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**Datasheet for the decision
of 26 May 2014**

Case Number: T 0067/11 - 3.3.04

Application Number: 04728755.2

Publication Number: 1623997

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Language of the proceedings: EN

Title of invention:

Recombinant antibodies and fragments recognising N-glycolyl-GM3 and use thereof in the diagnosis and treatment of tumours

Applicant:

Centro de Inmunologia Molecular

Headword:

Humanized antibodies / CENTRO DE INMUNOLOGIA MOLECULAR

Relevant legal provisions:

EPC Art. 56

Keyword:

Inventive step - (yes)

Decisions cited:

Catchword:

-



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Case Number: T 0067/11 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 26 May 2014

Appellant: Centro de Inmunologia Molecular
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted on 26 July 2010 refusing European patent application No. 04728755.2 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman G. Alt
Members: B. Claes
M. Blasi

Summary of Facts and Submissions

- I. The appeal lies from the decision of the examining division to refuse European patent application No. 04728755.2 (hereinafter referred to as "application as filed") with the title "*Recombinant antibodies and fragments recognising ganglioside N-glycolyl-GM3 and use thereof in the diagnosis and treatment of tumours*" which was published as WO2004/094477. The examining division decided that the subject-matter of claims 1 to 9 of the request before it lacked an inventive step (Article 56 EPC).

Independent claim 1 of this request read:

"1. A recombinant humanized antibody comprising the constant region of the IgG1 human heavy chain and the constant region of the Ck human light chain and heavy and light chain variable regions derived from the murine 14F7 monoclonal antibody produced by the hybridoma with the deposit ECACC98101901, wherein the murine 14F7 monoclonal antibody comprises the sequences of the hyper variable regions (CDRs) of the heavy and light chains shown below:

HEAVY CHAIN

CDR1: SYWIH
CDR2: YIDPATAYTESNQKFKD
CDR3: ESPRLRRGIYYYAMDY

LIGHT CHAIN

CDR1: RASQISISNNLH
CDR2: YASQISIS
CDR3: QQSNRWPLT;

and the murine 14F7 monoclonal antibody comprises the sequences of the framework regions (FRs) of the heavy and light chains shown below:

HEAVY CHAIN

FR1: QVQLQQSGNELAKPGASMKMSCRASQYSFT
FR2: WLKQRPDQGLEWIG
FR3: KAILTADRSSNTAFMYLNSLTSEDSAVYYCAR
FR4: WGQGTTVTVSS

LIGHT CHAIN

FR1: DLVLTQSPATLSVTPGDSVSFSC
FR2: WYQQRTHESPRLLIK
FR3: GIPSRFSGSGSGTDFTLS1ISVETEDFGMYFC
FR4: FOAGTKLELKRA;

wherein the recombinant humanized antibody comprises point mutations in the FRs of the heavy and light chains to reduce immunogenicity."

Independent claim 3 was directed to a single chain fragment (scFv) derived from the murine antibody 14F7 with the same sequences of the hyper variable regions and the framework regions as defined in claim 1. Claims 5 and 6 referred to particular scFvs, i.e. 2Am scFv and 3Fm scFv (claim 5) and 7Bhk scFv and 7Ahl scFv (claim 6). Independent claim 7 was directed to a cell line expressing the recombinant antibody or the single chain Fv fragment of any of claims 1 to 6. Independent claim 8 was directed to a pharmaceutical composition for use in the treatment of malignant tumors comprising the recombinant antibody or the single chain Fv fragment of any of claims 1 to 6.

II. The following documents are cited in this decision:

D1: EP-A-0 972 782

D2: EP-A-1 013 761

D6: Kabat *et al.* (1991), *The Journal of Immunology*, Vol. 147, No.5, pages 1709-1719.

- D7: Winter *et al.* (1993), *Immunology Today*, Vol. 14, No. 6, pages 243-246.
- D14: Damschroeder *et al.* (2007), *Molecular Immunology*, Vol. 44, pages 3049-3060.
- III. With the statement of the grounds of appeal the appellant maintained the main request refused by the examining division and filed two auxiliary requests.
- IV. At a later date, the appellant filed a third auxiliary request and submitted four documents.
- V. In a communication pursuant to Article 17(1) RPBA the board expressed its preliminary and non-binding opinion that the subject-matter of the claims of all the requests on file lacked an inventive step (Article 56 EPC), was not clear and lacked support in the application (Articles 84 EPC) and that the invention was insufficiently disclosed in the application as filed (Article 83 EPC).
- VI. The appellant replied on 6 December 2013 and submitted auxiliary request IV (claims 1 to 5), further technical data and arguments in favour of inventive step.
- VII. In a brief telephone conversation with the representative, the rapporteur informed the appellant of the preliminary opinion of the board. Subsequently, the appellant withdrew the main request and the first three auxiliary requests. Auxiliary request IV was maintained as the sole request.

Claim 1 of this sole request read:

"1. A recombinant humanized antibody that recognizes the NGcGM3 ganglioside comprising the constant region of the IgG1human heavy chain and the constant region of the Ck human light chain and heavy and light chain variable regions from the murine 14F7 monoclonal antibody produced by the hybridoma with the deposit ECACC 98101901, wherein the murine 14F7 monoclonal antibody comprises the sequences of the hyper variable regions (CDRs) of the heavy and light chains shown below:

HEAVY CHAIN

CDR1: SYWIH
CDR2: YIDPATAYTESNQKFKD
CDR3: ESPRLRRGIYYYAMDY

LIGHT CHAIN

CDR1: RASQSI>NNLH
CDR2: YASQSI
CDR3: QQSNRWPLT

and the murine 14F7 monoclonal antibody comprises the sequences of the framework regions (FRs) of the heavy and light chains shown below:

HEAVY CHAIN

FR1: QVQLQQSGNELAKPGASMKMSCRASGYSFT
FR2: WLKQRPDQGLEWIG
FR3: KAILTADRSSNTAFMYLNLSLTSEDSAVYYCAR
FR4: WGQGTITVTVSS

LIGHT CHAIN

FR1: DLVLTQSPATLSVTPGDSVSEFSC
FR2: WYQQRTHESPRLLIK
FR3: GIPSRFSGSGSGTDFTLTIISVETEDFGMYFC
FR4: FGAGTKLELKRA;

wherein the recombinant humanized antibody is characterized by containing the sequence of the variable region of the heavy chain of the murine 14F7

monoclonal antibody and a light chain variable region whose sequence is the following:

Fr1 DIQMTQTPSSLSASLGDRVTISC
CDR1 RASQDISNYLN
Fr2 WYQQKPDGTVKLLIV
CDR2 YTSRLHS
Fr3 GVPSRFSGSGSGTDYSLTISNLEQEDIATYFC
CDR3 QQGNTLPPTF
Fr4 GAGTKLELK

or:

Fr1 DIQMTQTPSSLSASVGDRVTITC
CDR1 RASQSISSEFLN
Fr2 WYQQKPGKAPKLLIY
CDR2 AASNLQS
Fr3 GVPSRFSGRGSGTDFTLTISLQPEDFAAYYC
CDR3 QQGYTTPLTF
Fr4 GQGTKLELK

or:

Fr1 QSVVTQPPSASGGPGQSLTISC
CDR1 TGTSSDVGGYNHVS
Fr2 WYQQHPGKAPKLMYI
CDR2 DVSKRPS
Fr3 GVPHRFSGSKSGNTASLTVSGLQAEDEAVYYC
CDR3 SSYAGSNNLVF
Fr4 GGGTKVTVL"

Independent claim 2 related to a single chain Fv fragment wherein the sequences of the light chain variable region were as defined in claim 1. Independent claim 3 was directed to a cell line expressing the recombinant antibody or the single chain Fv fragment of claims 1 or 2. Independent claim 4 related to a pharmaceutical composition for use in the treatment of malignant tumors comprising the recombinant antibody or the single chain Fv fragment of claims 1 or 2. Claim 5 was dependent on claim 4.

VIII. The appellant's arguments can be summarised as follows:

Amendments (Article 123(2) EPC, clarity (Article 84 EPC), sufficiency of disclosure (Article 83 EPC) and novelty (Article 54 EPC)

The requests complied with all the requirements of Article 54, 83, 84 and 123(2) EPC.

Inventive step (Article 56 EPC)

At the filing date numerous options existed for antibody modifications, but the downsides of such modifications for a specific antibody were not predictable. In particular, it was well known in the art that the modification of antibodies or single chain fragments often resulted in a significant decrease of their affinity.

In view of the prior art, the skilled person would have avoided introducing point mutations in the framework regions (FR) of the antibody.

There were few examples of humanised antiganglioside antibodies, which could be due to the high complexity of the antigen-binding characteristics in the case of carbohydrate-binding antibodies.

The skilled person would have expected that the humanized antibody with the best affinity properties would be the variant with less mutations with respect to the murine antibody.

IX. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis

of the request submitted on 6 December 2013 as auxiliary request IV (see section VII).

Reasons for the Decision

1. The appeal is admissible.

Amendments (Article 123(2) EPC)

2. Present claim 1 has a basis in claims 1 and 2 and in example 7 on pages 19 to 20 of the application as filed. Moreover, it contains the additional feature that the antibody "recognizes the NGcGM3 ganglioside", which is based on page 1, lines 8 to 9 of the description as filed and numerous references to the same antigen throughout the same.
3. The specific sequences cited in claim 1 which were originally disclosed in example 7 correspond also to the fragments set forth in claims 12, 14 and 15 as filed. The board is satisfied that the choice of these three embodiments does not amount to an intermediate generalisation, since all the elements characterising each specific antibody are included in claim 1.
4. The three alternatives of claim 2 find their basis in claims 12, 14 and 15 as filed. Claims 3 to 5 are based on claims 17 to 19 as filed.
5. In view of the above considerations, amended claims 1 to 5 comply with the requirements of Article 123(2) EPC.

Clarity, sufficiency of disclosure and novelty (Articles 84, 83 and 54 EPC, respectively)

6. In its decision, the examining division did not express any negative opinion under Articles 54, 83 or 84 EPC.
7. The board considers the claims clear (Article 84 EPC) since a skilled person is in a position to unambiguously identify an antibody, fragment or pharmaceutical composition containing these falling within the scope of the claims. The board considers furthermore that the application sufficiently discloses the novel antibodies of claim 1 and the novel single chain Fv fragments of claim 2 (Articles 54 and 83 EPC). Hence, the board is satisfied that these requirements of the EPC are met.

Inventive step (Article 56 EPC)

8. The idea underlying the present invention is the provision of an antibody which recognizes the same antigen as the murine antibody 14F7 with the same affinity but which is less immunogenic when administered to patients (page 4, lines 11 to 13 of the description). This is achieved by the replacement of the constant regions of the murine antibody by particular human constant regions and by the replacement of specific "murine" amino acids in the variable FR regions by "human" amino acids (see page 5 of the description, line 17 to page 6, line 16). The invention is accordingly claimed in claim 1.

Closest prior art

9. The examining division considered document (D1) to represent the closest prior art. The board agrees,

since this document discloses the murine monoclonal antibody 14F7 (the original antibody on which the presently claimed modified antibody is based), pharmaceutical compositions comprising it and their use for treating cancer and is hence considered the most promising springboard among the documents cited.

Technical problem and its solution

10. The recombinant humanised antibody of claim 1 (or the single chain Fv fragment of claim 2) and the antibody disclosed in document (D1) differ in that the former has particular human heavy and light chain constant regions and that the FRs of the heavy and light chains contain certain specific point mutations. The technical effects of these differences are, according to the application as filed (see page 4, lines 11 to 13), reduced immunogenicity in humans as compared to the murine antibody of document (D1) while maintaining a similar affinity towards the antigen.
11. Starting from document (D1), the problem to be solved is thus the provision of an antibody with an affinity towards the N-glycolil GM3 ganglioside which is similar to that of 14F7 but which is less immunogenic when administered to human patients.
12. At the relevant date, it was generally accepted in the art that the replacement of mouse constant regions and variable domain regions by human sequences results in less immunogenic antibodies when administered to the patient (see e.g. paragraphs [0007] and [0008] of document (D2) and page 243, right-hand column, first and second full paragraph of document (D7)). Hence, concerning the overall reduction of immunogenicity, the

board is satisfied that the subject-matter of claims 1 and 2 solves this part of the problem.

13. With respect to the level of affinity to the target antigen, the board notes that although example 7 and Figure 8 identify the three specific light chain variable fragments defined in claims 1 and 2 as particularly relevant for the invention and demonstrate their specificity to the N-glycolil GM3 antigen, the application as filed does not include any particular experimental measurement of the binding affinity of said fragments. However, on the basis of the facts before it, the board has no reason to doubt that they conserve the binding affinity to the antigen of the murine antibody. Indeed, the data submitted by the appellant on 16 December 2013 demonstrate and confirm that for the three specific single chain Fv fragments defined in the claimed subject-matter (ScFv 3Fm, ScFv 7Bhk and ScFv 7Ahl), the dissociation constant is indeed similar or lower than the value of the murine 14F7 antibody, which was used as a reference. The smaller the dissociation constant of an antibody, the more tightly it is bonded to the antigen. Hence, the values obtained indicate that the affinity towards the N-glycolil GM3 antigen of ScFv 3Fm, ScFv 7Bhk and ScFv 7Ahl is the same or higher than that of the reference murine 14F7 antibody.
14. The board is therefore satisfied that the subject-matter of claims 1 and 2 provides a solution to the problem formulated in point 11 above.

Obviousness

15. The examining division argued its inventive step objection in relation to the subject-matter of the

claims before it (see section I) starting from the disclosure of the murine antibody 14F7 in document (D1) and combining it with the common general knowledge of the skilled person about humanization of antibodies as reported in documents (D2) or (D7). The examining division held that, at the priority date of the application, the skilled person would have been able to obtain and select recombinant humanised antibodies based on the murine 14F7 antibody by replacing the mouse constant regions by human sequences and providing point mutations in the FRs of the heavy and light chains applying standard knowledge and technology and accordingly would also be able to obtain a cell line expressing those antibodies and pharmaceutical compositions containing them and to use those compositions for cancer treatment. Moreover, in the absence of any particular technical effect linked to the specific mutations cited in the claims before it, the claimed antibody sequences were regarded as arbitrary selections which could not render the antibodies inventive over the prior art.

16. The claims now before the board (see section VII) comprise extensive amendments as compared to the claims considered by the examining division (see section I). In particular, the general reference to point mutations in the FRs of the heavy and light chains in former independent claim 1 (and claim 3) has been replaced by three specific embodiments with defined specific sequences of the light chain variable region. These amendments aim at addressing the objections under Article 56 EPC raised by the examining division in the impugned decision.

17. The drawbacks of rodent monoclonal antibodies (and their consequential limited clinical utility) that had

motivated the development of humanised monoclonal antibodies, namely a short blood half-life and their immunogenicity in humans were well known in the art (see e.g. document (D7), page 243, left-hand column, lines 11 to 16). Hence, the board agrees with the examining division that the preparation of humanised chimeric antibodies by recombining variable domains of murine antibodies with constant domains of human antibodies in order to reduce the immunogenicity of the murine antibodies was part of the standard knowledge and technology.

18. It was also generally known in the art that the process of humanisation of murine antibodies could result in a significant loss of binding affinity to the antigen. Document (D7) reviews this point on pages 243 and 244 in the part titled "*Building humanized antibodies*" and mentions several strategies to recover at least part of the lost affinity, by methodologies including framework substitutions, chain shuffling or random mutations. The board notes however that document (D7) does not disclose any preference for a particular approach but rather that any of these strategies may be of general application for all antibodies.
19. The board considers therefore that, at the relevant date, the skilled person had a clear incentive to attempt humanisation of a potentially interesting therapeutic antibody.
20. While the possibility of carrying out mutations in framework residues is clearly suggested in the prior art as a possible route to improve antigen recognition on page 244 of document (D7), for the purpose of assessing obviousness of the claimed subject-matter, it still needs to be determined whether the prior art

provides clear instructions to the skilled person to obtain the specific mutations recited in the claims, i.e. whether such mutations were obvious possibilities for the person skilled in the art faced with the problem defined in point 11 above.

21. In this context, the following facts and considerations are of relevance:

21.1 Studies in the prior art in respect of the contribution of the variable domains of the heavy and light antibody chains on the specificity of antibody-antigen binding warn the skilled person that "*a single amino acid change may seriously disrupt site structure and in some instances abolish binding*" (see document (D6); in particular lines 18 to 21 of the abstract).

21.2 Based on the documents available to the board, it would appear that, even years after the filing of the present application no methodical study of the effects of humanisation on antibody binding, activity, stability and biophysical properties have been undertaken in the art and even less that a 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 (see document (D14), page 3049, right-hand column). This appears all the more true for humanised anti-ganglioside antibodies, of which only very few examples had been successfully prepared at the relevant date of the application.

21.3 The technical data submitted by the appellant (see point 12 above) relates to five antibody fragments with various particular point mutations in the FR region. The three which are now claimed have the desired

binding affinity similar to the murine monoclonal antibody 14F7, whereas two further embodiments with slightly different mutations have a dissociation constant much higher than that of 14F7, i.e. they have less affinity for the antigen. These results would therefore seem to corroborate the general warning in the prior art that minor structural differences involve significant functional consequences which the skilled person could not have predicted.

22. Accordingly, and taking into account the above facts and considerations, the board judges that in the present context the possibility of mutating some residues in the framework region was likely to have been considered by the skilled person when attempting the humanization of the 14F7 antibody with the aim of maintaining the affinity to the antigen. It would appear, however, that the prior art failed to provide the skilled person with any pointer towards any specific set of mutations which would do the job.

23. In accordance with the case law of the boards of appeal, a course of action is considered obvious within the meaning of Article 56 EPC if for the skilled person its results are clearly predictable or there is a reasonable expectation of success that the results be achieved. It has been established, and this board agrees, that such a reasonable expectation of success implies the ability of the skilled person to predict rationally, on the basis of the knowledge existing before a research project was started, the successful conclusion of the said project within acceptable time limits. The more unexplored a technical field of research is, the more difficult it is to make predictions about its successful conclusion and consequently, the lower the expectation of success is

(see Case Law of the Boards of Appeal of the EPO, 7th edition 2013, section I.D.7.1).

24. Accordingly, the expectation of success is inversely related to the difficulty of predicting the successful resolution of a technical problem and is inherently low in cases like the present one where minor changes (point mutations) bring about major differences on the final technical effect and where there is no guidance from the prior art concerning the impact of such possible changes. In the present case, and on the basis of the prior art and citations available, the board considers that the skilled person had no means to predict the impact of each possible mutation in the FR of the antibody on the final properties of the antibody or antibody fragment. In these technical circumstances, it is the attainment of an antibody (or antibody fragment) having indeed the desired characteristics which is considered surprising, not the theoretical possibility of achieving one.

25. Accordingly, the board judges that the subject-matter of claims 1 and 2 involves an inventive step on the basis of the prior art available. The same applies to the subject-matter of claim 3 to 5, which refer to the antibody or antibody fragments of claims 1 and 2.

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 on the basis of claims 1 to 5 of auxiliary request IV, filed with the letter dated 6 December 2013, after any necessary consequential adaptation of the description.

The Registrar:

The Chairwoman:



C. Rodríguez Rodríguez

G. Alt

Decision electronically authenticated