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Datasheet for the decision of 11 December 2020

Case Number: T 2731/17 - 3.3.04

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Publication Number: 2073842

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Title of invention:
Use of human cells of myeloid leukaemia origin for expression of antibodies

Patent Proprietor:
Glycotope GmbH

Opponent:
Laboratoire Français du Fractionnement et des Biotechnologies

Headword:
Antibody expression in myeloid leukaemia cells/GLYCOTOPE

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

Keyword:
Main request and auxilary request 1: amendments - allowable (no)
Auxiliary request 2: amendments - allowable (yes)
Auxiliary request 2: inventive step (yes)

Decisions cited:

Catchword:

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Beschwerdekammern

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Case Number: T 2731/17 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 11 December 2020

Appellant: (Patent Proprietor)	Glycotope GmbH Robert-Rössle-Strasse 10 13125 Berlin (DE)
Representative:	Hemsath, Lars König-Szynka-Tilmann-von Renesse Patentanwälte Partnerschaft mbB Postfach 11 09 46 40509 Düsseldorf (DE)
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Representative:	Regimbeau 20, rue de Chazelles 75847 Paris Cedex 17 (FR)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 16 October 2017 concerning maintenance of the European Patent No. 2073842 in amended form.**

Composition of the Board:

Chair	G. Alt
Members:	B. Rutz
	N. Obrovski

Summary of Facts and Submissions

I. The appeal by the proprietor ("appellant") lies from the opposition division's interlocutory decision to maintain European patent No. 2 073 842 ("the patent") in amended form on the basis of auxiliary request III. The patent is entitled "*Use of human cells of myeloid leukaemia origin for expression of antibodies*".

II. An opposition was filed against the patent. Article 100(a) EPC in combination with Article 54 EPC (lack of novelty) and Article 56 EPC (lack of inventive step), and Article 100(b) and 100(c) EPC were invoked as grounds for opposition.

III. The opposition division decided with regard to the main request, *inter alia*, that claims 1 to 18 and 39 to 43 related to subject-matter extending beyond the content of the application as filed (Article 123(2) EPC) because there was no "*basis for the selection of all these features in combination, as it would appear to amount to selections from at least two lists*" (see decision under appeal, point 3.2).

Claim 1 of auxiliary request I was held to be unclear (Article 84 EPC).

With regard to auxiliary request II the opposition division decided, *inter alia*, that the subject-matter of claims 37 to 42 lacked an inventive step. The opposition division reasoned with regard to claim 37 that, starting from the closest prior art, document D10, the technical problem of producing an antibody with improved properties could not be seen to have been solved over the whole scope of the claim. Without an

upper limit of the degree of sialylation in the claim the method lacked features to provide antibodies with any definable improved technical effect. Therefore, the problem to be solved had to be reformulated as that of providing an alternative method for the production of antibodies. The skilled person would find it obvious to select a host cell line, such as a myeloid cell line, in order to produce antibodies with desired properties of glycosylation, and to select those producing a high degree of sialylation, as in document D10.

IV. With the statement of grounds of appeal the appellant filed, *inter alia*, sets of claims of a main request and of seven auxiliary requests.

V. Claim 1 of the main request and of auxiliary request 1 reads:

"1. A method for producing a protein molecule composition, comprising
(a) introducing in a host cell which is an immortalized human blood cell at least one nucleic acid encoding at least a part of said protein; and
(b) culturing said host cell under conditions which permit the production of said protein molecule composition; and
(c) isolating said protein molecule composition;

wherein the host cell is selected from the group consisting of DSM ACC2858 (GT-2X), DSM ACC 2806 (NM-H9D8), DSM ACC 2807 (NM-H9D8-E6), DSM ACC 2856 (NMH9D8-E6Q12) or a cell or cell line derived therefrom;

wherein the derived cell or cell line is obtained by cultivation and cloning without prior mutation, performed by treatment with physical, chemical or

biological mutagens, or genetic engineering of DSM ACC2858 (GT-2X), DSM ACC 2806 (NM-H9D8), DSM ACC 2807 (NM-H9D8-E6) or DSM ACC 2856 (NM-H9D8-E6Q12), or

wherein the cell or cell line derived from DSM ACC 2806 (NM-H9D8), DSM ACC 2807 (NM-H9D8-E6) or DSM ACC 2856 (NM-H9D8-E6Q12) is capable of producing a protein molecule composition which
- comprises no detectable NeuGc;
- comprises α2,6-linked NeuNAc; and
- has an increased sialylation degree with an amount of NeuNAc on the total carbohydrate structures or on the carbohydrate structures at one particular glycosylation site of the protein molecule of the protein molecules in said protein molecule composition which is at least 15% higher compared to the same amount of protein molecules in at least one protein molecule composition of the same protein molecule isolated from ATCC No. CRL-9096 (CHOdhfr-) when expressed therein."

Claim 41 of auxiliary request 2 reads:

"41. A method for producing an antibody molecule composition, comprising
(a) introducing in a host cell which is an immortalized human blood cell of human myeloid leukeamia [sic] origin at least one nucleic acid encoding at least a part of said antibody; and
(b) culturing said host cell under conditions which permit the production of said antibody molecule composition; and
(c) isolating said antibody molecule composition;

wherein the host cell is selected to produce an antibody molecule composition having the following glycosylation characteristics:

- it comprises no detectable NeuGc;
- it comprises α2,6-linked NeuAc; and
- it has an increased sialylation degree with an amount of NeuNAc on the total carbohydrate structures or on the carbohydrate structures at one particular glycosylation site of the antibody of said antibody molecules in said antibody molecule composition which is at least 15% higher compared to the same amount of antibody molecules in at least one antibody molecule composition of the same antibody molecule isolated from ATCC No. CRL-9096 (CHOdhfr-) when expressed therein; and wherein the antibody is a therapeutic whole antibody."

VI. The opponent (respondent) did not reply to the appeal.

VII. The board summoned the parties to oral proceedings as requested and informed them of its preliminary opinion in a communication pursuant to Article 15(1) RPBA 2020.

VIII. Oral proceedings before the board took place on 11 December 2020 in the absence of the respondent, as announced beforehand. At the end of the oral proceedings, the chair announced the board's decision.

IX. The following documents are cited in the present decision:

D4 Running Deer, J. and Allison, D.S. "*High-level expression of proteins in mammalian cells using transcription regulatory sequences from the Chinese hamster EF-1 alpha gene*", Biotechnology Progress, 2004, 20(3):880-9

D5 WO2005/080585

D10 Kaneko Y., Nimmerjahn F., Ravetch J.V. "*Anti inflammatory activity of immunoglobulin G resulting from Fc sialylation*", Science, 2006, 313(5787): 670-3

X. The appellant's arguments, submitted in writing and during oral proceedings, may be summarised as follows:

Main request and auxiliary request 1
Amendments (Article 123(2) EPC) - claim 1

The disclosure of the features relating to the glycosylation characteristics "comprises α2,6-linked NeuNAc" and "has an increased sialylation degree with an amount of NeuNAc [...]" on pages 12 and 13 of the application as filed was directed to the host cells used in the production method. This was apparent from the passages on page 13, second paragraph, page 8 second paragraph, page 11, sixth paragraph, and page 12, fifth paragraph. Furthermore, claim 2 as filed defined the host cell as "*selected to produce a protein composition having at least one of the following glycosylation characteristics*". Claim 3, which referred back to claim 2, further defined those glycosylation characteristics. Claim 11 as filed recited the host cell lines now mentioned in claim 1.

The feature "comprises no detectable NeuGc" was inherent to human cells and thus needed no explicit basis; see sentence bridging pages 5 and 6 in the application: "*Most rodent cells express for example N-glycolylneuraminic acid ('NeuGc') an alternative for N-*

acetylneuraminic acid ('NeuNAc') not present in humans, which is immunogenic in humans and/or the immunogenic galactose alpha(1-3) galactose modification ('Gal alpha1-3Gal'), and/or they lack important carbohydrates such as the important alpha2-6 linked NeuNAc or they lack bisecGlcNAc.".

The cells derived from the deposited cell lines NM-H9D8, NM-H9D8-E6 and NM-H9D8-E6Q12 produced proteins having a sialylation degree which was at least 15% higher than that of the same protein produced in CHO cells. The application as filed explicitly disclosed on page 23, seventh paragraph, that these deposited cell lines and cells derived therefrom had high sialylation activity. The degree of sialylation was disclosed on page 12, fifth paragraph, and also in claim 3 of the application as filed.

The specific combination of the three glycosylation features set forth in claim 1 was disclosed in the application as filed as one embodiment, in particular on page 14, penultimate paragraph. The second and third features in the passage on page 14 were mutually exclusive alternatives, i.e. one with and one without NeuNAc. The fact that cells derived from NM-H9D8, NM-H9D8-E6 and NM-H9D8-E6Q12 provided sialic acid residues, with NeuNAc being the relevant sialic acid in human cells, was evident from page 23, seventh paragraph. Therefore, a selection from two lists was not required to arrive at the combination of those glycosylation features.

The glycosylation features were also individually disclosed as properties of the derived cell lines on page 8, fourth paragraph, page 44, first paragraph, and page 23, seventh paragraph of the application as filed.

The product-by-process feature ("derived cell or cell line is obtained by") was based on the general definition of derived cells present in the application as filed on page 20, fourth paragraph and could thus also be applied to the specific host cells referred to in present claim 1, which had a basis in the originally filed claim 11, *inter alia*. It was allowable to omit the selection step mentioned on page 20 because "*the desired properties*" to be selected and mentioned in this passage were not defined and thus not limiting. Derivation of a cell or cell line implicitly involved selection because, after cultivation and cloning, a cell had to be selected for further use.

Auxiliary request 2
Inventive step (Article 56 EPC) - claim 41

Document D10 was the closest prior art. It disclosed the production of the antibody 6A6, which was directed against platelets, in the human embryonic kidney cell line HEK293 (see page 671, middle column). The method according to claim 41 differed from the disclosure of document D10 in (i) the use of a different host cell line (human blood cells of myeloid leukaemia origin instead of human embryonic kidney cells), in (ii) that therapeutic antibodies were recombinantly produced (anti-platelet antibody 6A6 was not used in therapy) and in (iii) that the antibodies had a different glycosylation pattern (HEK293 cells used in document D10 had low sialylation activity).

These differences provided an improvement in circulation half-life of the antibodies *in vivo* and an improvement in anti-tumour activity while maintaining

antibody-dependent cellular cytotoxicity (ADCC) activity and antigen binding.

The opposition division's reasoning that the claimed method did not define an upper limit for the sialylation degree and thus could result in antibodies which had reduced ADCC activity (referring to document D10 and results therein) was refuted. The claimed method comprised a natural upper limit for the degree of sialylation of the produced antibody composition because the enzymes of the host cells had a limited ability to sialylate antibodies and the glycosylation site Asn297 in the antibody was relatively inaccessible (see paragraphs [136] and [137] on page 38 of the statement of grounds of appeal). This had to be contrasted with the method in document D10, which involved the selection of sialylated antibodies using lectins and yielded antibody compositions enriched in sialic-acid content.

The objective technical problem was therefore that of providing a production method for therapeutic antibodies which resulted in antibodies having improved properties (see paragraph [114] of the statement of grounds of appeal).

Faced with this technical problem the skilled person starting from document D10 would at best have tried to further reduce the degree of sialylation obtained by using HEK293 cells because document D10 explicitly taught that a high degree of sialylation significantly deteriorated the biological activity of antibodies. The skilled person did not have any incentive or motivation to develop the technology described in document D10 in the direction of the claimed subject-matter.

Requests

XI. The appellant requests 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 the sets of claims of one of seven auxiliary requests.

Reasons for the Decision

Main request and auxiliary request 1
Amendments (Article 123(2) EPC)- claim 1

1. Claim 1 of the main request differs from the claims as granted in that the "derived cell or cell line" is further defined as follows:

(i) "obtained by cultivation and cloning without prior mutation, performed by treatment with physical, chemical or biological mutagens, or genetic engineering of DSM ACC2858 (GT-2X), DSM ACC 2806 (NM-H9D8), DSM ACC 2807 (NM-H9D8-E6) or DSM ACC 2856 (NM-H9D8-E6Q12)", or

(ii) "capable of producing a protein molecule composition which
- comprises no detectable NeuGc;
- comprises α2,6-linked NeuNAc; and
- has an increased sialylation degree ..."

2. As regards the features under (ii) above, the board is persuaded by the appellant's line of argument that they find a basis in the application as filed for the reasons set out in paragraphs 1 to 5 of section X. above.

3. The board finds, however, that the product-by-process feature in claim 1 (point (i) above) cannot be directly and unambiguously derived from the application as filed.

4. In the passage on page 20, fourth paragraph, cited by the appellant, the process step "*selection of those cells or cell lines derived from said host cell with the desired properties*" is inextricably linked to the previous steps "*cultivation and cloning*".

5. The board is not persuaded by the appellant's argument that the selection step has no limiting effect because "*the desired properties*" could relate to any property. While agreeing that "*selection*" does not necessarily imply a reduction in number, the board is of the view that, in a method, the step of selection for "*desired properties*" requires additional considerations by the skilled person and, in particular, a decision on what to select.

6. Moreover, the skilled person would derive from the whole of the application that those "*desired properties*" are related to the aim of the invention, namely to provide a method for "*the production of protein molecule compositions and in particular antibody molecule compositions having increased activity and/or increased yield and/or improved homogeneity and a human glycosylation*" (see Summary, page 1 of the application). The omission of the selection step from the definition of the derived cells or cell lines thus results in the subject-matter extending beyond the content of the application as filed.

7. This conclusion also applies to claim 1 of auxiliary request 1, which is identical to claim 1 of the main request.

Auxiliary request 2
Added subject-matter (Article 123(2) EPC)

8. Claim 1 has been amended by deleting the product-by-process feature from the claim. Claim 1 thus fulfils the requirements of Article 123(2) EPC.

Inventive step (Article 56 EPC) - claim 41

9. In the decision under appeal the opposition division held that claim 37 of auxiliary request II before it, which is identical to claim 41 of present auxiliary request 2, lacked an inventive step.

Closest prior art and difference

10. The appellant did not dispute the opposition division's choice of document D10 as the closest prior art.

Document D10 is a scientific study of the role of differential sialylation of immunoglobulin G (IgG) for the switch between innate anti-inflammatory activity and adaptive pro-inflammatory effects upon antigenic challenge (see Abstract). It discloses the production of the monoclonal anti-platelet IgG antibody 6A6 in the human embryonic kidney cell line 293 (HEK293) (see page 671, middle column). The resulting antibody composition with a low level of sialylation (see Figure 1) was subjected to lectin affinity chromatography to enrich sialic-acid-containing antibody species by 60 to 80 times. In an assay for *in vivo* platelet clearance, a comparison between the low-

sialylated and the highly sialylated antibody composition revealed an inverse correlation with sialylation, i.e. increased sialylation resulted in reduced biological activity (see page 671, middle column).

11. The difference between the claimed method and the method disclosed in document D10 is that the host cell for the production of the antibodies is an immortalised human blood cell of human myeloid leukaemia origin which is selected to produce antibodies with the glycosylation characteristics as detailed in the claim.

12. The board does not agree with the appellant that the characterisation of the produced antibody as "therapeutic" in the claim represents a further difference. The monoclonal antibody produced in document D10 has a physiological target ("anti-platelet") and thus, although its use in therapy is not disclosed in document D10, it cannot be excluded from being therapeutically useful.

Technical effect of the difference

13. Next, the effect of the difference identified in point 11. above is to be determined. In this context, the opposition division interpreted the method according to claim 41, in which the sialylation degree is not (explicitly) defined by an upper limit, to include embodiments such that the antibody molecules produced showed "*an undefined high level of sialylation* [which] *would not produce the same positive technical effects as demonstrated for their host cell lines*" (see decision under appeal, point 5.38).

14. The board, however, agrees with the appellant that this is not a correct interpretation of the claim because the claimed method is in fact characterised by an inherent, natural upper limit for the degree of sialylation of the produced antibody composition. This limit is imposed by the maximum ability of host cells to produce sialylated antibodies, which maximum ability is, *inter alia*, due to the limited sialylation efficiency of the respective cellular enzymes and the relative inaccessibility of the glycosylation site Asn297 in the antibody.

15. The method for enriching sialylated antibodies by lectin affinity selection disclosed in document D10 (see point 10. above) is thus fundamentally different from the method according to claim 41 and results in an antibody composition with a much higher sialylation content than is naturally achievable.

16. The board hence concludes that, in contrast to the method disclosed in document D10, the claimed method yields an antibody composition with a sialylation degree that is such that the antibodies retain their biological activity.

17. The board further notes that using a host cell which is "an immortalized human blood cell of human myeloid leukaemia origin" makes it possible to directly produce an antibody composition with an increased degree of sialylation in the natural (optimal) range and removes the need for further enrichment steps which risk excessively increasing the sialylation degree with potentially detrimental effects on biological activity, as disclosed in document D10.

18. In view of this conclusion and the reasoning below (see points 21. to 24.) the board does not consider it necessary to analyse whether the further advantageous properties invoked by the appellant, i.e. higher circulation half-life and higher therapeutic activity *in vivo*, are achieved over the whole scope of the claimed method.

19. The effect of the difference is thus that no additional selection steps are required and that the highly sialylated antibody composition produced retains biological activity.

Objective technical problem

20. Hence, in contrast to the appellant's opinion (see section X. above, penultimate paragraph), the objective technical problem consists in providing a method for producing a highly sialylated antibody composition which retains biological activity without the need for further selection steps.

Obviousness

21. As detailed in point 10. above, document D10 studies the role of sialylation in the immune system.

The document discloses that a composition of monoclonal antibody 6A6 expressed in HEK293 cells lost biological activity when enriched for sialic-acid-containing antibody species by lectin affinity chromatography. Document D10, however, does not provide any disclosure for the skilled person seeking to solve the problem defined in point 20. above on how to modify the disclosed method so as to obtain an antibody

composition with a high degree of sialylation which retains biological activity.

Document D10 does not disclose, for example, what degree of sialylation is actually required for a biologically active antibody composition or which cell lines might be useful for their production.

Furthermore, since document D10 highlights the problems associated with increased sialylation, it instead discourages the skilled person from using cell lines which achieve increased sialylation.

22. Documents D4 and D5 have been cited in the opposition proceedings in relation to inventive step.

Document D4 discloses the use of K562 cells (of human myeloid leukaemia origin) for the expression of the model protein secreted alkaline phosphatase; however, sialylation or glycosylation are not disclosed in document D4, and antibody expression is only mentioned in CHO cells (see D4, page 887, left column).

Document D5 discloses precursor cell lines of some of the cell lines used according to the method in claim 41 (e.g. NM-F9 cells), but discloses that those cells "*lost the ability to sialylate in a way that can be reconstituted by metabolic complementation*" (see paragraph bridging pages 14 and 15). Document D5 thus describes an alternative approach for achieving increased sialylation, namely metabolic complementation.

23. In the light of the teaching of document D10, taken on its own or in combination with the teaching of document D4 or D5, the skilled person would not arrive at the

claimed solution to the objective technical problem. In particular, the method disclosed in document D10 uses HEK293 cells and selection for sialylated antibody species by lectin affinity chromatography and results in an antibody composition with *decreased* biological activity. There is no indication in the prior art which would have caused the skilled person to modify the method disclosed in document D10 by using immortalised human blood cells of human myeloid leukaemia origin to arrive at a method producing a highly sialylated antibody composition which *retains* biological activity, without the need for further selection steps.

24. The subject-matter of claim 41 thus involves an inventive step.

Further grounds referred to in the notice of opposition: Article 100(a) in combination with Article 54 EPC and Article 100(b) EPC

25. The respondent did not reply to the appeal and did not attend the oral proceedings. The board saw no reason to examine any of the further grounds for opposition of its own motion either.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent with the following claims and a description to be adapted thereto:

   - Claims 1 to 47 of auxiliary request 2 filed with the statement of grounds of appeal.

The Registrar: The Chair:

D. Magliano G. Alt

Decision electronically authenticated