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**D E C I S I O N**  
**of 3 May 2001**

**Case Number:** T 0669/97 - 3.3.4

**Application Number:** 85904274.9

**Publication Number:** 0194276

**IPC:** C12N 15/13

**Language of the proceedings:** EN

**Title of invention:**  
PRODUCTION OF CHIMERIC ANTIBODIES

**Patentee:**  
CELLTECH THERAPEUTICS LIMITED

**Opponent:**  
Aventis Pharma Deutschland GmbH  
Roche Diagnostics GmbH

**Headword:**  
Chimeric antibodies/CELLTECH THERAPEUTICS LTD

**Relevant legal provisions:**  
EPC Art. 123(2), 54, 83, 56

**Keyword:**  
"Main request - added matter - no"  
"Sufficiently disclosed - yes"  
"Novel -yes"  
"Inventive step - yes"

**Decisions cited:**  
T 0142/84, T 0002/83, T 0225/84, T 0532/88, T 0630/92,  
T 0971/92

**Catchword:**

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Case Number: T 0669/97 - 3.3.4

**D E C I S I O N**  
**of the Technical Board of Appeal 3.3.4**  
**of 3 May 2001**

**Appellants I:**  
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**Decision under appeal:** Interlocutory decision of the Opposition Division of the European Patent Office posted 24 April 1997 concerning maintenance of European patent No. 0 194 276 in amended form.

**Composition of the Board:**

**Chairman:** U. M. Kinkeldey  
**Members:** F. L. Davison-Brunel  
S. C. Perryman

## Summary of Facts and Submissions

I. The appeal lies from the decision of the Opposition Division issued on 24 April 1997 whereby the European patent No. EP-A-0 194 276 with the title "Production of chimeric antibodies" was maintained in amended form pursuant to Article 102(2) EPC.

Claims 1 and 10 as granted read as follows:

"1. A process for the production of a chimeric antibody comprising at least part of an Ig molecule and at least part of a non-Ig protein, in which both parts are capable of functional activity, the process comprising:

a) preparing an expression vector including a suitable promoter operably linked to a DNA sequence comprising a first part which encodes at least the variable region of the heavy or light chain of an Ig molecule and a second part which encodes at least part of a non-Ig protein;

b) transforming an immortalised mammalian cell line, which secretes an isolated Ig light or heavy chain respectively complementary to the part of the Ig molecule encoded by the first part of the vector prepared in step (a), with the prepared vector; and

c) culturing said transformed cell line to produce the chimeric antibody."

"10. A chimeric antibody comprising at least part of an Ig molecule and at least part of a non-Ig protein in which both parts are capable of functional activity, the antibody comprising:

a) a first polypeptide chain comprising: (i) at least the variable region of the heavy or light chain of an Ig molecule; and (ii) at least part of a non-Ig protein, **the parts (i) and (ii) being linked together by either a direct peptide bond or by an intervening peptide sequence;** and

b) a second polypeptide chain comprising at least the variable region of a complementary light or heavy chain respectively of an Ig molecule,

the first and second chains being associated together so as to form an antigen binding site."

(emphasis added by the Board)

Dependent claims 2 to 9 were directed to further features of the process of claim 1. Dependent claims 11 to 14 and 17 were directed to further features of the chimeric antibody of claim 10. Dependent claims 15, 16 and 18 were directed to various uses of said chimeric antibody.

II. The decision of the Opposition Division was appealed by the Patentees (Appellants I) and Opponents 2 (Appellants II). Opponents 1 also appealed the decision but withdrew their opposition with letter dated 17 May 1999. Opponents 3 are party to the proceedings as of right pursuant to Article 107 EPC.

III. The Board sent a communication under Article 11(2) of the rules of procedure of the Boards of appeal, summoning the parties to oral proceedings and setting out the Board's preliminary non-binding opinion.

IV. Appellants II and Opponents 3 informed the Board that they would not attend the oral proceedings.

V. Oral proceedings were held on 3 May 2001. Appellants I filed a new main request with 17 claims. Claims 1 to 9 were as granted. Claims 11 to 17 corresponded to granted claims 11 to 13, 15 to 18 respectively. Claim 10 corresponded to granted claim 14 and read as follows:

"10. A chimeric antibody comprising at least part of an Ig molecule and at least part of a non-Ig protein in which both parts are capable of functional activity, the antibody comprising:

a) a first polypeptide chain comprising: (i) at least the variable region of the heavy or light chain of an Ig molecule; and (ii) at least part of a non-Ig protein, **the parts (i) and (ii) being linked together by a specifically cleavable linker sequence;** and

b) a second polypeptide chain comprising at least the variable region of a complementary light or heavy chain respectively of an Ig molecule,

the first and second chains being associated together so as to form an antigen binding site."(emphasis added by the Board)

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

(5): WO 84/00382,

(6): Sharon, J. et al., Nature, vol. 309, pages 364 to 367, 24 May 1984,

(9): Boulianne, G. et al., Nature, vol. 312, pages 643

to 646, 3 December 1984,

(10): EP-A-0 120 694,

(19): Spooner et al., Molecular Immunology, Vol. 31,  
No. 2, pages 117 to 125, 1994.

VII. The arguments in writing and during oral proceedings by Appellants I insofar as they are relevant to the present decision are as follows:

*Article 123(2) EPC*

Support for the subject-matter of claim 10 could be found on page 5, 4th paragraph of the application as filed.

*Article 83 EPC*

Document (19) which, according to Appellants II, showed that the invention was not sufficiently disclosed should not be allowed into the proceedings for the following reasons:

- it was not accepted into the proceedings by the Opposition division for being late-filed and, thus, the arguments based on it were raised for the first time in Appellants II' appeal statement.
- it was published ten years after the priority date and, therefore, was not relevant to the situation at that date.

Were it to be accepted by the Board into the proceedings, it would not affect sufficiency of



disclosure. It described a process for expressing in mammalian cells a construct encoding a fusion protein between an immunoglobulin (Ig) heavy chain and ricin A. It showed that the expression of said construct led to cellular death, which implied that the ricin part of the fusion protein was active. This result did not lead to the conclusion that chimeric Ig-ricin fusion proteins could not be produced. The skilled person would know how to express the chimeric gene in a controlled manner so as to obtain the fusion protein, that is, to grow the cells to the required density under such conditions that said chimeric gene would not be expressed and, then, to change the growth conditions so as to express it.

Document (15) disclosed that fusion proteins comprising an Ig chain and alpha-galactosidase could not be obtained by transfection of mammalian cells with the corresponding chimeric DNA constructs. The author identified the reason therefor as being a very poor transfection efficiency of said construct but did not pursue the experiment. This was, thus, an occasional failure. Document (15) did not provide satisfactory evidence that the invention as claimed in claim 1 could not be carried out. In contrast, the patent in suit provided three examples of the production of active Ig-nonIg chimeric molecules.

Appellants II listed a number of cases where one would not expect chimeric Ig molecules to be recovered in active form but no examples were provided. Mere allegations did not discharge the burden of proof placed on the Opponent to show that in these cases, functional chimeric Igs could not be produced.

At the priority date, immortalized cell lines producing no light chain or no heavy chain were readily available as shown in document (6) and document (9), respectively.

*Article 54 EPC*

Document (6) did not disclose a process for producing by recombinant DNA (rDNA) techniques, a chimeric **DNA molecule** comprising at least part of an Ig molecule and at least part of a non-Ig protein. The last paragraph in the document was only concerned with devising chimera between Ig and non-Ig **proteins** by chemical linking of both types of proteins. Document (6) was not detrimental to the subject-matter of claim 1.

Document (10) was not detrimental to the novelty of claim 10 under Article 54(3)(4) EPC as it did not disclose a chimeric Ab comprising an Ig part and a non-Ig protein **linked together by a specifically cleavable linker sequence**.

*Article 56 EPC*

The closest prior art was document (6) which described the production by rDNA techniques of a chimeric Ig in mouse myeloma cells. On page 367, it was stated: "It would be interesting to compare X-ray crystallographic studies of the  $V_H C_{\alpha} - V_{\alpha} C_{\alpha}$  dimer... Such structural studies might provide insights into designing antibody molecules consisting of  $V_H$  and  $V_L$  attached to other molecules of interest such as enzymes and toxins." Thus, the objective problem to be derived from document (6) was clearly how to design antibody (Ab) molecules

attached to other proteins of interest. The fact that document (6) plainly stated that this was to be carried out on the basis of the crystal structure of the  $V_H C_{\delta} - V_{\delta} C_{\delta}$  dimer implied that the solution set forth in document (6) involved the chemical linking of the toxin or enzyme to the Ig molecule. There was no suggestion at all in document (6), of using rDNA technology to produce the chimeric molecule.

VIII. The arguments submitted by the other parties in writing, insofar as they are relevant to the present decision, are essentially as follows:

*Article 83 EPC*

Document (19) published ten years after the priority date showed that a process according to claim 1 applied to the production of a chimeric Ig-ricin protein did not in fact lead to any chimeric protein being obtained wherein the ricin part had retained activity. In the same manner, document (15) taught that Ig-alpha galactosidase fusion proteins could not be produced in mammalian cells. Furthermore, it was to be expected that fusion proteins which comprised a non-Ig part known to be lethal to mammalian cells on its own, would equally be lethal to mammalian cells. Accordingly, the process of claim 1 could not be reproduced over the whole scope of the claim.

At the priority date, there was no examples in the prior art of a generally available cell line producing an Ig heavy chain only.

*Article 54 EPC*

Claim 1 lacked novelty over document (6) as all the features of the claimed process were already disclosed in said document. While the disclosure content of document (6) was directed to making chimeric antibodies, there was no reason to confine this technology to said chimeric antibodies. The skilled person would rather have understood that it could be applied to the production of other types of chimera in view of the disclosure on page 367, 3d paragraph that: "Such structural studies might provide insights into designing antibody molecules consisting of  $V_H$  and  $V_L$  attached to other molecules of interest such as enzymes and toxins."

Document (10) was detrimental to the novelty of a claim to a chimeric antibody wherein the Ig part of the molecule was said to be linked to the non-Ig part of the molecule by an intervening sequence.

*Article 56 EPC*

Document (6) disclosed a process for the production of chimeric Abs having the same features as those in claim 1 of the contested patent with the possible exception that a chimeric Ab containing a non Ig protein in addition to the  $\hat{e}$  light chain constant region was not actually produced.

The technical problem underlying claim 1 could be seen as producing a chimeric Ab wherein the non-Ig protein is added to the  $\hat{e}$  constant domain in the chimeric polypeptide.

There could be no doubt that document (6) was concerned with obtaining chimeric Ig-Ig molecules by means of

recombinant DNA (rDNA) technology. Furthermore, it was concluded on page 367 that one could design antibody molecules that "consist of  $V_H$  and  $V_L$  attached to other molecules of interest such as enzymes and toxins." Starting from this teaching, it was obvious to try to produce these latter chimeric Ig-nonIg molecules by the rDNA technique and the skilled person had a reasonable expectation of success to produce them. Thus, the subject-matter of claim 1 could hardly be inventive.

IX. Appellants I requested as main request that the decision under appeal be set aside and that the patent be maintained on the basis of claims 1 to 17 and page 5 of the description as submitted at the oral proceedings on 3 May 2001 and pages 3,4 and 6 to 9 of the description and the Figures as granted or as auxiliary request that the appeal of Appellants II be dismissed.

Appellants II requested that the decision under appeal be set aside and the patent be revoked.

## **Reasons for the Decision**

### *Main request*

#### *Article 123(2); claim 10*

1. Claim 10 corresponds to granted claim 14 when dependent on granted claim 10. The subject-matter of the claim finds support on page 3, line 20 to page 6, line 5 of the published version of the application as filed, a specifically cleavable linker sequence between the Ig and non Ig parts of the chimeric Ab being specifically disclosed on page 5, lines 18 to 22. The requirements of Article 123(2) EPC are fulfilled.

*Article 83 EPC in relation to the subject-matter of claim 1:*

2. In the patent in suit, examples are given that functional Ig-nonIg proteins may be obtained by rDNA techniques. The feasibility of reproducing these examples was not challenged. Appellants II instead sought to show that the requirements of Article 83 EPC were not fulfilled over the whole scope of the claim by providing specific examples whereby, in their opinion, the claimed process failed to lead to the production of functional chimeric Ig-nonIg proteins. They cited documents (19) and (15).
  
3. Document (19), published some ten years after the priority date, was submitted at the appeal stage. In accordance with the case law of the Boards of Appeal (inter alia, T 142/84, OJ EPO 1987, 112), the main criterion for deciding whether a late filed citation mentioned for the first time in appeal proceedings should be taken into account is its relevance. The teachings of document (19) appear to be that some ten years after the priority date, the process of claim 1 could not be carried out for the production of functional Ig-ricin A fusion proteins. Prima facie, this teaching is relevant to sufficiency of disclosure. In addition, since sufficiency of disclosure must exist from the day, and at any time after, a patent application has been filed, the date when experiments were carried out is not important as such. Only the validity of the experiments and whether insufficiency can be deduced from them is relevant. Document (19) is, thus, accepted into the proceedings as being relevant.
  
4. Document (19) discloses the isolation of a chimeric DNA construct encoding an Ig heavy chain-ricin A fusion

protein and its transfection into a light chain secreting mouse plasmacytoma cell line. The only transfectants obtained are those where the transfected ricin A gene has been genetically inactivated. The authors conclude: " antibody-ricin A chain fusions intoxicate mammalian cells that express them... Whilst **this means that production of recombinant ricin A chain based Igs in mammalian cells is not feasible**, it is encouraging that ricin A chain retains its toxicity as part of an antibody fusion protein."

5. Appellants I, however, challenged this conclusion. In their written submissions dated 6 July 1998, they argued that methods were known at the priority date for the production of molecules likely to kill the cells in which they were expressed, such methods involving a controlled expression of the "lethal genes", the transcription of which would only be triggered after a sufficient amount of cells had been obtained. The other parties failed to present any counter-arguments or experiments to substantiate insufficiency. In the absence of any evidence to the contrary, the Board accepts Appellants I' argument as plausible and concludes that the teaching of document (19) is not detrimental to sufficiency of disclosure.
  
6. Appellants II cited a number of other proteins likely to be lethal to mammalian cells arguing that the corresponding Ig chimeric proteins would equally be lethal. Without any experimental evidence being produced, the Board considers this statement as a mere allegation which is not sufficient to destroy sufficiency of disclosure. And, besides, Appellants I' argument with regard to the feasibility of producing the Ig-ricin A protein (see point 5 above) would also

apply to the production of other potentially lethal chimeric molecules.

7. Document (15) is concerned, in particular, with the expression in mammalian cells of Ig- $\alpha$  galactosidase protein fusions. It is stated on page 85 that this specific chimeric protein could not be produced in mammalian cells because the transfection efficiency of the corresponding chimeric DNA into said cells was too low. The authors suggest that further transfection experiments should be carried out to show whether or not it is possible to produce it. Thus, the observed lack of transfection is to be seen as an occasional failure and the ensuing negative result with regard to protein production does not allow any conclusion as to the feasibility of obtaining active Ig- $\alpha$  galactosidase molecules. Document (15) does not allow the conclusion that the disclosure of the patent is insufficient.
  
8. Finally, it was argued that mammalian cell lines necessary to carry out the process of claim 1 were not available at the priority date. Document (6), page 364, right-hand column and document (9), page 643, right-hand column, respectively, provide evidence that mutant cell lines encoding the Ig light chain (J558L) or the Ig heavy chain (igk14) were available at the priority date. It was argued by Appellants I in their written submissions dated 6 July 1998 that the ordinary skilled person was able at the priority date to isolate a desired chain loss variant from a hybridoma cell line. In the absence of any evidence to the contrary from the other parties, it is concluded that mammalian host cells necessary to carry out the process of claim 1 were available at the priority date.



9. For the reasons mentioned in points 2 to 8 supra, the Board accepts sufficiency of disclosure.

*Article 54 EPC*

*Claim 10*

10. Document (10) (EP-A-0 120 694) in the name of the same Patentee as the patent-in-suit was cited under Article 54(3)(4) EPC against the novelty of granted claim 10. There is no evidence that this patent document is not enabling with regard to a process for the production of chimeric Igs in yeasts (see also decision T 400/97 of 23 May 2000). It discloses in a generic manner chimeric Igs having a non-Ig peptide moiety attached to the Ig part of the molecule (claim 33, page 10, lines 1 to 15 of the published version of the application as filed). Yet, there is no disclosure of the production of chimeric Ig molecules, both parts of which are linked by a sequence which is specifically cleavable. Accordingly, document (10) does not destroy the novelty of claim 10 of the main request now on file which is directed to such chimeric Igs.
11. Document (6) explicitly discloses a recombinant DNA process for the expression of a **chimeric Ig** in mouse myeloma cells but it does not explicitly disclose how to produce **chimeric Ig-nonIg molecules**. In fact, chimeric Ig-nonIg molecules are solely mentioned in the penultimate paragraph of the document where it is observed that useful information can probably be drawn from X ray crystallographic studies on chimeric Igs for designing chimeric Ig-nonIg molecules. However, the paragraph is silent as to which process to use to produce the Ig-nonIg molecules once they have been designed. Document (6) is not novelty destroying to the

subject-matter of claim 1.

12. Novelty over documents (10) and (6) can, thus, be acknowledged.

*Article 56 EPC*

*Claims 1 to 3*

13. It was argued that the problem of providing a rDNA process for producing Ig-nonIg chimeric molecules could be derived in an obvious manner from document (6). This document discloses producing a functional dimer between the variable region of a mouse heavy chain and the constant region of a mouse  $\epsilon$  light chain. The chimeric Ig is obtained by transfection with a recombinant vector expressing the chimeric DNA encoding  $V_H$  and  $C_{\epsilon}$ , of mouse myeloma cells which secrete only a  $\kappa$  light chain. In the penultimate paragraph it is disclosed that: "It would be interesting to compare X-ray cristallographic studies of the  $V_H C_{\epsilon}$ - $V_{\epsilon} C_{\epsilon}$  dimer and of the Fab fragment of the 36-65 protein. The dimer may be a good candidate for crystallisation because of its increased symmetry compared with Fab fragments ... Such structural studies might provide insights into designing antibody molecules consisting of  $V_H$  and  $V_L$  attached to other molecules of interest such as enzymes and toxins."
14. In the Board's judgment, this penultimate paragraph is concerned with further studies of the structure of proteins, whether it be the structure of the  $V_H C_{\epsilon}$ - $V_{\epsilon} C_{\epsilon}$  dimer as obtained by X-ray crystallography or the potential structures to be given to chimeric Ig-nonIg proteins, these latter being devised on the basis of the knowledge acquired on the dimer. The authors do not

suggest any way of producing the Ig-nonIg proteins once their structures have been defined. At the priority date, chimeric Ig-nonIgs were produced at the protein level by covalently linking the two parts of the chimera (document (5), pages 5 and 6). Thus, it is only with hindsight knowledge of the present invention that the penultimate paragraph may be combined with the results section relating to the production of Ig-Ig chimeras, in order to reach the conclusion that document (6) suggests producing Ig-nonIg molecules by a rDNA process. Document (6) does not constitute a satisfactory start point from which to define the problem solved by the subject-matter of claim 1.

15. Document (5) discloses the isolation of a chimeric Ig-nonIg molecule: TA-1-ricin A and, thus represents the closest prior art. The isolation process is a process of organic chemistry, by which a covalent link is introduced directly between the two parts of the chimeric protein.
16. Starting from the closest prior art, the problem to be solved can be defined as the provision of an alternative method of producing functional chimeric Ig-nonIg proteins.
17. The solution provided by each of claims 1 to 3 is a biological process where the link between the two parts of the protein is obtained indirectly by firstly joining together the DNAs encoding them and, secondly, expressing the hybrid DNA thus formed in the relevant host to produce the chimeric protein. From the examples provided in the patent specification, the Board is satisfied that functional chimeric Ig-nonIg molecules have been obtained.

18. In the Board's judgment, the skilled person would not have thought of combining the teaching of document (5) with that of document (6) or of any other documents on file to get to the above mentioned solution because document (5) is only concerned with the specific TA-1-ricin A molecule and does not suggest that the method of organic chemistry might be unsatisfactory. If, nonetheless, documents (5) and (6) were simultaneously taken into consideration, then, the provided solution would still remain unobvious. The teaching of document (6) is that binding the V part of an immunoglobulin molecule to a C part of another Ig molecule does not alter the functional properties of said V part. It is not possible to derive therefrom any expectations that a protein whose functional properties are different from that of an immunoglobulin (for example an enzyme) would still be active as part of an Ig chimera. The rDNA processes of claims 1 to 3 are inventive.

*Claim 10*

19. This claim is addressed to a chimeric antibody wherein the two parts are linked together by a specifically cleavable linker sequence. There is no evidence on file that such a molecule could be obtained by any other means than the inventive processes of claims 1 to 3. The subject-matter of claim 10 is, thus, also regarded as inventive.
20. The requirements of Article 56 EPC are fulfilled.

**Order**

**For these reasons it is decided that:**

1. The decision under appeal is set aside.
2. The matter is remitted to the first instance with the order to maintain the patent on the basis of the main request submitted at the oral proceedings on 3 May 2001.

The Registrar:

The Chairwoman:

U. Bultmann

U. Kinkeldey