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Datasheet for the decision of 24 April 2015

Case Number: T 0500/12 - 3.3.08

Application Number: 05700620.7

Publication Number: 1716233

IPC: C12N15/10, C12N15/62, C12N15/65, C12N5/10, C12Q1/68

Language of the proceedings: EN

Title of invention:
REGULATED STOP CODON READTHROUGH

Patent Proprietor:
Maxygen Holdings Ltd.
Maxygen Aps

Opponent:
Novartis AG

Headword:
Aminoglycoside antibiotic stop codon modulator suppressor/ MAXYGEN

Relevant legal provisions:
EPC Art. 56
EPC R. 84(1)

Keyword:
Request for continuation of appeal proceedings (granted)
Sole request - Inventive step (no)

Decisions cited:

Catchword:

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Case Number: T 0500/12 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 24 April 2015

Appellant: (Opponent)	Novartis AG Lichtstrasse 35 4056 Basel (CH)
Representative:	Roth, Carla König-Szynka-Tilmann-von Renesse Patentanwälte Partnerschaft mbB Postfach 11 09 46 40509 Düsseldorf (DE)
Respondent: (Patent Proprietor 1)	Maxygen Holdings Ltd. c/o Close Brothers (Cayman) Limited, 103 South Church Street, P.O. Box 1034 GT Grand Cayman (KY)
Respondent: (Patent Proprietor 2)	Maxygen Aps Agern Allé 1 2970 Hoersholm (DK)
Representative:	Hallybone, Huw George Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 13 December 2011 concerning maintenance of the European Patent No. 1716233 in amended form.**

Composition of the Board:

Chairman	M. Wieser
Members:	P. Julià
	D. Rogers

Summary of Facts and Submissions

I. European patent number 1 716 233 was maintained by the opposition division in amended form in accordance with an Auxiliary Request 2. The opposition division considered the Main Request and Auxiliary Request 1 not to fulfil the requirements of Article 54 EPC. All requests were filed at oral proceedings before the opposition division on 1 November 2011.

II. An appeal was lodged by the opponent (appellant). In the statement setting out its Grounds of Appeal, the sole argument put forward by the appellant concerned a lack of inventive step of the request upheld by the opposition division (Article 56 EPC). As an auxiliary measure, oral proceedings were requested.

III. In reply to appellant's Grounds of Appeal, the respondents (patentees) referred only to their arguments submitted with a letter dated 28 February 2011 in reply to opponent's Notice of opposition. A copy of this letter was enclosed to the respondents' submissions. The respondents requested oral proceedings as an auxiliary measure.

IV. In a letter dated 6 May 2014, the respondents informed the board of their intention not to take further action in relation to the appeal proceedings and not to attend the payment of the national renewal fees for the patent. The request for oral proceedings was withdrawn.

V. In a communication issued on 30 June 2014, the board, with reference to Rule 84 (1) EPC, sought to clarify the appellant's intentions as regards the continuation of the appeal proceedings. The board informed the respondents that the appeal proceedings could not be

terminated by informing the EPO that the opposed patent was surrendered.

VI. The appellant requested to continue the appeal proceedings. The respondents did not reply to the board's communication and did not file any submissions regarding appellant's request to continue the appeal proceedings.

VII. The board issued a communication pursuant to Rule 100(2) EPC informing the parties that appellant's Request to continue the appeal proceedings was granted (cf. "Case Law of the Boards of Appeal of the EPO", 7th edition 2013, IV.C.6.2, page 849). The board further noted the following:

i) the sole issue discussed in appellant's statement of Grounds of Appeal concerned Article 56 EPC (cf. point II *supra*);

ii) in its reply to appellant's Grounds of Appeal, the respondents did not address any of the appellant's arguments but referred only to their submissions made at an early stage of the first instance procedure (cf. point III *supra*);

iii) in the board's preliminary, non-binding opinion, the combination of documents D1 and D3 (*infra*) made obvious the subject-matter of the claims of Auxiliary request 2, upheld by the opposition division (Article 56 EPC). Therefore, the board intended to set aside the decision under appeal and to revoke the patent.

VIII. None of the parties replied to the communication of the board.

IX. Auxiliary Request 2, upheld by the opposition division, contained 11 claims. Independent claims 1-3, 5 and 9 were directed to five different methods. **Claim 1** read as follows:

"1. A method for screening or selecting cells expressing a desired level of a polypeptide, comprising:

a) providing a plurality of eukaryotic cells each comprising an expression cassette comprising a first polynucleotide encoding the polypeptide, at least one stop codon downstream of the first polynucleotide, and a second polynucleotide encoding a cell membrane anchoring peptide downstream of the stop codon;

b) cultivating the cells in the presence of a termination suppression agent under conditions that allow expression of the polypeptide, wherein the termination suppression agent is an aminoglycoside antibiotic; and

c) using FACS to select at least one cell expressing the polypeptide fused to a cell membrane anchoring peptide."

Claims 2 and 3 were directed to a method for evaluating recombinant polypeptide expression in a population of cells and to a method for screening or selecting at least one cell expressing a polypeptide with a desired binding affinity to a ligand from cells expressing a library of polypeptide variants. Both claims comprised steps a), b) and c) similar to steps a), b) and c) of claim 1.

Claims 5 and 9 were directed to a method for alternately expressing i) a soluble, untagged polypeptide or ii) a membrane-bound polypeptide from a single cell or cell line; and to a method for alternately expressing i) a membrane-bound, untagged polypeptide or ii) a membrane-bound, tagged polypeptide from a single cell or cell line. Both claims comprised steps a), b), c), similar to steps a), b) and c) of claim 1, and an additional step d) in which the selected cell was cultivated in the absence of a termination suppression agent to obtain expression of the desired polypeptide.

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

D1: WO 03/014361 (publication date: 20 February 2003);

D3: M. Manuvakhova et al., RNA, 2000, Vol.6, pages 1044 to 1055.

XI. The submissions of the appellant, insofar as they are relevant to the present decision, may be summarized as follows:

Article 100(a) EPC; Article 56 EPC

The closest prior art document D1 disclosed a method for screening/selecting cell clones expressing a high level of a desired polypeptide by using an expression cassette that was based on stop codon read-through. In this cassette, the gene coding for the polypeptide of interest and a selectable marker were translationally linked so that a stop codon read-through resulted in the production of a fusion protein. In that way, host cells comprising the expression cassette were selected

by positive selection on expression of the fusion protein. Approximately 0.1% of the polypeptide was naturally expressed as fusion protein (natural rate of stop codon read-through) and the rest was expressed as a polypeptide without the fusion partner. The amount of fusion protein could be modulated by using stop codon suppressor mechanisms (modulation of read-through efficiency). The use of the stop codon setting provided a strict dependence of the expression of the selectable marker gene (which enabled a positive selection) on the expression of the gene of interest. Document D1 disclosed drug resistance genes as selectable markers but, as possible alternative markers, referred to reporter genes, such as the Green Fluorescence Protein (GFP). Cells were then selected by identifying these cells expressing the reporter gene, e.g. Fluorescence Activated Cell Sorting (FACS) when using GFP. In Example 1 of document D1, GFP was not expressed as a selectable marker but as a protein of interest. However, this disclosure showed that GFP expressing cells could be selected by FACS as commonly known in the art.

For selecting high producer cells expressing secreted proteins, the expression cassette disclosed in document D1 comprised a further element ensuring that the selectable marker remained inside the host cell. This additional element, namely a stop transfer sequence (transmembrane anchor, membrane spanning domain, "M"), was located downstream of the stop codon and upstream of the selectable marker. In the transmembrane expression vector disclosed in Example 7 of document D1 (pSEAPstopMneo), the selectable marker neo was located intracellularly when the expressed polypeptide SEAP was displayed on the cell surface. Selection was performed

by adding the aminoglycoside antibiotic G418 which at high concentration functions as selection agent.

The use of the word "*comprising*" for defining the elements of the expression cassette in claim 1 allowed the presence of additional elements downstream of the membrane anchor. Indeed, the opposed patent explicitly disclosed the presence of a reporter gene arranged downstream of the membrane anchor gene. Thus, document D1 disclosed all steps of the method of claim 1 except the use of an aminoglycoside antibiotic to increase stop codon read-through, which at low concentrations functions as stop codon suppressor. However, document D1 taught other stop codon suppression options.

Starting from document D1, the objective technical problem was the provision of a FACS based screening/ sorting method where the expression of the membrane anchored variant was conditionally increased. The solution presented by claim 1, namely the use of an aminoglycoside antibiotic as a termination suppression agent (instead of the genetic elements disclosed in document D1) to conditionally increase stop codon suppression, was obvious for a skilled person.

A person skilled in the art would not have been limited to the stop codon suppression options mentioned in document D1. It was well-known in the prior art, as shown by document D3, that the extent of stop codon read-through achieved depended on the concentration of the aminoglycoside antibiotic in the culture medium. Indeed, document D3 analyzed the efficiency of different aminoglycoside antibiotics to induce stop codon read-through by testing several antibiotics in a series of read-through constructs. Document D3 taught that the level of stop codon read-through could be

varied depending on the type and concentration of the termination suppression agent used (dose-response curves). Moreover, cells did not die if a low concentration of aminoglycoside antibiotic was used. Therefore, claim 1 was obvious in the light of document D1 in combination with document D3.

XII. The respondents did not file any submissions in reply to the appellant's arguments, but referred only to their submissions filed in reply to the Notice of Opposition (cf. points III and VIII *supra*).

XIII. The appellant (opponent) requested that the decision under appeal be set aside and that the patent be revoked.

XIV. In their reply to appellant's Grounds of Appeal, the respondents (patentees) requested that the appeal be dismissed. The request for oral proceedings was withdrawn.

Reasons for the Decision

Appellant's request for continuation of the appeal proceedings

1. In reply to the respondents' notice that they did not intend to take further action in relation to the appeal proceedings, that they did not pay the national renewal fees due in January 2014 and that they did not intend to pay these renewal fees within the available grace periods, the appellant requested the continuation of the appeal proceedings (Rule 84(1) EPC; cf. points V and VI *supra*).

2. The appellant provided evidence from several national patent registers showing that the opposed patent had not lapsed in all Contracting States and that it was still in force. The appellant further indicated that the annuity fees could be paid not only by the patentees but also by a third party. Furthermore, annuity fees could still be validly paid with surcharge until 31 July 2014 in many of the Contracting States and, even if annuity fees were not paid with surcharge in due time, reinstatement periods had also to be considered.

3. In the light thereof, the board granted appellant's request to continue the appeal proceedings and informed the parties accordingly in its communication pursuant to Rule 100(2) EPC (cf. point VII *supra*).

Extent of the appeal proceedings

4. As stated in the communication pursuant to Rule 100(2) EPC, the sole substantive issue in appeal proceedings concerns inventive step (Article 56 EPC; cf. point VII *supra*).

5. The respondents were informed by the board in a communication that their reply to appellant's Grounds of Appeal, which solely refers to their submissions made in the first instance proceedings in reply to the Notice of opposition (cf. point VII *supra*), is not considered to be a straightforward and direct reply to the arguments put forward by the appellant or to the reasons provided by the opposition division in the decision under appeal. According to the case law of the Boards of Appeal, references to a party's first instance submissions cannot normally replace an explicit account of a party's legal and factual reasons

in appeal proceedings. Neither the board nor other parties are expected to make investigations of their own in order to consider the merits of a party's case in appeal proceedings (cf. "Case Law", *supra*, IV.E. 2.6.4.a), page 963).

6. The respondents did not reply to the board's communication (cf. point VIII *supra*).

Article 100(a) EPC; Article 56 EPC

7. Document D1 was identified and acknowledged at the first instance proceedings as the closest prior art document (cf. page 8 of the decision under appeal). The board agrees.

7.1 Document D1 discloses a construct as defined in step a) of claim 1 (cf. *inter alia*, on page 13, second paragraph of document D1). The construct is a recombinant gene expression vector comprising a gene of interest (encoding a secreted protein), a translational stop signal and a stop transfer sequence, encoding an internal hydrophobic (transmembrane, cell membrane anchoring) peptide, translationally linked to a selectable marker gene (cf. Examples 2, 6-7, claims 6, 11 and Figure 9 of document D1). Document D1 further refers to the use of Green Fluorescence Protein (GFP) as a suitable selectable marker (cf. page 7, fifth paragraph of document D1). FACS sorting by GFP is a well-known method described in the prior art (step c) of claim 1).

7.2 Document D1 explicitly refers to a correlation between expression of a polypeptide of interest and read-through efficiency. It is in this context that the document refers to "*modulating*" said efficiency and to

several possible alternatives to do so (cf. page 8, first paragraph to page 9, first paragraph of document D1). Moreover, a preferred range of stop codon efficiency is also explicitly disclosed in this context (cf. page 9, second paragraph of document D1).

8. Starting from this closest prior art, the objective technical problem is formulated as "*the provision of a FACS based screening/sorting method where the expression of a membrane anchored variant is conditionally increased*" (cf. page 8, third paragraph from the bottom in the decision under appeal; see also page 22, point 3.3 of appellant's Grounds of Appeal). It has not been disputed that the claimed subject-matter, which contemplates an expression cassette with a selectable marker/reporter gene (GFP) downstream of the membrane anchor gene (cf. *inter alia*, page 11, column 19, paragraph [0062] of the patent), is a solution to this problem.

9. As acknowledged in the decision under appeal, steps a) and c) of claim 1 are disclosed in document D1 but not step b), in which an aminoglycoside antibiotic is used as a termination suppression agent. It remains thus to be assessed whether this step b) of claim 1 is derivable from document D1 in an obvious manner.

10. There are several prior art documents on file showing the ability of aminoglycoside antibiotics to mediate suppression of stop codons in mammalian translation systems (cf. page 25, second paragraph from the bottom of appellant's Grounds of Appeal).

10.1 Document D3 shows that read-through efficiency can be easily "*modulated*" by different factors, such as the sequence context of the stop codon, the specific

aminoglycoside antibiotic (suppressor agent) and the level or concentration of the antibiotic, which has to be low enough so that the translation system is not inhibited and translation is supported. The knowledge that the level of polypeptide expression can be easily altered by changing the concentration of aminoglycoside antibiotic in the medium renders the use of this type of suppressor agent obvious to a skilled person.

10.2 The fact that document D1 explicitly suggests several alternatives for "*modulating*" the read-through efficiency (cf. pages 8 and 9 of document D1), does not teach away from alternatives, especially when these alternatives, such as the use of aminoglycoside antibiotics, are readily available from the prior art and described therein as having advantageous properties.

10.3 In the same sense and contrary to the decision of the opposition division (cf. page 7, point 3 of the decision under appeal), the board considers that the disclosure in document D1 of aminoglycoside antibiotics as resistance markers (cf. page 7, third and fourth paragraphs of document D1), would not have hindered a skilled person trying to solve the technical problem underlying the invention from using other alternative gene markers, such as those already cited in document D1, in particular GFP (cf. page 7, fifth paragraph of document D1).

10.4 Therefore, the combination of documents D1 and D3 renders the claimed subject-matter obvious, contrary to the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated