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**Datasheet for the decision  
of 12 July 2019**

**Case Number:** T 0528/18 - 3.3.04  
**Application Number:** 02792048.7  
**Publication Number:** 1464702  
**IPC:** C07K14/705, C07K16/00,  
C07K1/107, C07K16/36  
**Language of the proceedings:** EN

**Title of invention:**  
Method of stabilizing protein

**Applicant:**  
Chugai Seiyaku Kabushiki Kaisha

**Headword:**  
Method for producing a stabilised antibody/CHUGAI

**Relevant legal provisions:**  
EPC Art. 83  
EPC R. 103(1)(a)

**Keyword:**  
Main request, first to fifth auxiliary request: sufficiency of disclosure - (no)  
Reimbursement of appeal fee - (no)

**Decisions cited:**

**Catchword:**



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Case Number: T 0528/18 - 3.3.04

**D E C I S I O N**  
**of Technical Board of Appeal 3.3.04**  
**of 12 July 2019**

**Appellant:** Chugai Seiyaku Kabushiki Kaisha  
(Applicant) 5-1, Ukima 5-chome,  
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Tokyo, 115-8543 (JP)

**Representative:** Bassil, Nicholas Charles  
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**Decision under appeal:** **Decision of the Examining Division of the  
European Patent Office posted on 19 July 2017  
refusing European patent application No.  
02792048.7 pursuant to Article 97(2) EPC.**

**Composition of the Board:**

**Chair** G. Alt  
**Members:** R. Morawetz  
P. de Heij

## **Summary of Facts and Submissions**

- I. The appeal of the applicant (appellant) lies from the examining division's decision refusing European patent application No. 02 792 048.7. The application was filed as an international application under the PCT and entered the European phase on 5 July 2004 (hereinafter the "application as filed" or "application").
- II. In the decision under appeal, the examining division held that claim 1 of the main request before it lacked clarity (Article 84 EPC) and inventive step (Article 56 EPC). The subject-matter of claim 1 of the auxiliary request was held to extend beyond the content of the application as filed (Article 123(2) EPC).
- III. With the statement of grounds of appeal, the appellant filed sets of claims of a new main request and of new first to fifth auxiliary requests. They requested acceleration of the appeal proceedings because the duration of the proceedings before the examining division was considered to have been excessively long.
- IV. Claims 1 and 3 of the main request read as follows:
- "1. A method for producing a stabilized antibody, which comprises the step of substituting a glycine that is located adjacent to the C-terminal side of an asparagine with another amino acid, wherein the antigen-binding activity of the stabilized antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution.
3. The method of claim 1 or 2, wherein the asparagine exists in the complementary determining region (CDR)."

V. Claims 1 and 3 of the first auxiliary request read as follows (all emphases below added by the board to indicate amendments compared with the main request):

"1. A method for producing a stabilized antibody, which comprises the step of substituting a glycine that is located adjacent to the C-terminal side of an asparagine with another amino acid, wherein the stabilized antibody has reduced susceptibility to deamidation, and wherein the antigen-binding activity of the stabilized antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution.

3. The method of claim 1 or 2, wherein the asparagine exists in the complementary determining region (CDR)."

VI. Claims 1 and 3 of the second auxiliary request read as follows:

"1. A method of producing ~~a stabilized~~ an antibody with reduced susceptibility to deamidation, which comprises the step of substituting a glycine that is located adjacent to the C-terminal side of an asparagine of the antibody with another amino acid, wherein the antibody has a reduced susceptibility to deamidation relative to the antibody before the amino acid substitution and wherein the antigen-binding activity of the stabilized antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution.

3. The method of claim 1 or 2, wherein the asparagine exists in the complementary determining region (CDR)."

VII. Claim 1 of the third auxiliary request reads as follows:

"1. A method for producing a stabilized antibody, which comprises the step of substituting a glycine that is located adjacent to the C-terminal side of an asparagine with another amino acid, wherein the amino acid being deamidated exists in the complementary determining region (CDR) and wherein the antigen-binding activity of the stabilized antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution."

VIII. Claim 1 of the fourth auxiliary request reads as follows:

"1. A method for producing a stabilized antibody, which comprises the step of substituting a glycine that is located adjacent to the C-terminal side of an asparagine with another amino acid, wherein the stabilized antibody has reduced susceptibility to deamidation and wherein the asparagine exists in the complementary determining region (CDR), and wherein the antigen-binding activity of the stabilized antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution."

IX. Claim 1 of the fifth auxiliary request reads as follows:

"1. A method of producing ~~a stabilized~~ an antibody with reduced susceptibility to deamidation, which comprises the step of substituting a glycine that is located adjacent to the C-terminal side of an asparagine of the antibody with another amino acid, wherein the asparagine exists in the complementary determining

region (CDR), and wherein the antibody has a reduced susceptibility to deamidation relative to the antibody before the amino acid substitution, and further wherein the antigen-binding activity of the stabilized antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution."

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

D8 WO 2010/129904 (11 November 2010)

D10 WO 2012/170607 (13 December 2012)

D11 Declaration of Dr. Izumi Sugo,  
dated 28 November 2017

D12 Chothia C. *et al.*, J. Mol. Biol. (1992),  
vol. 227, pages 799 to 817

D14 Presta L. *et al.*, Thromb. Haemost. (March 2001),  
vol. 85, pages 379 to 389

D15 Cacia J. *et al.*, Biochemistry (1996), vol. 35,  
pages 1897 to 1903

XI. The board scheduled oral proceedings and issued a communication pursuant to Article 15(1) RPBA, in which the board informed the appellant that it had decided to accelerate the appeal proceedings as requested. The board indicated that in its preliminary opinion, *inter alia*, the disclosure in relation to claim 1 of auxiliary requests 4 and 5, both relating to a method for producing an antibody with reduced susceptibility to deamidation wherein the asparagine exists in the CDR of the antibody, and wherein the antigen-binding

activity of the stabilised antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution (see sections VIII and IX above), did not comply with the requirements of Article 83 EPC. In this context, the board introduced two documents into the appeal proceedings, documents D14 and D15.

- XII. In response, the appellant gave its opinion on, *inter alia*, the board's objection for lack of sufficiency of disclosure of the invention as claimed in claim 1 of auxiliary requests 4 and 5.
- XIII. Oral proceedings were held on 12 July 2019. At their end the chair announced the board's decision.
- XIV. The appellant's arguments, submitted in writing and during the oral proceedings and as far as relevant to the present decision, are summarised as follows:

*Main request and first to fifth auxiliary request*

*Sufficiency of disclosure (Article 83 EPC) - claim 1*

Although the skilled person was aware of the dangers of replacing amino acid residues in the complementary determining regions (CDRs) of antibodies, the application demonstrated that the replacement of a glycine (Gly) that was located adjacent to the C-terminal side of an asparagine (Asn) in a CDR region could result in an antibody that had improved stability with respect to its reduced susceptibility to deamidation whilst retaining much of its antigen-binding activity.



The application provided guidance in that the method was restricted to replacing the glycine in an Asn-Gly sequence present in a CDR of an antibody. The skilled person had only a finite number of potential amino acids with which to substitute the Gly in an Asn-Gly sequence. The skilled person would use their knowledge of the structure of the antibody to select a suitable substitution from this short list of amino acids, further guided by the examples in the application which demonstrated suitable replacements so that the antibody retained good binding activity.

Thus, both the selection of the substituting amino acid and the implementation of the chosen substitution were a matter of routine. According to established case law a reasonable amount of trial and error and occasional failures were permissible when it came to assessing sufficiency of disclosure. Therefore, it was not an issue that there would be antibodies where none of the substitutions would result in an antibody with the claimed properties since certainty of success was not required.

Substitutions which resulted in binding activity below 70% of the antigen-binding activity before the substitution were outside the scope of the claims.

In addition to the application, page 120 of document D8 and paragraph [0260] of document D10 also provided evidence that substitutions carried out according to the present invention could be effective in increasing stability whilst maintaining binding affinity.

*Reimbursement of the appeal fee*

The duration of the proceedings before the examining division was excessive and amounted to a substantial procedural violation justifying the reimbursement of the appeal fee.

- XV. The appellant requests that the decision under appeal be set aside and that a patent be granted on the basis of the set of claims of the main request or, alternatively, on the basis of the set of claims of one of the first to fifth auxiliary requests, all claim requests filed with the statement of the grounds of appeal, and further that the appeal fee be reimbursed because of a substantial procedural violation in the proceedings before the examining division.

**Reasons for the Decision**

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

*Main request and first to fifth auxiliary request*

*Sufficiency of disclosure (Article 83 EPC)- claim 1*

2. One embodiment falling within the scope of claim 1 of all claim requests is a method for producing a stabilised antibody which comprises the step of substituting a glycine (Gly) that is located adjacent to the C-terminal side of an asparagine (Asn) with another amino acid, wherein the asparagine exists in the complementary determining region (CDR) of the antibody, and wherein the antigen-binding activity of

the stabilised antibody is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution (see sections IV to IX).

3. Under Article 83 EPC, a European patent application must disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. In interpreting Article 83 EPC it has been established in the jurisprudence of the Boards of Appeal that the claimed invention must be sufficiently disclosed on the filing date (Case Law of the Boards of Appeal of the EPO, 8th edition 2016, II.C.1.) based on the application as a whole (*ibid.*, II.C.2.), in consideration of the common general knowledge of the skilled person (*ibid.*, II.C.3.). At least one way of carrying out the claimed invention must be disclosed, but this disclosure is sufficient only if it allows the invention to be performed in the whole range claimed (*ibid.*, II.C.4.2., II.C.4.4 and II.C.6.1.2). Furthermore, the disclosure must be reproducible without undue burden. Where the person skilled in the art has to find out by trial and error which compound, if any, meets the parameter set out in the claim, this constitutes an undue burden, even if it involves routine experimentation (*ibid.*, II.C.5.7.).
  
4. Thus, the criterion that needs to be assessed in determining whether the disclosure of the invention is sufficient is whether the application as filed provides the skilled person, in the light of their common general knowledge, with all the information necessary for carrying out the claimed invention (here: a method for stabilising any antibody comprising an Asn-Gly sequence in one of its CDRs, wherein the antigen-binding activity of the stabilised antibody is 70% or

higher than the antigen-binding activity of the antibody before the amino acid substitution) without undue burden.

5. In developing a method to suppress deamidation of Asn without affecting the antibody's activity, the application focuses on an anti-human tissue factor (TF) antibody with an Asn-Gly sequence in the CDR2 region (see page 2, line 29 to page 3, line 5). The application provides one relevant example (Example 2), in which one specific amino acid in the heavy chain CDR2, namely Gly at position 55 (Gly55), of the anti-TF antibody has been substituted with 19 different amino acids and the binding activity of the resultant mutants measured. The results are shown in Figure 11 of the application.
6. The board notes that not all replacements resulted in a binding activity which was "*70% or higher than the antigen-binding activity of the antibody before the amino acid substitution*". Consequently, the nature of the amino acid replacing the Gly seems to be critical.
7. The application does not provide any further examples or technical guidance for substitutions of glycines in Asn-Gly sequences occurring in CDR regions of any antibody that would help the skilled person find substitutions that result in an antibody as claimed, i.e. a stabilised antibody with antigen-binding activity which is 70% or higher than the antigen-binding activity of the antibody before the amino acid substitution.
8. As regards the available common general knowledge (see point 3 above), the appellant acknowledges (see document D11, point 6) that at the priority date of the

application *"it was known to persons skilled in the art that Gly residues readily form a flexible structure and play an important role in maintaining the canonical structure of antibody. It was believed that replacing a Gly residue in the variable regions (responsible for antigen-binding activity) with another amino acid would prevent the antibody from maintaining the structure that could retain the binding activity because it was thought that glycine residues in the CDR sequences may play a critical role in maintaining the canonical structure of an antibody (see, for example, Chothia et al., 1992, J. Mol. Biol., 227:799-817 [note by the board: document D12 in the appeal proceedings], in particular page 803, left hand column, lines 3 to 4; page 809, right hand column, lines 3 to 5; and page 825, left hand column, section headed "(d) Canonical structures in human expressed Vh segments)."*

- 8.1 Moreover, document D14, cited in the application on page 1, lines 32 to 33, discloses that simultaneously changing Asp in CDR-L2 and Asn in CDR-H2 to Ala in antibody D3 reduces the binding-activity of the antibody 4-fold (see page 386, right hand column, fourth paragraph).
  
- 8.2 Document D15 discloses that changing Asp in an Asp-Gly sequence located in a CDR decreases the relative binding affinity significantly (see page 1901, paragraph bridging columns, Table 4). The authors conclude that their studies *"demonstrate sensitivity of antigen recognition to subtle sequence changes in the CDRs and the tradeoffs incurred in engineering out a labile sequence"* (see page 1903, left hand column, end of first paragraph).

9. The board concludes that it can be inferred from the evidence on file (see points 8 to 8.2) that on the priority date of the application it was part of the common general knowledge of the skilled person that the substitution of an amino acid within a CDR of an antibody was likely to negatively affect the antigen-binding activity of the antibody.
10. Indeed, the application also states that "[g]enerally, an antibody is inactivated by amino acid substitution in the CDR" (see page 12, lines 32 to 33).
11. In the board's view, in these circumstances, providing a single example (see points 5 to 7) which shows that the antigen-binding activity of a specific antibody is retained after some particular substitutions, even when taken together with the general information in the application and the common general knowledge, cannot be considered to provide the information necessary to allow the skilled person to perform the claimed method for any antibody without undue burden, for the following reason:
12. The skilled person does not have at their disposal, either from the application as filed (see points 5 to 7) or from common general knowledge (see points 8 to 9), any information that would reliably lead them to the amino acid substitutions which result in an antibody fulfilling the functional requirements of the claim. Accordingly, for each and every antibody comprising an Asn-Gly sequence in one of its CDRs, the skilled person has to identify each time, by trial and error, which amino acid replacement will result in antigen-binding activity of the stabilised antibody which is 70% or higher than the antigen-binding activity of the antibody before the amino acid

substitution - without any guarantee that a substitution that fulfils the functional requirements of the claim will be found at all. On the contrary, given the common general knowledge (see points 8 to 9), the skilled person's expectation must be that most substitutions will not be successful (see also point 10 above).

13. The fact that the specific amino acid to be substituted - Gly in an Asn-Gly sequence - is disclosed, and that the number of amino acids that can be incorporated into polypeptides is finite, does not make the skilled person's task any easier because testing is still necessary for each of the possible substitute amino acids. The board is also not persuaded by the appellant's argument that once the skilled person had the information as to which amino acid to substitute, namely the Gly in an Asn-Gly sequence occurring in the antibody's CDR, selecting of the substituting amino acid and implementing the chosen substitution were a matter of routine. Even if the testing required in the present circumstances (see point 12 above) can be done by routine methods, it is considered to place an undue burden on the skilled person seeking to carry out the claimed invention because there is absolutely no guarantee that a substitution that works will be found for any antibody.
  
14. Given the wording of the claim, the appellant's submission that substitutions which result in binding activity below 70% of the antigen-binding activity before the substitution are outside the scope of the claims is correct, but misses the point when considering whether the requirements of Article 83 EPC are fulfilled. As explained above, the point is that the skilled person does not know beforehand whether any

of the possible amino acid substitutions will result in an antibody with binding activity above 70% of the antibody before the substitution.

15. Finally, in the circumstances of the present case the appellant cannot rely on post-filed evidence, i.e. documents D8 and D10, because the invention must be sufficiently disclosed at the effective date (see point 3 above).
16. From the above the board concludes that the application as filed does not provide the skilled person, in light of their common general knowledge, with all the information necessary for carrying out the claimed invention over the entire breadth of claim 1 of all claim requests without undue burden.
17. Therefore, none of the claim requests is allowable.

*Reimbursement of the appeal fee*

18. Pursuant to Rule 103(1)(a) EPC the appeal fee will be reimbursed in full where the board deems an appeal to be allowable, if such reimbursement is equitable by reason of a substantial procedural violation.
19. Since the appeal is not allowable (see point 17 above), the appellant's request for reimbursement of the appeal fee has to be refused.



## Order

### For these reasons it is decided that:

1. The appeal is dismissed.
2. The request for reimbursement of the appeal fee is refused.

The Registrar:

The Chair:



S. Lichtenvort

G. Alt

Decision electronically authenticated