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Datasheet for the decision of 6 October 2020

Case Number: T 0031/19 - 3.3.04

Application Number: 11711386.0

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Title of invention:
Disulfide stabilised antibodies and fragments thereof

Applicant:
UCB Biopharma SRL

Headword:
Disulfide stabilised antibodies/UCB Biopharma

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - (yes)

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T 1732/10

Catchword:

Europäisches Patentamt
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Case Number: T 0031/19 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 6 October 2020

Appellant: (Applicant)	UCB Biopharma SRL Allée de la Recherche 60 1070 Brussels (BE)
Representative:	Hambleton, Bernadette Angelina Sterling IP Orion House Bessemer Road Welwyn Garden City AL7 1HH (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 8 August 2018 refusing European patent application No. 11711386.0 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman	G. Alt
Members:	O. Lechner
	L. Bühler

Summary of Facts and Submissions

I. The appeal filed by the applicant (appellant) lies from the examining division's decision refusing the European patent application No. 11 711 386.0. The application was filed as an international application under the PCT and published as WO 2011/117648. The title of the application is "*Disulfide stabilised antibodies and fragments thereof*".

II. In the decision under appeal the examining division decided to refuse the European patent application for the sole reason that neither the main request nor auxiliary request 1 (the sole auxiliary request) complied with the requirements of Article 56 EPC.

Claim 1 of the main request read:

"1. An antibody fragment consisting of a Fab, a Fab', or a $F(ab)_2$, wherein the light chain variable region, V_L and the heavy chain variable region, V_H are linked by a disulfide bond position between a pair of cysteine residues selected from the group consisting of V_H37 and V_L95, V_H44 and V_L100, V_H44 and V_L105, V_H45 and V_L87, V_H55 and V_L101, V_H100 and V_L50, V_H100b and V_L49, V_H98 and V_L46, and V_H105 and V_L43; and wherein the numbering is according to Kabat."

III. The reasoning in the decision under appeal may be summarised as follows:

Document D6, or alternatively either of documents D7 or D8, represented the closest prior-art document.

The problem formulated by the applicant - avoiding the formation of inappropriate multimeric molecules after purification by a process known as "breathing" - was not disclosed in the application as filed and could therefore not be accepted.

Even if the problem formulated by the applicant was accepted, there was no evidence on file that it was solved by the claimed antibody fragments.

The difference between the disclosure in document D6 and the claimed subject-matter was that the V_H and V_L chains of the Fab fragment were "*linked by a disulphide bond position between a pair of cysteine residues selected from the group [...]*".

There was no "*special technical effect linked to that difference, as the constructs have not been tested*".

The technical problem was "*seen as the provision of an alternative Fab having modification in the variable region*".

Without the need to solve any particular problem, the claimed disulfide bond-stabilised Fab fragments had to be considered arbitrary modifications that could not involve an inventive step in view of the teachings of any of documents D12 to D14.

IV. With their statement of grounds of appeal, the appellant filed a new main request along with new auxiliary requests 1 to 3 and arguments in favour of inventive step.

V. The main request differed from from the main request considered in the decision under appeal (see section II

above) in that (i) the expression "selected from the group" was inserted after "antibody fragment" and before "consisting of", (ii) a new claim 2 was added and (iii) the numbering and back-references in claims 2 and 3 were adapted to take account of the additional claim.

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

"1. An antibody fragment selected from the group consisting of a Fab, a Fab', and a $F(ab)_2$, wherein the light chain variable region, V_L and the heavy chain region, V_H are linked by a disulfide bond position between a pair of cysteine residues selected from the group consisting of V_H37 and V_L95, V_H44 and V_L100, V_H44 and V_L105, V_H45 and V_L87, V_H55 and V_L10l, V_H100 and V_L50, V_H100b and V_L49, V_H98 and V_L46, and V_H105 and V_L43; and wherein the numbering is according to Kabat."

VII. The board issued a communication pursuant to Article 15(1) RPBA in which it *inter alia* provided its preliminary opinion on the issue of inventive step.

Like the examining division, the board considered document D6 to be the closest prior art and considered the difference to be essentially the same as in the decision under appeal. In its decision, the examining division had taken the view that there was no special technical effect linked to that difference. The board was of the preliminary view that the experimental data filed during the examination proceedings could not support any improvement over the relevant subject-matter in the closest prior-art document, yet an effect could be acknowledged on the basis of common general knowledge in that the fragments were "*more robust*". However, for want of any comparison with the closest

prior art, the board agreed with the examining division and formulated the technical problem as providing alternative Fab, Fab' or $F(ab)_2$ antibody fragments.

In the board's preliminary view, the claimed subject-matter was an arbitrary choice from a host of possible solutions, so none of the pending requests appeared to involve an inventive step.

VIII. In their reply to the board's preliminary opinion, the appellant observed, *inter alia*, that providing an alternative solution could also be inventive, and argued that the claimed solution did not constitute an arbitrary choice from a host of possible solutions.

IX. Oral proceedings were held on 6 October 2020 in the absence of the appellant, as announced beforehand. At the end of the oral proceedings the chair announced the board's decision.

X. The following documents are referred to in the present decision:

D1: WO 2011/030107

D7 (D23): Röthlisberger D. et al.: "*Domain interactions in the* Fab fragment: a comparative valuation of the single-chain Fv and Fab format engineered with *variable domains of different stability*", Journal of Molecular Biology (2005), vol. 347, No. 4, pages 773-789

D11: Wörn A. et al.: "*Stability engineering of antibody single-chain Fv fragments*", Journal of Molecular Biology (2001), vol. 305, No. 5, pages 989-1010

D12 (D22): Reiter Y. et al.: "*Engineering antibody Fv fragments* for cancer detection and therapy: disulfide-*stabilized Fv fragments*", Nature Biotechnology (1996), vol. 14, pages 1239-1245

D13: Reiter Y. et al.: "*Engineering interchain Disulfide* bonds into conserved framework regions of Fv fragments: Improved biochemical characteristics of recombinant immunotoxins containing disulfide- *stabilized Fv*", Protein Engineering (1994), vol. 7, No. 5, pages 697-704

D14: Jung S.H. et al.: "*Design of interchain disulfide bonds in the framework region of the Fv fragment of the monoclonal antibody B3*", Proteins: Structure, function and genetics (1994), vol. 19, No. 1, pages 35-47

XI. The appellant's arguments as far as relevant for the present decision may be summarised as follows

Main request

Inventive step (Article 56 EPC)

Document D6 represented the closest prior art.

It described mutations primarily in the variable regions of the antibody fragments to facilitate expression in *E. coli*. In the heavy chain the mutations were selected from leucine, tyrosine, proline, alanine, isoleucine, asparagine, serine, valine, tryptophan and aspartic acid. In the light chain the mutations were selected from tryptophan, histidine, alanine, lysine,

leucine, isoleucine, glutamic acid, asparagine and arginine (see Tables II and III).

None of the amino acids was replaced with cysteine.

The differences between the claimed subject-matter and the disclosure in document D6 were the number of mutations, i.e. two versus numerous, and the position and type of mutation, i.e. two cysteines are introduced, one in the variable heavy (V_H) domain and one in the variable light (V_L) domain to enable the formation of a disulfide bond.

The inventors aimed to tackle the problem of promiscuous pairing, known as "breathing", of the V_H and V_L domains between proximal Fabs and the formation of higher-order aggregates after purification and formulation. This was a new problem not identified by any prior art. The technical effect of the differences was to fix the relationship between the V_H and V_L domains to minimise/eliminate this phenomenon.

The technical problem starting from document D6 was to improve the physical stability of the purified antibody.

The claimed solution to this problem, i.e. the introduction of a V_H-V_L interchain disulfide (S-S) bond within a Fab molecule, provided a generically applicable solution which did not require any antibody sequence-specific optimisation.

There was no teaching in any of documents D7, D8, D10 and D11 to D14 that introducing an S-S bond between the V_H and V_L domains within a Fab was beneficial to prevent aggregation after purification.

Requests

XII. The appellant requested 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 one of the sets of claims of the first to third auxiliary requests, all filed with the statement of grounds of appeal. The appellant also requested reimbursement of the appeal fee.

Reasons for the Decision

Admissibility of the appeal

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

Main request

Subject-matter of the application

2. The application provides antibody-derived, modified antigen-binding fragments (Fabs). A Fab consists of two amino acid chains, each comprising a variable domain (V) and a constant domain (C). A Fab is thus formed by a variable light chain (V_L) plus a constant domain (V_L-C_L), and a variable heavy chain (V_H) plus a constant domain (V_H-C_H). In many Fabs the two chains are held together by the interaction of the amino acid side chains extending between the interface of the V_H and V_L and C_H and C_L domains. In addition, the C_H and C_L domains are connected by an interchain S-S bond.

The invention in this case aims at stabilising the interaction of the V_L-C_L and V_H-C_{H1} chains by introducing a non-naturally occurring interchain S-S bond between the V_H and the V_L domain.

Claim construction

3. The board construes the wording of claim 1 as defining subject-matter which encompasses a Fab, Fab' or $F(ab)_2$ fragment linked by "a", i.e. one single, S-S bond. Such a V_H/V_L-stabilised Fab fragment may form part of another antibody molecule or antibody fragment (see description, page 3, paragraph 1).

The modifications need to be made within the V_H/V_L domains of a directly linked V_H-C_{H1}/V_L-C_L pair (see description, page 2, line 19, and page 9, penultimate paragraph). Therefore, the definition in the claims does not cover dual variable domain immunoglobulins (DVD-Igs), which consist of an IgG molecule with additional V_H-V_L pairs (normally representing additional binding entities) attached N-terminally by peptide linkers to each V_H-V_L pair of the IgG unit, resulting in a tetravalent binder.

Inventive step (Article 56 EPC)

Closest prior art

4. Like the examining division and the appellant, the board also considers document D6 to be the most promising starting point for assessing inventive step given that it deals with stabilising Fab molecules.

5. This scientific article discloses stabilising Fabs by mutating 3 to 11 amino acids in the variable fragment (Fv) regions to facilitate expression in *E. coli*. In the heavy chain the mutations were selected from leucine, tyrosine, proline, alanine, isoleucine, asparagine, serine, valine, tryptophan and aspartic acid. In the light chain the mutations were selected from tryptophan, histidine, alanine, lysine, leucine, isoleucine, glutamic acid, asparagine and arginine (see Tables II and III). However, none of the amino acids was replaced with cysteine, nor was one inserted.

Poor Fab expression is linked to poor intrinsic stability observed in terms of misfolding and non-functionality (see abstract). The mutations described in Tables II and III resulted in an increased melting temperature (T_m) of the Fab molecule (i.e. in a more stable Fab molecule), higher yields of expressed Fabs and higher levels of properly folded and functional Fabs (see abstract).

Technical effect and problem to be solved

6. The board agrees with the appellant that the subject-matter of claim 1 differs from the disclosure in the closest prior-art document D6 on account of the number of mutations, i.e. only two versus numerous, and the position and type of mutation, i.e. two cysteines are introduced, one in each of the V_H and V_L domains to enable the formation of a S-S bond between those two domains. A further difference is the type of antibody fragment, i.e. claim 1 relates not only to Fab antibody fragments, but also to Fab' or $F(ab)_2$ fragments.

7. The following analysis concentrates on the Fab fragments.

8. Regarding the effect of the difference, the examining division stated that "*[t]here is no special effect linked to that difference, as the constructs have not been tested*" (emphasis by the examining division).

The examining division therefore formulated the technical problem "*as the provision of an alternative Fab having modification in the variable region*".

9. On the basis of the application, the appellant formulated the technical effect linked to the differences (see point 6 above) as fixing the relationship between the V_H and V_L domains so as to minimise/eliminate promiscuous pairing of the variable domains and the consequent formation of aggregates.

Consequently, the technical problem was formulated as "*to improve the physical stability of the purified antibody*".

10. With regard to the examining division's reasoning that no special technical effect could be acknowledged because "*the constructs have not been tested*", the board notes that any kind of evidence, i.e. not necessarily tests, can be offered for substantiating the effect of the difference.

11. With regard to the appellant's line of reasoning, the board notes that when formulating the technical problem only those effects for which there is evidence that they are achieved vis-à-vis the closest prior art are taken into consideration (see Case Law of the Boards of Appeal, 9th edition, 2019, I.D.4.1, page 188, paragraph 5).

The board has seen no evidence of any kind pointing to the fact that the stability of the claimed Fab molecules with an engineered S-S bond linking the V_H and V_L domains is improved compared with that of the Fab disclosed in document D6.

Hence, the effect and the problem cannot be formulated as suggested by the appellant.

12. In the board's view, one effect that can be recognised as being achieved by the differences set out in point 6 above is that stated in the application on page 1, lines 16 and 17, namely that the modifications make the antibody fragments "*more robust*". This effect can be accepted on the basis of common general knowledge about the function of S-S bonds in proteins.

13. For want of any evidence regarding the robustness compared with antibody fragments disclosed in document D6, the objective technical problem to be solved is to be formulated as providing alternative stabilised Fab molecules.

Obviousness

14. The question to be answered when assessing the obviousness of the claimed subject-matter is whether or not the skilled person, starting from document D6 and faced with the problem of providing alternative stabilised Fab molecules, would have modified the approach disclosed in document D6 such as to arrive at the claimed subject-matter.

15. To answer this question, documents D7 and D11 to D14 were considered most relevant.

16. Document D7, a scientific article, analyses domain interactions in Fab molecules and provides a comparative evaluation of the scFv and the Fab format engineered with V-domains of different stabilities.

17. Pages 774, right-hand column, paragraph 2 states: "*Early studies of the stability of a particular Fab fragment, compared to that of the light chain alone, showed a significant stabilization of the light chain through interactions with the heavy chain. Within the context of a disulfide-linked Fab fragment, the V_HV_L heterodimer and the C_HC_L heterodimer were found to behave as two distinct folding units. The C_HC_L heterodimer was found to be significantly more stable than the V_HV_L heterodimer for the particular antibody under study.*"

According to page 779, left-hand column, paragraph 3, both the C_H and the C_L domains are required in order to further stabilise both V- and C-domains within a Fab.

Page 781, right-hand column, paragraph 2 states that "*[...] the interchain disulfide bond at the C-terminal ends of the constant domains plays an important role in the stabilization of the Fab fragment (Figure 5) [...]*".

The authors of document D7 conclude on page 786, left-hand column, last paragraph, that:
"*Especially weak variable domains experience a considerable stabilization to make them more usable for practical application. This effect is mostly due to kinetic stabilization provided by the C_HC_L heterodimer and especially the disulfide-linked $C_HC_L^{SS}$ heterodimer. At equilibrium, the stabilizing effect is smaller. Conversely, with very stable variable domains, constant*

domains without an intradomain disulfide bond become a liability. This is remedied only by the re-introduction of the interdomain disulfide bond. The question of which format is "better", Fab fragment or scFv fragment, does not have a unique answer, but depends on the stability of the variable domains involved, as well as on the importance that is placed on production yields, as Fab fragments containing the interdomain disulfide bond tend to give lower yields in bacterial production than either Fab fragments without disulfide bond or scFv fragments."

18. Thus, document D7 teaches that one of the key elements in stabilising the interaction of the two chains in a Fab molecule is the S-S bond between the C_H-C_L chains. The document does not teach introducing a further S-S bond, nor does it suggest introducing any such bond in the variable domains. Moreover, introducing S-S bonds is also reported to have some negative effects.

19. Review article D11 describes stability engineering of single-chain Fvs (scFv), stating that one of the most widely used strategies to stabilise the V_H-V_L interface is to engineer an interface S-S bond into the contact surface between the two domains (see page 1001, right-hand column, last paragraph). In this context it is also explained that the most frequently used modification is the combination of introducing cysteines in positions H44 and L100 (see page 1001, left-hand column, lines 3 *et seq.*). However, document D11 also reports some problems associated with an engineered S-S bond as a means for stabilising the V_H-V_L interface (see page 1001, right-hand column, paragraph 3).

20. Document D12 summarises the design strategy, construction and production of various S-S-stabilised Fv antibody fragments (dsFv) and dsFv-fusion proteins. It also discusses the biochemical features of dsFvs in comparison with scFvs, the effect of S-S stabilisation on Fv binding and activity, and various applications of dsFvs and dsFv-immunotoxins for tumour imaging and treating solid tumours in animal models (see abstract and Figures 1 and 4).

21. Document D13 discloses S-S-stabilised Fv fragments in which an S-S bond has been introduced at one of the positions V_H44-V_L100, V_H44-V_L105 and V_H111-V_L48. This was shown to stabilise various Fvs while at the same time retaining full binding capacity and specificity. Recombinant immunotoxins comprising S-S-stabilised Fvs are reported to be indistinguishable in binding and specificity from their scFv-immunotoxin counterparts, and are more stable in human serum and more resistant to thermal and chemical denaturation than the scFv immunotoxin (see abstract and Figure 2).

22. Document D14 reports that Fv fragments are usually stabilised by being produced as an scFv in which the two chains are linked by means of a short polypeptide linker. An alternative strategy is to connect the two chains by means of an interchain S-S bond. Two sites identified for introducing the S-S bond are V_H44-V_L100 and V_H105-V_L43. Immunotoxins comprising the dsFv are found to be just as active as the scFv and considerably more stable. The authors expect the S-S bond at these sites to stabilise the Fv fragment of most antibodies and the antigen-specific portion of the T-cell receptors, which are homologous (see abstract).

23. In view of the above, documents D11 to D14 all relate to stabilising Fv and scFv - and not Fab - fragments by introducing an interdomain S-S bond at some of the claimed positions as an alternative to the peptide linker in an scFv molecule (see e.g. document D11, page 1001, left-hand column, last paragraph to right-hand column, paragraph 3).

None of these documents prompts the skilled person to introduce a V_H-V_L interdomain S-S bond into Fabs in order to increase their stability.

24. The question is whether a skilled person would have transferred the knowledge gained with S-S-stabilised Fv fragments to Fab molecules.

25. In this regard the board notes that the authors of document D7 were aware of Fvs stabilised by a disulphide bond - as evidenced by their reference 60 (document D11 in these proceedings). Nevertheless, after having performed extensive analyses of the domain interactions within Fab molecules, the authors suggest re-introducing the S-S bond at the C_H-C_L interface - not within the variable regions (see page 786, left-hand column, last paragraph).

26. As such, the board considers that, in the light of documents D7 and D11 to D14, the skilled person faced with the problem of providing alternative stabilised antibodies would not have modified the teaching in document D6 such as to arrive at the embodiment of the claimed invention being analysed here, i.e. Fab fragments (see point 7 above).

27. The board's reasoning above with respect to Fab fragments also applies to the claimed Fab' and $F(ab)_2$

fragments, since the Fab unit represents the essential component of both of these antibody fragments too.

28. Consequently, the subject-matter of claim 1 involves an inventive step (Article 56 EPC).

Reimbursement of the appeal fee

29. In its letter of 9 March 2020, the appellant made the following request for the first time in the appeal proceedings and without any further explanation: "*We also request reimbursement of the appeal fee*" (see page 7, last sentence before the signature).

30. It has been established by the jurisprudence in relation to claim requests, that any claim requests filed without substantiation should not be deemed submitted until the date they are substantiated unless the reasons for their filing are self-explanatory (see decision T 1732/10, point 1.5 followed by many other decisions; see Case Law of the Boards of Appeal, 9th edition 2019, V.A.4.12.5). Given that the purpose of substantiation is so that a board understands why a request has been submitted, the board considers that the case law referred to above is also applicable to requests for reimbursement of the appeal fee.

31. Since, in the case in hand, the request for reimbursement of the appeal fee was not substantiated either when it was made or later, and the reasons for its filing were not self-explanatory, this request does not form part of these appeal proceedings. As a consequence, the board need not decide on the matter.

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 for further prosecution.

The Registrar: The Chair:

L. Malécot-Grob G. Alt

Decision electronically authenticated