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D E C I S I O N
of 29 January 2004

Case Number: T 0886/00 - 3.3.4

Application Number: 89311731.7

Publication Number: 0368684

IPC: C07K 13/00

Language of the proceedings: EN

Title of invention:
Cloning immunoglobulin variable domain sequences

Applicant:
MEDICAL RESEARCH COUNCIL, et al

Opponent:
-

Headword:
Immunoglobulin variable domain sequences/MEDICAL RESEARCH COUNCIL

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (yes)"

Decisions cited:
G 0009/92, G 0004/93, G 0008/93, G 0001/99, T 0023/86, T 0856/92, T 0149/02

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0886/00 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 29 January 2004

Appellant I: (Proprietor of the patent)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 29 May 2000 concerning maintenance of European patent No. 0368684 in amended form.

Composition of the Board:

Chairwoman: U. Kinkeldey
Members: M. Wieser
R. Moufang

Summary of Facts and Submissions

I. The Patent Proprietors (Appellants I) and the Opponents (Appellants II) lodged appeals against the interlocutory decision of the Opposition Division on the amended form in which European patent No. 0 368 684 can be maintained.

II. The present decision refers to the following documents:

(5) Nature, vol. 302, 1983, pages 575 to 581

(8) Progr. in Biotechnology, vol. 5, 1988, pages 231 to 246

(9) WO-A-88/06 630

(13) J.Immunology, vol. 141, No. 6, 1988, pages 2063 to 2071

(14) WO-A-88/01 649

(15) Biochemistry, vol. 31, 1992, pages 1270 to 1279

(16) J.Mol.Biol., vol.265, 1997, pages 161 to 172

(33) Declaration Dr Rabbitts, filed 9 January 1998

(34) Declaration Prof. Plückthun, filed 19 March 1998

III. The patent had been opposed under Article 100(a) EPC on the ground of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC). After the expiry of the opposition period, lack of enablement of disclosure

(Article 100(b) in connection with Article 83 EPC) had been raised as a new ground of opposition.

IV. The Opposition Division (OD) had a main request, claims 1 to 32 as granted, and four auxiliary requests before them. Claim 1 of the main request read:

"A method of cloning sequences (target sequences) each containing a sequence encoding at least part of an immunoglobulin variable domain, which method comprises providing a sample repertoire of nucleic acid containing target sequences, and using forward and back primers in the process of copying and cloning of the target sequences for expression of a repertoire of proteins each comprising at least part of an immunoglobulin variable domain, the forward primer being specific for a sequence at or adjacent the 3'end of the sense strand of each of the target sequences, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of each of the target sequences."

V. The OD examined whether the new ground of opposition was *prima facie* relevant for the maintenance of the patent. They found that the term "immunoglobulin variant domain" was clear in the context of the patent and that the invention was disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. They decided not to allow the late filed ground according to Article 100(b) EPC into the proceedings (cf point (13) of the decision).

VI. The OD decided that claims 1 to 31 of the main request were novel according to Article 54 EPC, which was not disputed (cf point (16) of the decision). Furthermore, in point (21) of their decision they came to the conclusion that these claims involved an inventive step (Article 56 EPC). Document (8), which like the patent in suit, used the PCR technique for cloning of antibody sequences, was considered as closest state of the art. The OD found, that the problem underlying the patent, namely the provision of a method allowing the one-step cloning of a large number of different immunoglobulin variable domain sequences in a manner that their subsequent functional expression was possible, could not be derived in an obvious way, either from document (8) alone or in combination with one of documents (9), (13) or (14).

VII. Claim 32 of the patent as granted was found by the Opposition Division to lack novelty (Article 54 EPC).

VIII. Claims 1 to 31 of the first auxiliary request before the OD were identical to claims 1 to 31 of the main request. Claim 32 read:

"An expression library comprising a repertoire of nucleic acid sequences for expression of a repertoire of proteins each comprises an immunoglobulin variable domain."

The OD came to the conclusion that the requirements of Articles 123(2) and (3) EPC were met; in detail column 16, lines 5 to 18 of the original application was considered to be the basis for the amended claim (point (19) of the decision). Novelty (Article 54 EPC),

which was not disputed, was acknowledged in point (20) of the decision.

IX. However, the claim was found to lack an inventive step (Article 56 EPC) in the light of documents (9) and (14).

X. When considering claim 32 of the second auxiliary request before them, the OD came to the same result.

XI. Claim 32 of the third auxiliary request before the OD (claims 1 to 31 thereof were identical to claims 1 to 31 of the main request) read:

"An expression library comprising a repertoire of third CDR sequences, said sequences being located in an otherwise invariant VH gene."

The claim was found by the OD to be based on example 7 as originally filed and its scope was held to be limited compared to the scope of claim 32 as granted. Thus the requirements of Articles 123(2) and (3) EPC were met (cf point (26) of the decision). Novelty of the claim, which was not disputed, was acknowledged in point (27) of the decision. Finally, the OD decided that claim 32 of the third auxiliary request met the requirements of Article 56 EPC. Document (14) was considered as being the closest state of the art. The problem to be solved was defined as being the actual provision of an expression library. The solution claimed, i.e. the provision of an expression library comprising a repertoire of third CDR sequences, was not considered to be obvious, as no prior art document, special attention was paid to documents (5) and (9), provided evidence to choose one of the CDRs, let alone

especially the third one, as a basis for mutation (cf point (28) of the decision).

The OD decided to maintain the patent on the basis of the third auxiliary request.

XII. On 17 October 2003 the Appellants II withdrew their opposition.

XIII. The Appellants I requested as main request that the decision under appeal be set aside and that the patent be maintained on the basis of the following documents:

Description: Pages 3 to 5 and 7 to 27 as granted, and page 6, filed on 27 November 2003.

Claims: 1 to 31 as granted, 32 and 33 as filed on 27 November 2003

Figures: 1 to 9, 10a, 10b, 11 to 13, 14a, 14b, 15 to 20, 21a, 21b, 21c, 22 and 23 as granted.

Claim 32 of Appellant's I main request is identical to claim 32 of the first auxiliary request before the Opposition Division (see *supra* section VIII), claim 33 is identical to claim 32 of the third auxiliary request before the Opposition Division (see *supra* section XI).

XIV. The submissions by the Appellants I may be summarised as follows:

Claim 32 was not obvious over documents (9) and (14). Document (14) did not address the problem underlying

the patent in suit but was concerned with the improvement of the binding affinity of a single scFv to the same antigen, without contemplating changing the target antigen of said scFv. Document (9) was considered to be speculative and not enabling.

Reasons for the Decision

1. The appeal of the Appellants I (Patent Proprietors) meets the requirements of Articles 106 to 108, Rules 1(1) and 64 EPC and is thus admissible.

2. By withdrawing their opposition, and thereby their appeal, the Appellants II (Opponents) ceased to be a party to the appeal proceedings in respect of substantive issues (cf decision of the Enlarged Board of Appeal G 8/93 OJ EPO 1994, 887).

3. Claims 1 to 31 and 33 of Appellant's I main request are identical to claims 1 to 32 of the amended form in which the Opposition Division maintained the patent in suit (see sections IV, XI and XIII above).

4. In G 9/92 and G 4/93 (OJ EPO 1994, 875; confirmed in G 1/99, OJ EPO 2001, 381, point 4.1), the Enlarged Board of Appeal has decided that in cases where the patent proprietor is the sole appellant, the board may not challenge the maintenance of the patent as amended in accordance with the interlocutory decision ("die Fassung des Patents gemäß der Zwischenentscheidung in Frage stellen" in German and "contester le texte du brevet tel qu'approuvé dans la décision intermédiaire" in French). For example in decisions T 856/92

(8 February 1995) and T 149/02 (25 July 2003), the competent boards of appeal have relied on this holding when confronted with a request of the proprietor (and sole appellant) consisting partly of claims which were identical with claims accepted by the Opposition Division in its interlocutory decision maintaining the patent in amended form. They took the view that the boards had no power to challenge such identical claims (see T 856/92, point 2; T 149/02, point 2).

5. The present case does not require a decision as to whether the legal approach expressed in decisions T 856/92 and T 149/02 is to be followed, since the board agrees with the conclusions of the Opposition Division (see sections V, VI and XI above) relating to claims 1 to 31 and 33 of Appellant's I present main request and considers that these claims meet the requirements of the EPC. Thus, even if the principle of prohibition of *reformatio in peius* did not restrict the power of the board as much as suggested in the above-mentioned decisions T 856/92 and T 149/02, the board is not inclined to challenge the patentability of claims 1 to 31 and 33 of the present main request.

6. The board also sees no reason to diverge from the appealed decision in so far as it concluded that claim 32 of the present request (= claim 32 of the first auxiliary request before the Opposition Division, see section VIII above) met the requirements of Articles 123(2) and (3) and of Article 54 EPC. Therefore, the only remaining issue to decide is, whether claim 32 is based on an inventive step according to the requirements of Article 56 EPC, a

question which has been answered by the Opposition Division in the negative (see section IX above).

7. Claim 32 refers to an expression library comprising a repertoire of nucleic acid sequences. Said sequences, upon expression, result in a repertoire of proteins each comprising an immunoglobulin variable domain.

In accordance with the problem solution approach, the Boards of Appeal have repeatedly pointed out that the closest prior art for assessing inventive step is a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, i.e. the minimum of structural modifications (cf Case Law of the Boards of Appeal of the European Patent Office, 4th. edition, 2001, English version, page 102).

8. When applying these criteria the board comes to the conclusion that document (14) represents the closest state of the art.

This document refers to the production of single chain Fv antibody fragments (scFvs), which are proteins comprising the variable domains of the light and heavy chains of an antibody linked by a covalent linker. In order to improve the binding affinity of a single scFv, which may be produced according to example 2, document (14) suggests on page 67, lines 26 to 32 the following:

"Once the strain carrying the single chain building molecule gene has been constructed, the same can also be subjected to mutagenesis techniques using, chemical agents or radiation, as is well known in the art. From the colonies thus obtained, it is possible to search for those producing binding molecules with increased binding affinity."

This suggestion is not supported by a worked example.

9. The library according to claim 32 comprises a **repertoire** of nucleic acid sequences for expression of a **repertoire** of proteins each comprising an immunoglobulin variable domain.

10. The term **"repertoire"** is a term of art, whose meaning was discussed during the whole proceedings.

According to established case law of the boards of appeal, the description and the drawings, as understood by a skilled person helped by his technical knowledge, shall be used to interpret the claims (cf decision T 23/86, OJ EPO 1987, 316).

11. Two declarations of technical experts have been filed in this respect by the parties. Prof. Plückthun in document (34) agrees with the opinion expressed by Dr Rabbitts in document (33) saying that this term has to be understood as meaning "..a range of differing antibody specificities which approximates to or resembles that seen in an animal", but adds, that in his understanding the term is not limited to a collection of biomolecules found in nature, but could also mean ".. libraries of molecules with certain

biological activities or specifications that do not occur in nature ..", possibly created by recombinant DNA technology.

12. The Board agrees with the author of document (34) in so far as the term "**repertoire**" in claim 32, when interpreted in the light of the description can have both meanings. This position was also adopted by the Opposition Division in point (15) of their decision.

13. According to document (14) the polynucleotide sequences coding for scFvs are preferably expressed in transformed E.coli (see page 64 to 65 and examples). Other possible hosts are mammalian cells (page 66) and yeast cells (page 67). The only results of an expression experiment are presented in example 2, whose last sentences read:

"This plasmid was transformed into an E.coli host. The strain containing this plasmid has been induced, and the single chain protein produced as >2% of total cell protein."

According to page 67, the strain carrying the single chain building molecule gene is subjected to mutagenesis techniques using chemical agents or radiation.

If, as was convincingly argued by Appellants I, the E.coli genome is assumed to consist of approximately 4 million nucleotides in contrast to approximately 700 nucleotides coding for an immunoglobulin variable domain, then, statistically the probability is only 1/5000 that a mutation will occur in the region coding

for such domain. Thus, the vast majority of mutagenic events will be to the genome of the host cell or to the vector carrying the scFv gene. The probability of achieving a mutation in the single chain protein with the method suggested in document (14) may be even lower, as some of the mutations at the nucleic acid level will not result in amino acid changes due to the degeneracy of the genetic code.

The board concludes that a skilled person following the suggestion on page 67 of document (14) will not obtain an expression library according to claim 32.

14. Thus, document (14) is considered to define the wish to create a functional expression library only, without however providing the technical means to realize this goal.

The problem to be solved is considered to be the actual provision of such library. This problem has been solved by the patent in suit as shown in examples 1 to 7 by applying the method according to claim 1 (see section IV above).

In order to determine whether the actual provision of a library, whose production is suggested in theoretical terms on page 67 of document (14), is based on inventive step, it has to be asked if the skilled person would have derived the necessary technical means from the state of the art in an obvious way.

15. The Opposition Division considered document (9) to be a reliable source providing the technical information required. They concluded that the skilled person

starting with a scFv expressing strain of document (14) and aiming to produce therefrom an expression library according to claim 32, would have been able to solve this problem with a reasonable expectation of success by following the teaching of document (9).

16. Document (9) discloses the surface display of a single chain antibody domain (SCAD) as a fusion product with a protein normally appearing on the surface of an organism and random mutation of the expression product in order to obtain a repertoire of differing clones. The mutation step is described on page 3, lines 17 to 20 of the description, which reads: "The next step (step 1010) consists of generating, from the one SCAD displayed and encoded in the organism, a diverse population of SCADs by varying the DNA sequence encoding the SCAD by mutation techniques." Document (9) proposes on page 4 to replace the CDRs of SCAs by any constant residue. The only guidance given is, that the number of such residues can be determined by analysis of natural antibody sequences, computer modelling of the framework or by trial and error.

The only example on pages 7 and 8 is purely theoretical and refers to the production of a single SCAD in phage lambda. It is stated that phage lambda is assembled in the reducing environment of the cytoplasm of infected E.coli cells, where one would not expect disulfide bonds to form (page 7, lines 19 to 21). The importance of disulfide bonds formed by cysteine residues for the proper folding of functional antibody molecules is well known in the art and is acknowledged in document (9) on page 7, lines 23 to 24: "..., reduced cysteines will greatly destabilize folding of a SCA." In order to

solve this problem, document (9) proposes the following:

"Therefore, to get proper folding of SCAD inside a cell, one mutates the SCAD gene to change all or some of the CYS's to SER, THR, ALA or GLY" (page 7, lines 24 to 27).

17. However, there is evidence in the art published a number of years even after the priority date of the patent in suit, that following these instructions results in the formation of non-functional SCADs having lost their antigen-binding ability.

Document (15) discloses experiments wherein each of the cysteine residues of the variable domain of a specific Fv antibody fragment is replaced by a series of different amino acids and consequences for the expression in E.coli. The results showed that, while the wild type fragment behaved normally, none of the mutated single chain Fv fragments was detected in Western Blot experiments or isolated by affinity chromatography (page 1274, right column).

Document (16) reports that the stability of a single chain Fv fragment of a natural antibody missing a cysteine residue in the VH domain can be increased above that of other, unrelated scFv fragments, when the cysteine residue is reintroduced and the disulfide bridge thus restored.

18. In the light of this teaching in the post published art, the board concludes that the technique suggested by document (9), namely to change all or some of the

cysteine residues of a single chain antibody fragment, would merely generate a diverse population of non-functional sequences without functional binding domains.

Accordingly, the board is convinced that the theoretical disclosure of document (9) does not contain the technical information that would allow a skilled person to solve the underlying problem, namely to actually provide an expression library according to claim 32, a goal that has been already defined as wish in the closest state of the art document (14). To arrive at the claimed subject-matter, thus, was not obvious.

Since the required technical information is not contained in any other prior art document either, the subject-matter of claim 32 is considered to be based on an inventive step and to meet the requirement of Article 56 EPC.

Order

For these reasons it is decided:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the main request:

Description: Pages 3 to 5 and 7 to 27 as granted, page 6, filed on 27 November 2003;

Claims: 1 to 31 as granted, 32 and 33 filed on 27 November 2003;

Figures: 1 to 9, 10a, 10b, 11 to 13, 14a, 14b, 15 to 20, 21a, 21b, 21c, 22 and 23 as granted.

The Registrar: The Chairwoman:

P. Cremona U. Kinkeldey