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D E C I S I O N
of 11 November 1997

Case Number: T 0510/92 - 3.3.4

Application Number: 84102712.1

Publication Number: 0121157

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Language of the proceedings: EN

Title of invention:
Novel human interferon-gamma polypeptide

Patentee:
KYOWA HAKKO KOGYO CO., LTD., et al

Opponent:
Boehringer Ingelheim GmbH

Headword:
Gln^{9}-interferon variant/KYOWA HAKKO KOGYO

Relevant legal provisions:
EPC Art. 54(3), (4), 56

Keyword:
"Inventive step (no)"

Decisions cited:
T 0233/90, T 0153/95, T 0495/92

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0510/92 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 11 November 1997

Appellant: (Opponent)
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Representative:
-

Respondent: (Proprietor of the patent)
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Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office dated 10 April 1992 concerning maintenance of European patent No. 0 121 157 in amended form.

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: R. E. Gramaglia
W. Moser

Summary of Facts and Submissions

I. European patent No. 0 121 157 was granted with the title "Novel human interferon-gamma polypeptide" with eight claims based on European application No. 84 102 712.1.

Claim 1 read as follows:

"A recombinant plasmid containing a tryptophan promoter and downstream thereof a DNA fragment encoding a human interferon-γ polypeptide having the amino acid sequence illustrated in Fig. 3"

Claim 2 related to specific recombinant plasmids and claims 5 to 8, to microorganisms containing these plasmids. Claims 3 and 4 related to a process for producing a human interferon-γ polypeptide whereby the polypeptide is expressed from the recombinant plasmids of claim 1 or 2.

II. A notice of opposition was filed. Grounds were presented for the revocation of the patent in its entirety under Article 100(a) EPC (lack of novelty, lack of inventive step) and Article 100(b) EPC (insufficiency of disclosure), relying inter alia on the following documents:

(1): EP-A-0 112 976
(3): Gray et al., Nature, volume 295, pages 503 to 508 (1982)
(5): Derynck et al., Nucl. Acids Res., volume 10, number 12, pages 3605 to 3615 (1982)

(7): Nishi et al., J. Biochem., volume 97, pages 153 to 159 (1985)

III. At the beginning of oral proceedings before the opposition division, the request to revoke the patent for insufficiency of disclosure (Article 100(b) EPC) was withdrawn.

The Opposition Division considered the requirement for novelty to be fulfilled because documents (1) and (3) even if taken together as a single document did not unavoidably lead to the subject-matter of the patent in suit.

Document (3) was retained as closest prior art as it disclosed an interferon-γ which differed from the wild-type interferon-γ in that it contained Gln rather than Arg in position 140.

Starting from this prior art, the opposition division held the view that the underlying technical problem was to be seen in preparing a second generation interferon-γ with improved biological properties and that the solution consisting in the interferon-γ of claim 1 which differed from the wild type interferon by the presence of Gln at position 9 satisfactorily solved that problem.

At the date of filing of the application the state of the art had not convincingly established the possible existence of a polymorphism of interferon-γ. Nor did it contain any suggestion as to the particular allelic variant of the patent in suit. Furthermore, the

properties of this variant tended to be slightly better than that of the wild-type interferon. For all these reasons, inventive step could be acknowledged.

The requirements for patentability were, thus, fulfilled for claims 1 to 8 submitted on 26 October 1990. This set of claim differed from the granted one in that in claim 3, the reference to claim 2 had been replaced with a reference to "plasmid pGKA-2 (FERM BP-496)" to be found in claim 2 as granted.

IV. The Appellant (Opponent) lodged an appeal against the decision of the opposition division, paid the appeal fee and filed a statement of grounds of appeal.

V. The Respondent (Patentee) filed a reply in answer to the statement of grounds of appeal.

VI. A communication was sent by the Board according to Article 11(2) of the Rules of procedure of the Boards of appeal setting out the Board's preliminary position.

VII. The Appellant informed the Board that he would not take part in the oral proceedings and requested that a decision be taken according to the state of the file.

VIII. The Respondent withdrew his request for oral proceedings.

IX. The submissions in writing by the Appellant can be summarized as follows:

- The assessment of novelty required that document (1) should be taken into account together with document (3) which was specifically mentioned in document (1). Thus, the teaching of the prior art was that a recombinant plasmid could be isolated which carried a DNA sequence encoding Gln^{9}-interferon-γ and, by reference to document (3), that the trp promoter was the promoter of choice to express this DNA sequence in *E. coli*. Accordingly, document (1) was novelty destroying for the subject-matter of claim 1.

- The two experiments provided by the Respondent in the course of examination to allegedly show that the claimed Gln^{9}-interferon-γ variant had unexpected properties when compared with the wild type interferon were not in any way conclusive:

- The first experiment was carried out to compare the specific activities of Gln^{9}- and wild-type interferons at 37°C. The second experiment purportedly showed the activation of both interferons-γ by trypsin at low concentration. In both cases, the specific activity of each interferon was calculated as the average between three values obtained from three independent measurements of antiviral activity. Yet, these values were so widely scattered in each case that making an average out of them could not be meaningful, nor, of course, could a comparison between these averages be meaningful.

- A further evidence of the unreliability of the

data was that the specific activity of either of the interferons at 0°C (control experiment) did not remain the same in both experiments.

- Finally, the specific activities obtained in experiment 2, if taken at their face value, would be fully expected as the wild-type interferon-γ contained one more site for trypsin cleavage than Gln^{9}- interferon-γ.

X. The Respondent replied as follows:

- Document (1) only contained a reference to document (3) in the background section of the description and could not be considered as suggesting that the Gln^{9}-interferon-γ must be expressed in the *E. coli* expression system disclosed in document (3). The subject-matter of the claims of the patent in suit was, thus, novel.

- The variations between the lowest and highest values in the independent measurements of antiviral activity were large but this did not only affect the calculation of the specific activity of the Gln^{9}-interferon-γ but also that of the Lys^{9}-interferon-γ. Although they existed, it was clear that Gln^{9}-interferon-γ showed superior antiviral activity over Lys^{9}-interferon-γ in the presence of serum at 37°C.

- The two control experiments were not run under the same experimental conditions which explained why

the specific activity of each interferon did not remain constant at 0°C.

- It was not foreseeable if and how the loss of a cleavage site would influence the specific activity of interferon-γ. It all depended on whether this cleavage site was situated in the active site of the molecule. Thus, the results obtained at various trypsin concentrations could not have been expected.

XI. The Appellant requested that the decision under appeal be set aside and that the patent be revoked for lack of novelty and inventive step. The Respondent requested implicitly that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

Novelty (Article 54(3) and (4) EPC)

2. The document of relevance to novelty under Article 54(3) and (4) EPC is document (1) which discloses a recombinant plasmid containing the SV40 early promoter and downstream thereof a DNA fragment encoding a human interferon-γ with the same sequence as reported for the interferon-γ of present claim 1 (Gln^{9}-interferon-γ). In the description, page 2, reference is made to document (3) as reporting "the cloning and expression of an IFN-γ cDNA in Escherichia coli ...". In fact, document (3) discloses a recombinant plasmid

containing the trp promoter and downstream thereof a DNA fragment encoding the human Gln^{140}-interferon-γ.

3. According to the case law of the Boards of appeal, combining documents of the state of the art is not permissible when considering novelty, except in very specific circumstances (T 0233/90 of 8 July 1992; T 0153/85, OJ EPO 1988, 1). Thus, in T 0153/85, the Board found that "where there is a specific reference in one prior document ("the primary document") to a second prior document, when construing the primary document (i.e. determining its meaning to the skilled man) the presence of such specific reference may necessitate that part or all of the disclosure of the second document be considered as part of the disclosure of the primary document.

4. In the present case, the Board is of the opinion that the knowledge of document (3) is irrelevant when it comes to determining what is meant in document (1) because this latter document is not concerned with providing a generic teaching on the isolation of recombinant plasmids containing a promoter and, downstream thereof, a DNA fragment encoding any interferon-γ, but rather with the isolation of a specific recombinant plasmid containing the SV40 early promoter together with the specific Gln^{9}-interferon-γ. Thus, the combination of documents (1) and (3) is irrelevant to the novelty issue dealt with here.

5. Document (1) discloses a recombinant plasmid which differs from the claimed plasmids in that it does not contain the trp promoter. None of the further documents

on file discloses the constructs as claimed. Novelty is thus acknowledged.

Inventive step (Article 56 EPC)

6. The closest prior art is document (3) which discloses a recombinant plasmid containing the trp promoter and downstream thereof a DNA fragment encoding a variant of the wild-type human interferon-γ. This variant which differs from the wild-type interferon by the presence of Gln instead of Arg at position 140 is produced in *E. coli* containing the recombinant plasmid.

Starting from this prior art, the underlying technical problem may be seen in the provision of an alternative interferon-γ.

The solution is provided by claim 1 and consists in a recombinant plasmid containing the trp promoter and downstream thereof a DNA fragment encoding the Gln^{9}-interferon-γ. The amino acid sequence depicted in Figure 3 differs from that of the wild-type interferon-γ by substitution of Lys at position 9 with Gln.

From reading example 3 of the patent specification, the Board is satisfied that Gln^{9}-interferon-γ is produced in *E. coli* containing the recombinant plasmid.

In the Board's view, the coupling of the DNA fragment encoding the interferon-γ variant to the trp promoter to ensure expression in *E. coli* as such cannot be considered inventive since that very same coupling has

been used in document (3) to the same effect. Neither can the provision of a further variant as such be inventive in the light of the fact that already document (3) shows that there are variants of interferon-γ.

Thus recognition of inventive step would require that the particular variant Gln^{9} can be found non obvious.

A reason for inventive step submitted by the Respondent was that the claimed Gln^{9}-interferon-γ had unexpected better activity.

The question of the inventive step of the Gln^{9}-interferon-γ variant has already been dealt with in case T 0495/92. The experimental evidence then submitted to demonstrate that the Gln^{9} variant had unexpected properties is the same as the one now on file. These facts have already been pointed out by the Board in the communication accompanying the summons to oral proceedings, so that the requirements of Article 113(1) EPC have been complied with.

7. Two experiments are submitted by the Respondent to show that Gln^{9}-interferon-γ has unexpected and advantageous properties. In both experiments, the biological activity tested is defined as the average between three independent measurements.

Experiment 1 compares the biological activity of the Gln^{9}-interferon-γ variant with that of wild-type interferon-γ under physiological conditions, ie, after incubation for 24 hours at 37°C. The Gln^{9} variant turns out to be 14% more active than the wild-type. Experiment 1 also shows that Gln^{9}-interferon-γ is 50% more active at 37°C than at 5°C, while the activity of the wild-type remains the same at both temperatures.

Experiment 2 is designed to compare the susceptibility of the Gln^{9} variant and the wild-type interferon-γ to trypsin activation. Both interferons are incubated with various amounts of trypsin before their biological activity is measured. It is found that Gln^{9}-interferon-γ is 27%, 6.5% or 18% more active than the wild-type at trypsin concentrations of 1/100, 1/200 and 1/400, respectively. At a trypsin concentration of 1/50, Gln^{9}-interferon-γ was 51% less active than wild-type interferon-γ.

8. In the Board's view, if recognition of inventive step is to be based on results of a comparison of the average biological activities of the Gln^{9} and wild-type interferons, these activities have to be intrinsically meaningful.

9. In Experiment 1, the values on which the average biological activity of the Gln^{9} and wild-type interferons is based are as follows: Gln^{9} variant: 248.3, 230.9, 284 U/ml; wild-type: 234.0, 233.8, 205.7 U/ml. Thus in each case, two of the experimental values are practically identical whereas the third one is

remarkably high (Gln^9 variant: 284) or remarkably low (wild-type: 205.7).

10. It is to be expected that independent experimental measurements of biological activity lead to different results. Biological assays are intrinsically variable because they involve live material, the behaviour of which is hardly exactly reproducible. This inherent characteristic of biological assays can, however, be taken care of in a standard manner in order to produce significant biological data, which is to repeat the assays and to discard any "stray value" which may occur.

11. It is apparent from the way the average values have been calculated in the experiments provided by the Respondent that the 14% increase in activity of the Gln^9 variant over the wild-type interferon is solely based on the experimental value for each interferon which obviously strays from the values otherwise obtained. The Board is not convinced by the argument of the Respondent that because the scattering of the experimental values used to determine the average biological activities occurs in the same manner for both the wild-type and variant interferons, it should not be given any importance. In the Board's view, if stray values are not eliminated in the calculation of averages, these averages are objectionable and their comparison meaningless.

12. The statistical data carried out and submitted by the Appellant at oral proceedings before the Opposition Division (Annexes 3 to 5) and unchallenged by the

Respondent indicate that many convergent measurements would be necessary to make 14% difference statistically relevant. The Respondent himself recognises in a post-published paper (document (7), page 159) that "the conversion from Lys to Gln at amino acid position 9 might not affect the specific activity of the HuIFN-γ".

13. The Respondent emphasizes that Gln^{9}-interferon-γ appears to be 50% more active after 24 hours at 37°C than after 24 hours at 5°C. This result is, however, also obtained by comparing averages calculated from too few and too far apart experimental values. Accordingly, by the same rationale as given above, the experiment does not show that Gln^{9}-interferon-γ has unexpected and advantageous properties.

14. For these reasons, the Board is not convinced by the results of Experiment 1 with regard to an enhanced biological activity of the variant compared to the wild-type.

15. Experiment 2 purportedly shows the activation of both interferons by trypsin at low concentrations.

16. Some determinations of average biological activity are fairly homogeneous: for example, the biological activity of the wild-type interferon at a trypsin concentration of 1/200 (1240.6 U/ml) is calculated from three independent measurements which vary by 4.7% at the most (1271.8 and 1214.5). Others, however, are widely scattered: the biological activity of Gln^{9}-interferon-γ at a trypsin concentration of 1/100 (887.7

U/ml) is calculated from three independent measurements which vary by as much as 39% (760.2 and 1059.3 U/ml), that of the wild-type interferon at a trypsin concentration of 1/400 (1309.2 U/ml) derives from measurements which vary by as much as 56% (1004 and 1566 U/ml).

17. As in Experiment 1, the interpretation of the data did not involve the elimination of the stray values. Moreover, too few repeats of each measurement were performed for the difference observed between average biological activities calculated therefrom to be significant.

18. Accordingly, the Board cannot find Experiment 2 any more conclusive than Experiment 1 as to the improved biological properties of Gln^{9}-interferon-γ compared to the wild-type interferon.

19. The Respondent has pointed out to the Board that there is a definite albeit small tendency for Gln^{9}-interferon-γ to show a better biological activity in the presence of trypsin at low concentrations. In his opinion, this effect should be acknowledged as unexpected even if the experiment was not sufficiently repeated to the extent that a statistical analysis could be performed. However, it is apparent from the results obtained that the more trypsin is added, the less active are the interferons (887, 1240 and 1553 U/ml at trypsin concentration of 1/100, 1/200 and 1/400 respectively). This observation is in direct contradiction with the knowledge that trypsin activates interferons. Thus, the biological meaning of the data is quite unclear and not conducive to drawing any conclusion as to the

properties of Gln^{9}-interferon-γ.

20. Thus, an unexpected improvement of Gln^{9}-interferon-γ, which would have supported an inventive step, given the fact that the provision of an alternative interferon-γ as such was obvious (see point 6 supra), cannot be seen.

21. Accordingly, the Board decides that inventive step may not be acknowledged to the subject-matter of any of claims 1 to 8 filed on 26 October 1990.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairwoman:

D. Spigarelli U. Kinkeldey