead immunoglobulin, the method comprising:
a) identifying one or more related immunoglobulins of common lineage with and that bind the same target antigen as the first lead immunoglobulin, said one or more related immunoglobulins being related to the first lead immunoglobulin, each immunoglobulin having been raised against a target antigen by immunisation of a transgenic non-human mammal comprising human immunoglobulin genes with the target antigen and said one or more related immunoglobulins being derived from

the same germline sequence by somatic hypermutation of a germline sequence in the transgenic non-human mammal;
b) comparing amino acid sequences of the first lead immunoglobulin and the one or more related immunoglobulins;
c) identifying, based on the sequence comparison, one or more sites at which there are variant amino acid residues between:

(i) the first lead immunoglobulin and the one or more related immunoglobulins, and/or

(ii) where the one or more related immunoglobulins is a plurality of immunoglobulins, between the plurality of immunoglobulins,

wherein the one or more sites at which there are variant amino acid residues comprise somatic hypermutation hot spots targeted during the immune response that are potential sites for modification of the first lead immunoglobulin;
d) selecting one or more sites for modification to replace an amino acid of the first lead immunoglobulin with the corresponding variant amino acid of one or more of the related immunoglobulins, based on the sequence comparison;
e) generating immunoglobulin sequences for the library based on the sequence of the first lead immunoglobulin, modified at one or more of the selected sites for modification; and (f) generating an immunoglobulin library comprising immunoglobulins having the sequences generated in step e), optionally further comprising step g) screening the immunoglobulin library to identify one or more immunoglobulins having desired biological properties."

Claim 1 of auxiliary request 1 reads as follows (changes in relation to the main request highlighted by the board):

"1. A method of designing an immunoglobulin library for optimisation of a biological property of a first lead immunoglobulin, the method comprising:
a) identifying ~~one or more~~ at least 20 related immunoglobulins of common lineage with and that bind the same target antigen as the first lead immunoglobulin, said ~~one or more~~ at least 20 related immunoglobulins being related to the first lead immunoglobulin, each immunoglobulin having been raised against a target antigen by immunisation of a transgenic non-human mammal comprising human immunoglobulin genes with the target antigen and said ~~one or more~~ at least 20 related immunoglobulins being derived from the same germline sequence by somatic hypermutation of a germline sequence in the transgenic non-human mammal;
b) comparing amino acid sequences of the first lead immunoglobulin and the ~~one or more~~ at least 20 related immunoglobulins;
c) identifying, based on the sequence comparison, one or more sites at which there are variant amino acid residues between:
(i) the first lead immunoglobulin and the ~~one or more~~ at least 20 related immunoglobulins, and/or
(ii) ~~where the one or more related immunoglobulins is a plurality of immunoglobulins,~~ between the plurality of at least 20 related immunoglobulins,
wherein the one or more sites at which there are variant amino acid residues comprise somatic hypermutation hot spots targeted during the immune response that are potential sites for modification of the first lead immunoglobulin;
d) selecting one or more sites for modification to replace an amino acid of the first lead immunoglobulin with the corresponding variant amino acid of one or

more of the related immunoglobulins, based on the sequence comparison;
e) generating immunoglobulin sequences for the library based on the sequence of the first lead immunoglobulin, modified at one or more of the selected sites for modification; and
(f) generating an immunoglobulin library comprising immunoglobulins having the sequences generated in step e), optionally further comprising step g) screening the immunoglobulin library to identify one or more immunoglobulins having desired biological properties."

V. With the statement of grounds of appeal the appellant raised objections under Articles 56 and 83 EPC and filed new document D26 (see below).

VI. In reply, the patent proprietor (the "respondent") filed sets of claims of 39 auxiliary requests (auxiliary requests 1 to 18 as already filed during opposition proceedings) and new documents D27 and D28.

VII. The current board issued a communication pursuant to Article 15(1) RPBA providing its preliminary assessment of the appeal.

VIII. The parties replied.

IX. The oral proceedings before the board took place as scheduled on 5 July 2023 by videoconference.

At the end of the oral proceedings, the Chairwoman announced the board's decision.

X. Reference is made to the following documents:

D2: P. S. Chowdhury et al., Nature Biotechnology

(1999), vol. 17(6): 568-572

D3: V. K. Nguyen et al., The EMBO Journal (2000), vol. 19(5): 921-930

D4: T. R. Poulsen et al., J Immunol (2007), vol. 179: 3841-3850

D5: S. Seeber et al., PLOS ONE (2014), vol. 9(2) e86184, 14 pages

D6: M. Michaeli et al., J Clin Bioinformatics (2013), vol. 3(15), 6 pages

D7: W. J. E. Van Esch et al., Clin Exp Immunol (2003), vol. 131: 364-376

D8: J. Zheng et al., J. Biol Chem (2009), vol. 284(20): 13610-13619

D9: A. Burkovitz et al., FEBS Journal (2014), vol. 281: 306-319

D10: WO 00/73346 A1

D16: K. Y. F. Yau et al., Journal of Immunological Methods (2005), vol. 297: 213-224

D22: J. D. Berry et al., Chapter 15: Antibody Libraries from Immunized Repertoires, in Phage Display in Biotechnology and Drug Discovery, 1st edn, (2005): 529-657

D23: US 2002/0177170 A1

D25: Y. Safdari, Biotechnology and Genetic Engineering Reviews (2013), vol. 29(2): 175-186

D26: WO 2006/050491 A2

D27: Information relating to and including USPTO, "Information disclosure statement by applicant", No. 33803009 for application number 15/540400, filing date 28 June 2017, 7 pages in total

D28: G. Winter et al., Immunology Today (1993), vol. 14(6): 243-246

XI. The respondent's arguments, in so far as relevant to the decision, can be summarised as follows:

(a) Main request

Disclosure of the invention - Article 83 EPC - claim 1

None of the steps in claim 1 required the identification of a somatic hypermutation hot spot (SHH). The claim merely recited that "the one or more sites at which there are variant amino acid residues comprise somatic hypermutation hot spots targeted during the immune response that are potential sites for modification of the first lead immunoglobulin". This implied, as also found by the opposition division, that it was not necessary to determine SHHs, but that variations were identified by comparison. A person skilled in the art knew that such variations had arisen due to somatic hypermutation.

There was a vanishingly small possibility that some nucleic acid sequence changes were due to polymerase-

chain reaction (PCR)-cloning errors, but this had no impact on the person skilled in the art being able to carry out the claimed method. The vast majority, if not all, of the variant amino acid residues would have been introduced by somatic hypermutation *in vivo*. Changes introduced to an antibody sequence during somatic hypermutation *in vivo* that did not improve properties of the immunoglobulin would not be selected and maintained *in vivo*. This was well known in the art.

The person skilled in the art knew how to identify SHHs, as evidenced by documents D4 to D10.

Some of the appellant's objections were of a semantic nature and appeared rather to be clarity objections.

The skilled person knew that by following the claimed method it would be possible to arrive at immunoglobulins with optimised biological properties.

The appellant had not raised serious doubts substantiated by verifiable facts that would suggest that the invention could not be carried out by a skilled person. There was no evidence that a person skilled in the art was not able to identify SHHs when comparing only a few sequences.

(b) Auxiliary request 1

Disclosure of the invention - Article 83 EPC - claim 1

The amendment made in claim 1, requiring at least 20 variant immunoglobulins to be compared with the lead immunoglobulin as claimed, rendered all previous objections moot.

Admittance of documents D26 to D28

Document D26

Document D26 could and should have been cited in the first-instance proceedings given that the appellant knew about the *prima facie* relevance of this document. Article 12(2), (4) and (6) RPBA 2020 fully applied to the case in hand. The case law cited by the appellant in support of the admittance was not relevant since it was based on older, outdated versions of the Rules of Procedure of the Boards of Appeal.

The opponent was wrong in asserting that the opposition division had taken a new position in the decision under appeal and had cited Chowdhury et al. (1998). Point 48.4 of the decision under appeal merely cited verbatim the passage on page 10, lines 29 to 34 of document D10, which references Chowdhury 1998, but it did not introduce this document or its specific content into the opposition proceedings. Thus, this citation by the opposition division was not a justification for the late filing of document D26.

Document D25 had been admitted into the proceedings by the opposition division as it had been filed in direct response to document D23, which the opponent had filed late. Document D25 had been submitted prior to the oral proceedings and had been discussed at the oral proceedings. The opponent would have had ample opportunity to make its case regarding document D25. There was no legitimate reason why document D25 should necessitate the admission of document D26 into the proceedings.

Documents D27 and D28

If document D26 was admitted into the proceedings, documents D27 and D28 should also be admitted as a response to the late filing of document D26.

Inventive step - Article 56 EPC - claim 1

Closest prior art

Document D10/D2 or D23 represented the closest prior art.

Difference and objective technical problem
D10/D2 as the closest prior art

The claimed method differed from the disclosure in document D10 in that the immunoglobulins generated for the library were based on the actual *in vivo* blueprint in response to immunisation, i.e. the library comprised only SHHs and replacements selected by an *in vivo* affinity maturation process against the same target antigen. The same arguments could be equally applied to the disclosure in the related scientific article D2.

D23 as the closest prior art

The claimed method differed from the disclosure in document D23 in that the SHHs identified were dictated by the *in vivo* response and were specific to functionally related molecules because they had all been generated in response to the same antigen. The claimed method was an antigen-driven process and the immunoglobulins belonged to the same germline.

The claimed method led to the identification of immunoglobulins with higher specificity than the lead, enabling identification of SHHs that the methods of the art did not identify.

The objective technical problem was to provide an improved method for designing an immunoglobulin library for optimising a biological property of a first lead immunoglobulin.

Obviousness

None of the disclosure of the closest prior-art documents D10/D2 and D23, either alone or in combination with the teaching in document D3, D16, D9 or D22, rendered the claimed subject-matter obvious.

The subject-matter was not obvious even if starting from an objective technical problem of providing an alternative method.

XII. The appellant's arguments, in so far as relevant to the decision, can be summarised as follows:

(a) Main request

Disclosure of the invention - Article 83 EPC - claim 1

The patent showed that it was possible to identify SHHs by comparing the lead immunoglobulin sequence with at least 20 variants derived from the same germline (see Example 1 and Figures 1 and 4).
Claim 1 did not require more than two immunoglobulin sequences to be compared, or require the variant amino acid to be observed in more than one related sequence in order for it to be selected for modification. Nor

did it require the sites selected for modification to actually be SHHs.

First, there was no sufficiently clear and complete teaching in the patent and the common general knowledge on how to verify if variations in the amino acid sequence of immunoglobulins arose at an SHH by comparing only the amino acid sequences of two or a few immunoglobulins. Documents D4 to D10, cited by the opposition division to show that the skilled person was able to identify SHHs, did not form part of the common general knowledge.

Second, a library made in the claimed way would not allow a biological property to be optimised since step d) did not require that the one or more sites to be selected for replacement were an SHH; rather, it allowed any mutation to be included. According to the respondent, step c) of claim 1, stating "the one or more sites at which there are variant amino acids", did not require the identification of SHHs either. Sites of modification could also be due to, for example, PCR-cloning errors and would not result in an optimised antibody.

(b) Auxiliary request 1

Disclosure of the invention - Article 83 EPC - claim 1

The objection that a library made in the claimed way would not allow a biological property to be optimised since step d) did not require that the one or more sites to be selected for replacement were an SHH, as discussed in the context of the main request, applied *mutatis mutandis* to the invention of claim 1 of auxiliary request 1.

Admittance of documents D26 to D28

Document D26

Document D26 was *prima facie* relevant for assessing patentability and should therefore be admitted as it directly impacted the board's decision. The case in hand was similar to the situation in decisions T 1213/19, T 1817/15, T 1380/04 and T 238/92, in which late-filed documents had been admitted into the proceedings because of their *prima facie r*elevance. Document D26 should therefore be admitted for that reason alone.

Moreover, document D26 had been filed in response to the filing of document D25, which the patent proprietor had filed only a few days before the oral proceedings in opposition. The opponent/appellant did not have the chance to adequately react to this late-filed document during the opposition proceedings.

Document D25 had influenced the opposition division's decision, as evidenced by its modified reasoning concerning the importance of using the same germline when observing variations in immunoglobulins raised against the same target antigen leading to a more focused library (compare paragraphs 41.7 and 43.2 of the decision under appeal and paragraph 34 of the annex accompanying the opposition division's summons of 5 October 2020).

Moreover, the respondent had not been taken by surprise as it had been familiar with document D26, its content and its relevance to patentability as the respondent had cited the corresponding US publication

(US 2006/099204 A1) in an Information Disclosure Statement it had filed during proceedings in the corresponding US case.

1.1.1 The respondent had argued that review article D25 had been filed as evidence of the skilled person's common general knowledge in response to the appellant's filing of new document D23 with its response to the opposition division's preliminary opinion.

Document D26 was the effective counter-evidence to the reasoning given in the decision under appeal, which had been influenced by the new consideration of the teaching in Chowdhury 1998 cited in document D10, and the extremely late-filed document D25.

Documents D27 and D28

The appellant did not comment on the admittance of documents D27 and D28.

Inventive step - Article 56 EPC - claim 1

Closest prior art

Document D10 (or its scientific counterpart D2) or D23 represented the closest prior art.

Difference and objective technical problem

D10/D2 as the closest prior art

D10/D2 did not raise immunoglobulins in a transgenic non-human animal against the same target antigen, to identify naturally occurring variations within the same germline. Instead, the method of D10/D2 started from a

single lead immunoglobulin - SS(scFv) - and applied random sequence mutations based on the locations of predicted SHH sites to arrive at an immunoglobulin library, which is then screened.
By contrast, the claimed method used observed variations following immunisation of a non-human animal.

D23 as the closest prior art

The claimed subject-matter differed from the method in document D23 in that it obtained antibody sequences derived from the same germline sequence following immunisation of a transgenic non-human mammal, the antibodies having been raised against the same target antigen, while the method in document D23 obtained them from a synthetic (non-immune) library.

There was no technical effect associated with the respective differences because it had not been shown that a library generated according to the claimed method showed any improvement over the libraries designed according to the methods disclosed in the closest prior-art documents.

The objective technical problem was to provide an alternative method of designing an immunoglobulin library for optimising a biological property of a first lead immunoglobulin.

Obviousness

The claimed solution was obvious from the teaching in the closest prior-art document D10/D2 alone or in combination with the teaching in document D3 or the common general knowledge represented by document D22.

Alternatively, the claimed subject-matter was obvious starting from document D23 in combination with the common general knowledge represented by document D22.

It was known from the common general knowledge that there were only two ways of mimicking the natural hypermutation process, i.e. by motif-based SHH site prediction or by observing the SHH sites directly, the latter being part of the method of claim 1.

The skilled person knew from the common general knowledge that approaches relying on motif-based SHH prediction, as used in the methods of document D10/D2, were not representative of what occurred *in vivo*, so the skilled person had been motivated to adapt the method of document D10.

As evidenced *inter alia* by paragraphs [0550], [0555], [0556], [0663], [0770] and [0771], the authors of document D23 had appreciated the importance of trying to mimic affinity maturation and the importance of hot spots, and that creating a library containing a combination of the observed variants made it possible to generate an antibody with improved properties.

According to established case law (see the Case Law of the Boards of Appeal, 10th edn, 2022, I.D.4.5 and especially decisions T 1179/16 and T 148/10), the skilled person did not need a pointer when selecting an alternative without a technical effect.

Document D3 suggested combining amino acid variants at predicted SHHs with amino acid variants at observed SHHs in order to design a library.

Document D22 taught the skilled person that starting from immunoglobulin sequences from an immune library was preferred when trying to generate improved antibodies because the host would have already performed affinity maturation. It was also clear from document D22 that immune libraries were not useful for fishing for antibodies against antigens to which the host immune system had not been sensitised.

Thus, in combining the teachings of document D10/D2 with those of document D3 and the common general knowledge represented by document D22, it was obvious to include (all) observed sequence mutations with respect to the lead immunoglobulin instead of relying on motif-predicted SHHs, regardless of their origin, to form a new screening library. The skilled person knew from document D22 that immune libraries were superior to non-immune or synthetic libraries. Thus, the skilled person would have arrived at the method of claim 1 in an obvious manner.

XIII. From the written submissions, the board understands the parties' requests to be as follows:

(a) The appellant requests that
- the decision be set aside and the patent be revoked in its entirety
- that document D26 be admitted
- that auxiliary requests 19 to 39 not be admitted into the proceedings

(b) The respondent requests that
- the appeal be dismissed and that the opposition division's decision be maintained, i.e. on the basis of the set of claims according to the main request as filed on 13 May 2021 and maintained by

the opposition division or on the basis of one of auxiliary requests 1 to 39 as filed with the reply to the statement of grounds of appeal
- that document D26 not be admitted into the proceedings
- if document D26 were to be admitted into the proceedings, remittal of the case to the opposition division
- documents D27 and D28 be admitted in case document D26 was admitted
- document D28 be in any case admitted into the proceedings since it represented common general knowledge

Reasons for the Decision

2. Admittance of documents D26 to D28

2.1 Violation of the right to be heard and admittance of document D26

2.1.1 Under Article 113(1) EPC, decisions may only be based on grounds or evidence on which the parties concerned have had an opportunity to present their comments.

2.1.2 The appellant argued that it had not been able to respond adequately to the document Chowdhury 1998, on which the opposition division had relied in paragraph 48.4 of its decision but which had never previously been admitted or referred to at any stage of the opposition or examination proceedings. The conclusion drawn by the opposition division in view of Chowdhury 1998, i.e. that the authors of document D10 "*were of the opinion that immune libraries with improved members*

could only be achieved by focusing on variations that would not occur in nature" was completely incorrect. This position was not derivable from the opposition division's preliminary opinion.
Document D26 had been filed in direct response to the new position taken by the opposition division and the new evidence, i.e. Chowdhury 1998, cited by the opposition division.

2.1.3 Point 48.4 of the decision under appeal merely cites verbatim the passage on page 10, lines 29 to 34 of document D10, which references Chowdhury 1998. The opposition division's argument why the claims of the main request were inventive was based on what is taught in document D10 itself and not the document referenced in the cited passage.

The appellant/opponent itself mentioned in its letter of 13 May 2021, page 16, third-to-last paragraph that "*D10 describes a method for generating antibodies with higher affinity to a target antigen than that of a parental antibody (see D10, page 8 lines 30-31). D10 teaches the advantages of a targeted approach (D10, passage bridging pages 10 and 11)*". Thus, the opposition division has not referred to any new passage of document D10 (see point 48.4 of the decision under appeal).

In line with the discussion on the teaching in the closest prior-art documents in point 43.1 of the decision under appeal and covering the opponent's above-mentioned argument, the opposition division reasoned that document D10 (as well as documents D2 and D23) did not focus on naturally occurring variations but applied random sequence mutations. Paragraph 48.4 of the decision under appeal discusses the teaching of

document D10 but does not rely on the content of the Chowdhury 1998 reference itself, beyond the information provided in the relevant sentence on page 10, lines 29 to 31 of document D10.

The board therefore considers that the opposition division did not violate the right to be heard within the meaning of Article 113(1) EPC and that the argument made in paragraph 48.1 of the decision under appeal cannot be used to support the admittance of document D26.

2.2 Other reasons concerning the admittance of document D26

2.2.1 The board fails to see a clear link between the reference to review article D25 and the change in the opposition division's reasoning when comparing paragraph 34 of its preliminary opinion and paragraphs 41.7 and 43.2 of its final decision.

Document D26 may be of *prima facie* relevance; however, this is only one criterion to be considered by the board when deciding on the admittance of late-filed submissions. Other criteria to be considered include fairness and procedural economy.

2.2.2 In the board's opinion, the decisions referred to by the appellant, namely T 1817/15, T 238/92 and T 1380/04, were all taken in the context of earlier, outdated versions of the Rules of Procedure of the Boards of Appeal.

The appellant also referred to decision T 1213/19. In this case, the entrusted board applied the RPBA 2020 when deciding on the admittance of a document, as the appellant had filed the document only after filing the

statement of grounds of appeal. The board decided to admit the document on the basis of its *prima facie* relevance and the fact that the respondent had known the content of that document before the appeal was filed and had had sufficient time to assess its contents and react appropriately (see Reasons 5 and 7).

2.2.3 In the case in hand, it seems that the issue of whether the prior art itself provided a motivation to replicate the *in vivo* somatic hypermutation process and was not limited to identification of the SHHs by *in silico* means, as well as the general concept of identifying SHHs by analysing sequence data of immunoglobulins generated *in vivo*, had already been discussed in the notice of opposition (see points 9.21 and 9.22) and the patent proprietor's reply (see e.g. page 30, last three paragraphs et seq.).

As these issues have been extensively discussed since the start of the opposition proceedings, D26 could and should have been submitted during the opposition proceedings.

2.2.4 Consequently, document D26 is not admitted into the proceedings under Article 12(4) and (6) RPBA.

2.3 The request for admittance of documents D27 and D28 was made conditional on the admission of document D26. The respondent argued that document D28 should be admitted into the proceedings in any case since it represented the common general knowledge. However, the content of document D28 was invoked exclusively in the context of the respondent's discussion of document D26 in its reply to the statement of grounds of appeal.

Since document D26 was not admitted into the proceedings, the board considers it unnecessary to decide on the request to admit documents D27 and D28.

Main request

1. Claim construction

1.1 Claim 1, step a)

1.1.1 The appellant argued that claim 1 did not require active immunisation and that to put the claim into practice, it was necessary simply to identify related immunoglobulins.
The method of claim 1 encompassed a sequence comparison between the first lead immunoglobulin and one or more related immunoglobulins, i.e. including the comparison of only two or a few sequences. The scope of the expression "one or more sites" was open-ended and not restricted to only SHHs. Hence the method of claim 1 comprised identifying any mutation at any position. Step g) of claim 1 was optional and so was not limiting.

1.1.2 The board agrees that claim 1, step a) does not require active immunisation; however, the identified immunoglobulins need to have been raised against the same target antigen and derive from the same germline sequence as the first lead immunoglobulin. Claim 1 also requires the immunoglobulins to have been raised by immunisation of a transgenic non-human mammal comprising human immunoglobulin genes. Yet this wording does not necessarily imply that the final immunoglobulin comprises human sequences as suggested by the respondent.

1.2 Claim 1, step c)

Claim 1, step c) reads "*identifying, based on the sequence comparison, one or more sites at which there are variant amino acid residues* [...] *wherein the one or more sites at which there are variant amino acid residues comprise somatic hypermutation hot spots targeted during the immune response that are potential sites for modification of the first lead immunoglobulin;*" (underlining added by the board).

This wording requires that the "one or more sites at which there are variant amino acid residues" comprise SHHs (targeted during the immune response in the mammal) as potential sites for modification. Thus, the claimed method defines a step of identifying at least one site which has to include SHHs.

2. Disclosure of the invention - Article 83 EPC - claim 1

2.1 Identification of SHHs

2.1.1 Claim 1, step c) requires knowledge of the presence of at least one SHH at the identified variant site(s) (see point 1.2 above). Therefore, it is essential to know whether the person skilled in the art knew how to determine the presence of SHHs.

2.1.2 The application as filed does not provide any instructions on how to generally identify SHHs, nor is there any prior-art reference in that direction. The only method disclosed is the identification of SHHs by aligning multiple, i.e. 20 or more, sequences with the first lead immunoglobulin (see Example 1 and Figures 1 and 4).

The question arises as to whether the identification of SHHs in general was common general knowledge at the filing date without requiring the alignment of at least 20 or more sequences.

In relation to its decision that the subject-matter of claim 1 was sufficiently disclosed, the opposition division referred to documents D4 to D10 as showing that the skilled person would be able to identify SHHs (see paragraph 32 of the decision under appeal).

The appellant contested this and argued that there was no evidence that alignment of only two or a few sequences allowed SHHs to be identified. None of documents D4 to D10 represented the common general knowledge.

The respondent had not shown that identifying SHHs was part of the common general knowledge or that SHHs could be identified by comparing only two or a few sequences.

2.1.3 The board is thus not convinced that the skilled person would have been able to verify whether variations in the amino acid sequence of immunoglobulins were due to SHH maturation by comparing the amino acid sequences of two or only a few immunoglobulins. The board is thus not convinced that the skilled person would have been able to identify SHHs.

Since it has not been shown that the relevant part of the method of claim 1, i.e. the part relating to the comparison of the lead immunoglobulin with only two or very few immunoglobulins, ensures the presence of SHHs as required in step c), the board concludes that the patent application fails to disclose the claimed invention in a manner sufficiently clear and complete

for it to be carried out by a person skilled in the art.

Auxiliary request 1

3. Disclosure of the invention - Article 83 EPC - claim 1

3.1 For the reasons provided in the context of claim 1 of the main request (see point 2.1), the board is of the view that the claimed invention, involving the comparison of the first lead immunoglobulin with at least 20 related immunoglobulins of common lineage that bind the same target antigen as the first lead immunoglobulin, allows SHHs to be identified and PCR-cloning errors to be excluded. See also Figure 4 of the patent and Annex A of document D15, showing that aligning the lead immunoglobulin sequence with those of more than 20, specifically 35, other members of the same lineage makes it possible to identify amino acid positions that have been mutated during the immune response.

3.2 Does a library produced by the claimed method allow for optimisation?

3.2.1 The board agrees with the respondent that the observed variations in the immunoglobulins raised against the same antigen and deriving from the same germline can be expected to be mainly due to the *in vivo* affinity maturation process by somatic hypermutation. Occasional PCR-cloning-induced variations in a given sequence cannot be excluded but can be recognised and eliminated by comparing multiple sequences. The appellant has not provided any serious doubts substantiated by verifiable facts suggesting that the invention cannot be carried out by a skilled person.

3.2.2 Not all library members will have an optimised biological property. However, this is inherent to the nature of immunoglobulin libraries which need to be screened for members having the desired optimised biological property. On the basis of the selection of *in vivo*-matured immunoglobulins derived from a common lead germline sequence, it is credible that immunoglobulins with an optimised biological property in relation to the lead immunoglobulin will be present as members of the library and thus can be found. There is no evidence to the contrary on file.

Thus, the patent application discloses the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art as per Article 83 EPC.

Inventive step - Article 56 EPC - claim 1

The parties started their inventive step reasoning with document D2/D10 or D23 as the closest prior art. The board has no reason to deviate from this.

D10/D2 as the closest prior art

3.3 Document D10 (document D2 is the corresponding scientific article) discloses a method for improving the binding affinity of an immunoglobulin using a phage display library. The variant sequences differ from the parental/lead immunoglobulin on account of at least one random amino acid substitution, the amino acid being encoded by a codon comprising a nucleotide SHH-motif selected from the tetranucleotide A/G-G-C/T-A/T (Pu-G-Py-A/T) or the serine codons AGC or AGT. The amino acid substitution(s) can occur in any of the

complementarity-determining regions (CDRs) of the variable heavy (VH) and/or variable light (VL) chain (see page 4, lines 9 to 16 and page 10, lines 9 to 12). The method allows for the generation of Fvs with increased affinity from a small library of variants (see page 9, last paragraph). The method yielded a 15 to 55-fold affinity improvement from a small library of only about 8 000 independent clones (see page 11, paragraph 2).

Example 1 discloses the construction of phage libraries starting from the mesothelin-specific single-chain variable fragment (scFv) referred to as "SS scFv" (see page 3, last paragraph), which comprises in its VL CDR3 two hot spot motifs of the A/G-G-C/T-A/T type and one AGT serine codon. Lastly, residues 89, 93 and 94 were randomly replaced.

3.4 Document D10 implicitly uses the same germline because all the sequences are derived from a single lead immunoglobulin. The immune library made according to the method disclosed in document D10/D2 is also focused on SHHs, as required in the method according to claim 1. However, the immune library of document D10/D2 uses a random amino acid replacement, i.e. all 20 natural amino acids (including the original one) randomly appear in place of the original hot spot amino acid.

D23 as the closest prior art

3.5 Document D23 relates to methods for screening and identifying immunoglobulins with diverse sequences and high affinity to a target antigen by combining computational prediction and experimental screening of a biased library of antibodies (see paragraph [0003]).

The method makes it possible to efficiently generate and screen protein libraries for optimised proteins with desirable biological functions. The process is carried out computationally in a high-throughput manner by mining databases of protein sequences of all organisms, especially human. The method is used in designing antibodies that are diverse in sequence and yet functionally related to each other. On the basis of the designed antibody sequences, a library of antibodies can be constructed to include diverse sequences in the CDRs and/or humanised framework regions (FRs) of a non-human antibody. This library of antibodies can be screened against a wide variety of target molecules for novel or improved functions (see paragraphs [0023] and [0024]).

In one aspect, the method relates to the *in silico* selection of antibody sequences on the basis of the amino acid sequence of a region in a lead antibody, which is used to search protein sequence databases. The choice of the database depends on the specific functional requirement of the designed motifs. Databases for immunoglobulin sequences of various species or even unrelated sequences in Genbank, Swiss-Prot or Kabat can be used to design the CDRs. A library of diverse antibody sequences can be constructed and screened experimentally *in vitro* or *in vivo* for antibody mutants with improved or desired function(s), such as affinity (see paragraphs [0025], [0229] and [0346]). A conventional BLAST analysis may be employed to search for sequences with high homology to the CDR H3 sequence (see paragraph [0726]).

The method may comprise (pre-)selecting from the plurality of tester protein sequences at least two peptide segments that have at least 10% sequence

identity with the lead sequence, the selected peptide segments forming a hit library (see paragraphs [0087], [0118] and [0139]), and determining if a member of the hit variants library is structurally compatible with the lead structure template using a scoring function (see e.g. paragraphs [0119], [0141], [0174], [0186] and [0608]).

Paragraph [0552] states that: "*Given the availability of a high affinity complex structure as a template, the hit variant library can be computationally pre-screened to reduce the library size, yet remain functionally highly focused compared to traditional libraries generated through complete randomization of amino acids in each position of the lead antibody. Through prediction and construction of the hit variant library in silico, the whole process of protein evolution can be hastened, effectively mimicking the natural process of antibody affinity maturation in a high throughput manner.*"

Selecting antibodies from a highly diverse library allows for broad coverage of sequences, thereby maximising the chance of finding the optimal sequence(s). To avoid 3D structural incompatibility between the tester and the lead, it is suggested to use expressed protein sequences and filter out the incompatible sequences (see paragraph [0555], [0556], [0560], [0636] or [0663]).

Difference and objective technical problem

3.6 The claimed method differs from the method in document D10 in that:

- The variant immunoglobulins to be included in the library were raised against the same target

molecule in a transgenic non-human mammal comprising human immunoglobulin genes.
- The positions to be modified are selected on the basis of SHHs observed *in vivo* (i.e. not at motif-predicted sites) by comparing at least 20 immunoglobulins with the first lead immunoglobulin.
- The modification at the identified SHH(s) is limited to the variant amino acids observed in the pool of compared immunoglobulin sequences (i.e. does not use a random amino acid replacement).

3.7 The claimed subject-matter differs from the disclosure in the closest prior-art document D23 in that:
- The method compares at least 20 related immunoglobulins with the first lead immunoglobulin.
- All of the immunoglobulins have been raised against the same target antigen in a transgenic non-human animal.
- The positions to be modified are selected on the basis of SHHs observed *in vivo*.
- The modification at the identified SHH sites is limited to the variant amino acids observed in the pool of compared immunoglobulin sequences.

3.7.1 The appellant referred to paragraphs [0013] to [0015] of the patent as providing evidence that CDR homology was how related immunoglobulins were to be identified according to the invention, which was the same approach as used in document D23 (see paragraph [0726]).

3.7.2 The board does not agree. Claim 1, step a) relates to "identifying at least 20 related immunoglobulins of common lineage". In other words, two conditions need to be met:
i) the selection of "related" immunoglobulins which, according to paragraphs [0013] and [0015] to [0017],

requires at least one CDR or FR to have at least 70% homology when compared with the lead immunoglobulin
ii) the immunoglobulins need to be derived from a common, i.e. the same, germline sequence (see paragraph [0014])

Thus, the germline sequence classification is to be considered independent of the at least 70% CDR/FR homology-based "related" feature.

3.8 The objective technical problem starting from either document D10/D2 or D23 as the closest prior art can be formulated as to provide a method for designing an alternative immunoglobulin library for optimising a biological property of a first lead immunoglobulin.

Obviousness

3.9 In assessing the obviousness of the claimed subject-matter, the relevant question is whether or not, having regard to the state of the art, the skilled person faced with this objective technical problem would have modified the method disclosed in document D10/D2 or D23 and arrived at the claimed method.

3.10 Document D10 in combination with document D3 or D22

3.10.1 In its inventive step arguments, the appellant specifically pointed to part b) of claim 42 of document D10, which it asserted would make the skilled person realise that the method was not limited to the identification of the hot spots by *in silico* means. It was the replication of the *in vivo* somatic hypermutation process that was key, not the method by which somatic hypermutations were identified or the identity/provenance of the immunoglobulin itself

(reference was made to page 11, lines 16 to 19 and the sentence bridging pages 51 and 52).

3.10.2 However, part b) of claim 42 of document D10 cannot be read in isolation and follows part a), which explicitly refers to "*providing a nucleic acid molecule encoding an amino acid sequence of a VH or a VL domain of a parental antibody, the nucleic acid molecule comprising at least one parental hot spot codon comprising at least one nucleotide within a hot spot motif*" (underlining added by the board). In other words, the skilled person would also read part b) of this claim, in line with the general teaching of document D10, as requiring motif-based prediction of SHHs as a mandatory part of the method.

The paragraph bridging pages 10 and 11 of document D10 sets out other prior attempts to improve antibody affinity and explicitly mentions either random mutations of the CDR residues or mutating particular amino acids found from crystallographic analysis to affect antigen contact. Subsequently, the inventors propose targeting hot spots in the CDRs by random mutation to overcome some of the disadvantages in these methods, thereby teaching away from using the reported prior-art methods, including immunisation or DNA immunisation, for improving antibody affinity.

The board is of the opinion that on the basis of the teaching of document D10/D2 alone, a skilled person would not have arrived at the subject-matter of claim 1 in an obvious way.

3.10.3 With reference to document D3 and the common general knowledge, the appellant argued that the skilled person would know that the *in silico* modelling approach of

document D10 might not capture everything that happens *in vivo*, and so the skilled person would actually be motivated to identify the somatic hypermutations for a given antibody on the basis of a sequence analysis of what is actually observed *in vivo* after immunisation. The appellant also pointed to document D22 as teaching the use of immune libraries obtained from host immune systems sensitised against the target antigen.

3.10.4 Document D3 analyses the diversification of camel heavy-chain antibodies by investigating the germline variability and the specific mechanisms of diversification. The authors of document D3 recommend providing a (naive) library that contains all germlines and analysing the positions where mutations occur (see page 922, left-hand column, paragraph 1). It is also explained that the occurrence of hypermutational hotspots, such as the AGY and TAY (Y = C or T) sequences, corresponded to the highest variability at the CDR1 and CDR2 of cDNA sequences (see page 923, right hand column, "*Hypermutational hotspots imprinted in the germline*").
Thus, a motif-based prediction of SHHs appears to be a reasonable approach (at least for the CDR1 and CDR2).

However, there is no suggestion to use variants derived from the same germline obtained by immunisation with the same antigen, nor is there any hint towards restricting the positions to be mutated only to amino acids observed *in vivo*.

Consequently, document D3 cannot lead to the method of claim 1.

3.10.5 The book chapter D22 reviews antibody libraries from immune repertoires and teaches that a relatively small

immune library can be generated by using the potential of the immune system to enrich the antigen-binding B cells via clonal expansion and perform the affinity maturation (see paragraph bridging pages 549 and 550). Page 624 highlights the advantages of using immune libraries, such as from xenomice, for *in vitro* modifications including affinity maturation or humanisation of existing potent murine antibodies. Document D22 is not concerned with methods for designing antibodies using SHHs, and there is no pointer towards amending the method disclosed in document D10 and using immune libraries.

The paragraph bridging pages 10 and 11 of document D10, which discourages the use of the reported prior-art methods (including immunisation or DNA immunisation) to improve antibody affinity, actually points away from amending the method of document D10 in the direction of immunised libraries.

3.10.6 None of the other documents (e.g. document D16 or D9 relied on in the opposition proceedings) teaches replacing the amino acids at observed SHHs only with the variants observed during *in vivo* affinity maturation against the same target antigen.

3.10.7 In the board's view, on the basis of the teaching of document D10/D2 alone or in combination with the teaching of document D3 or D22, a skilled person would not have arrived at the claimed subject-matter in an obvious way.

3.11 Document D23 in combination with document D22

3.11.1 The appellant further argued that combining the teachings of document D23 with the skilled person's

common general knowledge as represented by document D22 would render the subject-matter of claim 1 obvious.

3.11.2 The book chapter D22 is summarised in point 3.10.5 above.

3.11.3 On the basis of the teaching in document D23, the skilled person would not consider using an immune library from xenomice as suggested in document D22, since document D23 emphasises creating an unfocused library in order to have as many variations as possible for screening towards a specific target antigen (see paragraph [0025], [0346], [0555], [0560], [0636] or [0650]).

3.11.4 There is no teaching in either document D23 or document D22 that the lead and variant immunoglobulins in the library have to be derived from the same germline by somatic hypermutation, or that the positions to be modified are selected on the basis of SHHs observed *in vivo*, or that the modification at the identified SHHs is limited to the variant amino acids observed in the pool of compared immunoglobulin sequences.

No document on file suggests modifying the methods described in document D23 so as to arrive at the method claimed according to claim 1. None of these documents discloses steps d) and e) of claim 1 of auxiliary request 1 on file.

3.12 The board concludes that the claimed subject-matter is not obvious from one of the two alternative closest prior-art documents D10/D2 and D23, either alone or together with any combination document discussed above. Thus, the claimed subject-matter involves an inventive step within the meaning of Article 56 EPC.

In view of this, it is not necessary to consider inventive step starting from an objective technical problem of providing an improved method.

Order

For these reasons it is decided that:

- The decision under appeal is set aside.

- The case is remitted to the opposition division with the order to maintain the patent according to the claims of auxiliary request 1 filed with the statement of grounds and description and drawings possibly to be adapted thereto.

The Registrar: The Chairwoman:

I. Aperribay M. Pregetter

Decision electronically authenticated