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Datasheet for the decision of 10 December 2021

Case Number: T 2454/16 - 3.3.08

Application Number: 06824300.5

Publication Number: 1974017

IPC: C12N5/0781, C12N5/10

Language of the proceedings: EN

Title of invention:
MEANS AND METHODS FOR INFLUENCING THE STABILITY OF ANTIBODY PRODUCING CELLS

Patent Proprietors:
Academisch Medisch Centrum bij de Universiteit van Amsterdam
Kling Biotherapeutics B.V.

Opponent:
STRAWMAN LIMITED

Headword:
Methods for increasing the replicative life span of antibody producing cells/ACADEMISCH MEDISCH CENTRUM - KLING BIOTHERAPEUTICS

Relevant legal provisions:
RPBA Art. 12(4)
EPC Art. 123(2), 83, 54, 56

Keyword:
Main Request - requirements of the EPC met (yes)

Decisions cited:
T 1811/13, T 0608/07, T 0464/05

Catchword:

Europäisches Patentamt
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Beschwerdekammern

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Case Number: T 2454/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 10 December 2021

Appellant: (Patent Proprietor 1)	Academisch Medisch Centrum bij de Universiteit van Amsterdam Meibergdreef 9 1105 AZ Amsterdam (NL)
Appellant: (Patent Proprietor 2)	Kling Biotherapeutics B.V. Meibergdreef 59 1105 BA Amsterdam (NL)
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Appellant: (Opponent)	STRAWMAN LIMITED Orchard Lea Horns Lane Combe Witney, Oxfordshire OX29 8NH (GB)
Representative:	Goodfellow, Hugh Robin 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 26 August 2016 concerning maintenance of the European Patent No. 1974017 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	C. Almberg

Summary of Facts and Submissions

I. European patent No. 1 974 017 is based on European patent application No. 06824300.5 (published as International patent application WO 2007/067046). The patent was opposed on the grounds of Article 100(a) to (c) EPC. An opposition division considered the main request to lack novelty and decided to maintain the patent in amended form on the basis of auxiliary request 1 with a description adapted thereto.

II. The patentees (appellants I) and the opponent (appellant II) lodged an appeal against the decision of the opposition division and submitted their statement of grounds of appeal.

III. Appellants I submitted a main request and auxiliary requests 1 to 4 with its statement of grounds of appeal, an auxiliary request 5 in reply to appellant II's statement of grounds of appeal and an auxiliary request 6 in reply to the board's preliminary opinion.

IV. Appellant II replied to appellants I's statement of grounds of appeal.

V. The parties were summoned to oral proceedings.

VI. In a communication pursuant to Article 17 RPBA 2020 sent in preparation of oral proceedings, the board provided observations on procedural issues and expressed a provisional opinion on some issues concerning Articles 123(2), 84, 83, 54 and 56 EPC.

VII. Appellants I withdrew its main request and auxiliary requests 1 and 2 with a letter dated 11 September 2020.

VIII. Appellant II informed the board with a letter dated 21 October 2021 that it would not attend the scheduled oral proceedings while relying on its written case.

IX. Oral proceedings were held on 10 December 2021 in the absence of appellant II. Appellants I withdrew auxiliary request 3 at the end of the oral proceedings.

X. Independent claims 1, 11, 22, 25, 27 and 28 of the highest ranking remaining request, auxiliary request 4 (filed with the statement of grounds of appeal) read as follows:

"1. A method for increasing the replicative life span of an antibody producing B cell, comprising enhancing Blimp-1 expression and increasing or maintaining the amount of BCL6 expression product as compared to a memory B cell or a naïve B cell within said antibody producing B cell by
- providing said antibody producing B cell with a nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof capable of increasing the replicative life span of an antibody producing B cell, and
- culturing said antibody producing B cell in the presence of a compound capable of increasing Blimp-1 expression, wherein said compound capable of increasing Blimp-1 expression comprises IL-21.

11. A method for producing an antibody producing B cell which is capable of replicating for at least one week, the method comprising:
- increasing an expression level of Blimp-1 in a B cell, as compared to a memory B cell or a naïve B cell, by providing said B cell with a nucleic acid sequence

encoding STAT3 or a functional part or a functional derivative thereof capable of upregulating Blimp 1 expression, and/or culturing said B cell in the presence of a compound capable of increasing Blimp-1 expression, wherein said compound capable of enhancing Blimp-1 expression comprises IL-21; and
- providing said B cell with a nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof capable of increasing the replicative life span of an antibody producing B cell.

22. An antibody producing B cell which is capable of replicating for at least nine weeks, wherein BCL6 and Blimp-1 are co-expressed, and wherein said cell comprises an exogenous nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof capable of increasing the replicative life span of an antibody producing B cell, and comprising an exogenous nucleic acid sequence encoding STAT3 or a functional part or functional derivative thereof capable of upregulating Blimp-1 expression.

25. A method for producing a B cell line comprising:
- obtaining an antibody producing B cell with a method according to any one of claims 1-21, and
- culturing said antibody producing B cell ex vivo.

27. A method for obtaining antibodies, comprising:
- obtaining an antibody producing B cell with a method according to any one of claims 1-21;
- culturing said antibody producing B cell ex vivo, and
- harvesting antibodies produced by said antibody producing B cell.

28. A method for producing antibodies capable of specifically binding an antigen of interest, the method

comprising:
- producing an antibody producing B cell that is capable of replicating for at least one week using a memory B cell capable of differentiating into a B cell which B cell produces antibodies capable of specifically binding said antigen of interest, in a method according to any one of claims 1-21, and
- obtaining antibodies produced by said antibody producing B cell."

Dependent claims 2 to 10, 12 to 21, 23, 24, 26 and 29 specify further features of the methods according to claims 1, 11, 25 and 28 or define embodiments of the product according to claims 22.

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

D1 WO 03/052083 (publication date 26 June 2003);

D2 A. Shvarts *et al.*, "A senescence rescue screen identifies *BCL6* as an inhibitor of anti-proliferative $p19^{ARF}$-p53 signaling." Genes & Development, vol. 16 (6), pages 681-6, (2002);

D4 K. Ozaki *et al.*, "Regulation of B Cell Differentiation and Plasma Cell Generation by IL-21, a Novel Inducer of Blimp-1 and Bcl-6." Journal of Immunology, vol. 173, (9), pages 5361-71, (2004);

D5 D.S. Mehta *et al.*, "IL-21 Induces the Apoptosis of Resting and Activated Primary B Cells." Journal of Immunology, vol. 170, (8), pages 4111-8, (2003);

D12 R. Reljic *et al.*, "Suppression of Signal Transducer and Activator of Transcription 3-dependent B Lymphocyte terminal Differentiation by BCL-6." The Journal of Experimental Medicine vol. 192, (12), pages 1841-8 (2000);

D13 F.A. Scheeren *et al.*, "STAT5 regulates the self-renewal capacity and differentiation of human memory B cells and controls Bcl-6 expression." Nature Immunology vol. 6, (3), pages 303-13, (2005);

D23 R. Ettinger *et al.*, Abstract 107, presentation at GARN, 15-18 September 2005, Vienna, Austria;

D24 WO 2005/052139 (publication date 9 June 2005);

D26 Antibody Engineering, Second Edition, Editor Carl A. K. Borrebaeck, Chapter 9, "Vectors and Approaches for the Eukaryotic Expression of Antibodies and Antibody Fusion Proteins", pages 267-293, 1995.

XII. The submissions made by the Patentees/Appellants I, insofar as relevant to the present decision, may be summarized as follows:

Auxiliary request 4
Article 123(2) EPC

Claim 1

The patent application as a whole disclosed that a long term culture of replicating antibody producing plasmablast-like cells capable of both proliferating and producing antibody was obtained when the amount of

BCL6 was maintained or increased and the amount of Blimp-1 was increased, as compared to the levels of a memory B cell or a naïve B cell (see page 6, line 16 to page 7, line 11 of the patent application). The BCL6 expression had to be maintained or increased in order to prevent the B cells from developing into non-proliferating plasma cells.

Basis for the first and second method steps of claim 1 could be found on page 10, lines 16 to 19 and on page 11, line 25 to page 12, line 6 of the patent application respectively. Further basis could be found on page 4, lines 16 to 24; page 19, line 20 to page 20, line 2; page 26, lines 13 to 18; page 30, lines 4 to 6; page 32, lines 7 to 9; figures 2 and 4; claims 2, 5, 8, 39. The reference to a nucleic acid sequence encoding STAT3 or a functional part or a functional derivative thereof could be found on page 33, lines 16 to 17 and claim 10 of the patent application).

The capability of a functional part or derivative of influencing the stability of an antibody producing cell, could either be found on page 15, lines 4 to 13 or on page 3, lines 25 to 26 and page 4, lines 23 to 30 of the patent application. The functional part or derivative of BCL6 was useful for stabilizing an antibody producing cell, so that the antibody producing cell remained in a certain developmental stage in which the cells continue to proliferate, thereby increasing the replicative life span of the antibody producing cell.

A basis for a compound that was capable of activating STAT3 or enhancing expression of STAT3 could be found in claims 1, 8 and 9, and on page 12, line 27 to page 13, line 5 of the patent application.

Claim 22

Claim 22 was amended to refer to an exogenous nucleic acid sequence encoding STAT3 or a functional part or functional derivative thereof capable of upregulating Blimp-1. Basis for this amendment could be found on page 33, lines 17 to 20 of the application as filed.

Claim 27

Claim 27 was based on page 30, lines 4 to 6 of the patent application. It referred to a method according to the invention, further comprising selecting and/or isolating, i.e. harvesting, or collecting an antibody of interest (see page 32, lines 7 to 9 of the patent application).

It was nevertheless essential, when deciding on issues of added subject-matter, to identify the actual teaching conveyed by the original disclosure, i.e. the technical information that the skilled person reading the original disclosure would have derived from its content (description, claims, drawings) considered in its entirety (see Examination Guidelines H-IV 2.2 and decision T 667/08, paragraph 4.1.4).

Article 83 EPC

The concept of "forbidden area" mentioned by appellant II was associated with the scope of the claims, i.e. Article 84 EPC, rather than with sufficiency of disclosure. The relevant question with respect to the sufficiency of disclosure was whether the patent provided sufficient information enabling the skilled person, when taking into account common general

knowledge, to reproduce the invention. "What is decisive for establishing insufficiency within the meaning of Article 83 EPC is whether the parameter, in the specific case, is so ill-defined that the skilled person is not able, on the basis of the disclosure as a whole and using his common general knowledge, to identify (without undue burden) the technical measures (eg selection of suitable compounds) necessary to solve the problem underlying the patent at issue." (see catchword of decision T 593/09).

The technical and active steps needed to put the invention into practice - i.e. expression of exogenous BCL6 and culturing in the presence of a compound capable of increasing Blimp-1 expression, such as IL-21 - are known to the skilled person and are also described in the patent application. All other techniques necessary to verify if the desired effect had been achieved were well established and/or disclosed in the Examples (e.g. proliferation assays, verifying BCL6 expression or antibody production) (see also paragraph 9.3.1. of the decision under appeal).

Examples 1 and 4 reported that BCL6-transduced B cells showed strong proliferation in the presence of IL-21. Despite the view provided in paragraph [0095] of the patent, there was no need to withdraw IL-21 from such cultures.

Decision T 464/05 was not applicable to the present situation. Indeed, it was undisputed that the patent underlying decision T 464/05 did not describe in detail a test method for measuring a weighted average mass vapor transmission rate (MVTR). The opponent provided evidence that for measuring the MVTR of a sample in accordance with the instructions in the patent in suit,

the results were very different depending on whether an air gap of 10 mm or of 25 mm was selected. Since, on the basis of the information available from the patent, the skilled person could indifferently use different air gaps, providing substantially different results, it was not able to determine whether the samples was in accordance with the claimed invention or not.

Article 54 EPC

Claims 25 to 29
The method of claims 25 to 29 referred to a method and not to a product-by-process. Claims 25 to 29 were dependent at least on independent method claims 1 and 11 (see paragraph 7.4 of the decision under appeal). Claims 25 to 29 were novel because of their dependence on claims 1 and 11.

Claims 1 and 11
Increasing the replicative life span of antibody producing cells in the claimed method was a functional feature missing in document D12, relying on short time frames (see Figures 1 to 4, 5A, 5E, 5F). The wording of claim 1 was identical to the method referred to in part F Chapter 4.13 of the Guidelines "method for remelting galvanic layers", which was held to be a functional feature of the method claimed.

The method of claims 1 and 11 comprised the step of enhancing Blimp-1 expression as compared to a memory B cell or a naïve B cell by culturing said cell in the presence of a compound capable of increasing Blimp-1 expression, which was missing in all the prior art methods (see document D13, Figure 7c and patent, Figure 3). Blimp-1 expression was not increased in STAT5- and BCL6-expressing B cells in the presence of IL-2 and

IL-4 (see document D13 and the patent in Figure 3, mRNA level). Thus, documents D1, D2 and D13 failed to disclose long term cultures of antibody producing cells that both proliferated and secreted antibody (see paragraphs [0011], [0014] and [0015] of the patent).

In document D12 no increase of the replicative life span of the BCL6 transduced BCL1 cells when cultured in the presence of IL-2/IL-5, as compared to the negative control, i.e. pHL6-GFP-transduced cells cultured without IL-2/IL-5, was disclosed. Since the vector transduction efficacy and the amounts of transduced cells and untransduced cells in the cultures were unknown and no statistical analysis was disclosed, it was unclear whether the difference observed in Figure 3 between the GFP fluorescence of the BCL6/Blimp-1 double transduced cells and the Blimp-1 single transduced cells reported after 15 days was significant at all. At best, the skilled person would have concluded from Figure 3 that the BCL6/Blimp-1 double transduced cells survived slightly longer before they died, but not that the method of document D12 was capable of increasing the replicative life span of antibody producing cells. The expression of Blimp-1 caused the GFP^+ fraction to fall, regardless of the presence of BCL-6. Blimp-1 transduced cells died and BCL6 could not prevent this. The GFP fluorescence of the control BCL1 cells transduced with pHL6-GFP was set to 100% (see Figure 3, o). While it would have been correct to compare the BCL6/Blimp-1 double transduced cells with BCL1 cells lacking exogenous BCL6, transduced with pHL6-GFP as negative control in Figure 3, there was no reason for selecting the Blimp-1 single transduced cells over the BCL6 single transduced cells as a starting point.

Article 56 EPC

Long term cell cultures containing cells that both replicate and keep producing antibody during prolonged periods of culture was achieved in the prior art only by using EBV immortalisation or hybridoma technology. Documents D1, D2, D12 and D13 were directed to B cell development only.

The technical problem to be solved arising from this teaching was the provision of cells that both replicate and produce antibody for prolonged periods of culture (see also decision under appeal item 8.3.3).

Paragraphs [0014] and [0015] of the patent made clear that the present invention was about long term culturing of cells capable of both proliferating and producing antibody. There was no reason to overemphasize paragraph [0011] of the patent and to disregard the antibody production achieved by the claimed method.

Examples 1 and 4 of the patent described the production of B cell cultures wherein the cells both replicated and produced antibody (see paragraph [0086], Fig.1).

Document D1 stated that the replicating B cell cultures had lost their capacity to produce Ig upon prolonged culture in the presence of IL-2 and IL-4 and had to be terminally differentiated for antibody production (see page 6, line 21 to page 7, line 2; page 11, lines 13 to 20 and page 39, lines 4 to 20). Hence the B cell cultures were not capable of proliferating and producing antibody simultaneously. Alternatively, document D13 disclosed a method of culturing BCL6 transduced B cells in the presence of IL-2 and IL-4. There was no hint in document D1 or document D3 to

replace IL-2 by IL-21 for promoting Blimp-1 expression. Indeed, IL-21 was known to be apoptotic or to strongly drive terminal differentiation into plasma cells, teaching away from using it to solve the technical problem identified above (see documents D4, D5, D23 and D24). There was no hint whatsoever in documents D1 or D13 that the technical problem could be achieved by increasing the expression of Blimp-1 in B cells, as compared to a memory B cell or a naïve B cell, with a nucleic acid sequence encoding STAT3 or a functional part or a functional derivative thereof capable of upregulating Blimp-1 expression.

Thus, claims 1 and 11 involved an inventive step over any of the documents D1, D2, D12 and D13 in combination with documents D23 or D24.

XIII. The submissions made by the Opponent/Appellant II, insofar as relevant to the present decision, may be summarized as follows:

Auxiliary request 4
Article 123(2) EPC

Claim 1

The method of claim 1 referred to increasing the life span of an antibody producing B cell instead of a memory B cell or a naïve B cell. The patent application provided no basis for a method in which the B cells were modulated regardless of their developmental stage, with the exception of memory B cells or naïve B cells.

The method of claim 1 required that the antibody producing B cell had increased levels of BCL-6 and Blimp-1 expression products "compared to a memory B

cell or a naive B cell". All the passages in the patent application mentioned that the manipulated cell in the method were either a memory B cell or a naïve B cell. A comparison with cells other than a memory B cell or a naïve B cell were not disclosed.

The embodiment described on page 19, line 20 to page 20, line 2 of the patent application was clearly limited first to an "ex vivo" method and second to "produce an antibody producing cell which was stable for at least one week". Both aspects were absent in present claim 1 so that the method was not necessarily an ex vivo method and could impart an increased replicative lifespan of less than one week.

Claim 11

For the reasons developed above for claim 1, the product of claim 11, comprising the feature of increasing Blimp-1 expression "as compared to a memory B cell or a naïve B cell", violated Article 123(2) EPC as well.

Claim 27

The patent application failed to disclose a method as defined in claim 27. The term "harvesting" of claim 27, was only found in the context of antibody producing B cells in claim 49 of the patent application. This term was different from the terms "isolating" or "producing" or "obtaining" used in the context of any kind of antibody producing cells (see e.g. page 30, lines 4 to 6 and page 32, lines 7 to 9 of the patent application). Even if the term "harvesting" was mentioned in the prior art section on page 1, lines 4 to 5, which cannot form an adequate basis for amendments, there was no

indication that these terms were synonymous and interchangeable.

Article 83 EPC

It was impossible to determine the limits of the scope of protection assigned to the claims of the patent as how the properties set out in the method of claim 1 must be determined were not defined.

Claim 1 failed first to set a reference point above which a replicative life span was increased, second it failed to determine what was the standard level of BCL6 and Blimp-1 expression in either a memory or a naïve B-cell and what type of expression target had to be compared (e.g. mRNA or protein or phosphorylation degree).

If the precise level of the standard was undefined, the amount of expression product in a test cell could not be determined to be above or below the standard. Thus, it was impossible for the skilled person to determine whether or not he/she worked within the "forbidden area of the claims" because the use of different methods or standards to determine the functional features of the claims could result in the determination of different relative expression levels or replicative life span.

The claims related to any B cells capable of producing antibodies and encompassed more than naïve or memory B cells. It was not credible that the same experimental protocols reported in the examples had the same effect on all B cell types. Besides, documents D23 and D24 showed that the time point of the addition of IL-21 to B cells was important. IL-21 was apoptotic in certain situations while in others it strongly induced terminal

cell differentiation. Reference was made to paragraph [0095] of the patent application. This evidence raised serious doubts based on verifiable facts that the skilled person could not determine how to increase the replicative life span of an "antibody producing B cell" grown in IL-21 containing media without undue burden.

Article 54 EPC

Document D26 anticipated the methods of claims 25 to 29. They all referred back to the method of claim 21, which itself referred back to any preceding method claims. Since claim 21 was not a method of increasing the lifespan of a cell or a method of producing a cell with increased replicative lifespan, but was a routine method of taking immunoglobulin genes from one cell and cloning them into another cell, the resulting cell obtained from the method of claim 21 could not be distinguished from any other antibody-expressing cell. The method of claims 25 to 29 including the step of producing/obtaining a cell by the method of claim 21 had to be interpreted to refer to a cell "obtainable" by that method, i.e. using a cell made in an earlier, disconnected method. Thus, document D26, which described the expression of an antibody from a cell containing antibody genes, anticipated the method of claims 25 to 29.

Documents D1, D2, D12 and D13 anticipated the subject-matter of claims 1, 11, 12, 18 to 20 and 26.

Document D1 described that BCL-6 was controlled indirectly, by influencing the level of STAT5 which was acting upstream of BCL-6 in the regulatory pathway. With the expression of a constitutively active form of STAT5b, it was possible to generate stable long term B

cell cultures which expressed a desired product, such as a protein, enzyme or antibody (paragraph bridging pages 6 and 7). Similar experiments were performed using a BCL-6 transgene (see pages 46 and 47). Cell culture similar to that disclosed in document D1 grew for more than one week, i.e. over 4 months (see document D2, page 684, left column, first sentence). The patent confirmed that the BCL-6 gene expression increased the replicative lifespan of transduced cells to several months (see paragraph [0086] of the patent). Thus, claims 1, 11, 12, 18 to 20 and 26 lacked novelty.

Document D2 described the introduction of transgene-expressed BCL-6 into primary tonsillar B cells (see page 683, right column, final paragraph). The cells were cultured with IL-2, a cytokine that the patent described as a compound capable of enhancing Blimp-1 expression, and could proliferate for 40 days and even for over 4 months (page 684, left hand column, first paragraph).

Document D12 disclosed a B cell line BCL1 transfected with a retroviral vector encoding BCL-6. The cell line was cultured in media comprising IL-2 and IL-5, undergoing exponential growth after 6 days, whereas without BCL6 expression, the IL-2 and IL-5 cultured cells became apoptotic. It was observed that BCL-6 could artificially be used to arrest terminal differentiation by overexpression of the BCL-6 gene. This arrest occurred even in the presence of cytokines such as IL-2 and IL-5 causing terminal differentiation (by the induction of the Blimp-1 protein). Ectopic expression of transgene BCL-6 was considered to result in expression levels at least equivalent to plasmablast. The retroviral vector encoding BCL-6 was also used to transfect splenic B cells which were then

cultured in media comprising IL-2, IL-4, IL-7 and IL-15. Expression of BCL-6 was shown to inhibit plasma cell terminal differentiation of primary B cells (see p.1844, column 1, second full paragraph). The expression of Blimp-1 mRNA was shown to increase in the BCL-6 transduced cell population (see Figure 4c). BCL-6 and Blimp-1 were transduced in a stepwise manner into the same cell resulting in a decrease of the GFP^+ fraction and thus of the percent viable cells (see page 1844, col.1, first paragraph, Figure 3). Thus, claims 1, 11, 12 and 18 to 20 and 26 lacked novelty.

Document D13 described the overexpression of STAT5b and reported that this increased the expression of BCL-6 and the proliferation of B cells (see abstract). The BCL-6 transgene was introduced into primary tonsillar B cells and cultured with IL-2 for 77 days (see Figure 6(b)). The cells grew over two months before the experiment was stopped (see page 307, col.1). The cells express antibodies on their surface and "consistently express soluble antibody" (page 310, left column, lines 32 to 36).

Article 56 EPC

Document D13 represented the closest prior art. Documents D1, D2 or D12 were mentioned as alternative closest prior art documents.

Document D13 disclosed a method comprising expressing a BCL-6 transgene in human B cells and then culturing them in the presence of IL-2, which induces Blimp-1.

The difference between document D13, or documents D1, D2 or D12, and the claimed subject-matter was that IL-21 was used to induce Blimp-1 expression, known in

the prior art to be capable of inducing antibody expression in antibody producing cells.

Since the subject-matter of claim 1 covered cells producing antibodies as well as cells that may turn into cells capable of expressing such antibodies (see [0011] of the patent), the technical effect of increasing the cell's antibody secretion will not be achieved when applying IL-21 on cells that do not yet produce antibodies but may be turned into cells producing and secreting antibodies (see [0086] of the patent).

The long term culture in the presence of IL-21 resulted furthermore in a loss of replicative properties necessitating a replacement of IL-21 by IL-2/IL-4 (see [0095] and [0121] of the patent). Thus, a method lacking this replacement step would not achieve the technical effect.

Since document D13 and the claims related to long term cultures of B cells that keep both, proliferating and producing antibody, the technical problem had to be reformulated into a less ambitious problem of providing an alternative method for increasing the replicative lifespan of B cells which comprise antibody genes, which in due course might be capable of developing into cells which express antibodies.

IL-21 was known in the prior art as an inducer of antibody expression (see documents D23 and D24 example 4, page 52, line 27 and paragraph bridging page 53 and 54).

The skilled person, faced with the technical problem identified above, was therefore motivated to add IL-21

as a potent alternative to IL-2/IL-5 (document D24) or as an improved alternative to IL-2/IL-10 (document D23) into the culture medium for inducing antibody expression and a robust immunoglobulin secretion.

The skilled person was therefore motivated to include IL-21 as a component of the medium for inducing antibody expression into the claimed methods of culturing antibody producing cells. The claimed methods lacked an inventive step.

The arguments for a lack of inventive step based on document D13 could also be based on one of documents D1, D2 and D12.

Document D1 related to the "maintenance" of antibody expressing cell cultures. Document D2 disclosed that "BCL6 expression also dramatically extends the replicative lifespan of primary human B cells in culture" (see documents D1, title; D2, page 684, column 1, first full paragraph, first sentence). Document D12 disclosed that BCL-6^+ BCL1 cells, transduced with pHL6-BCL-6-NEO, maintained their growth for over 2 weeks (see page 1844, top of left column). They all disclosed the same method as document D13. Hence, the claimed method lacked an inventive step over these documents as well.

XIV. Appellants I requested that the decision under appeal be set aside and the patent be maintained on the basis of auxiliary request 4.

XV. Appellant II requested in writing that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Auxiliary request 4 (Claims 1-29)
Admission of auxiliary request 4

1. Auxiliary request 4 submitted with appellants I's statement of grounds of appeal is identical to auxiliary request 3 underlying the decision under appeal, except that independent claims 1, 11, 22, 25, 27 and 28 refer to antibody producing B cells instead of antibody producing cells. A basis for this amendment can be found on page 5, lines 14 to 15 and page 7, lines 2 to 5 of the patent application as filed.

1.1 Considering also that no objection was raised against its admission, the board found no reason not to admit this claim request into the appeal proceedings (Article 12(4) RPBA 2007).

Article 123(2) EPC
Claim 1

2. In the decision under appeal, the opposition division considered that the claims of the main request were amended with respect to the claims as granted as outlined in amendments No. 1 to No. 11 in point 3.2.1. The amended claims resulted from the combination of dependent claims or from the deletion of alternative embodiments. They were held to comply with Article 123(2) EPC. Appellant II did not contest this finding.

2.1 Appellant II argued however that the method of claim 1 related to increasing the life span of an antibody producing B cell instead of a memory B cell or a naïve B cell. There was no basis in the patent application for a method that was not limited to a memory B cell or a naïve B cell.

2.1.1 The method also required that the antibody producing B cell had increased levels of BCL-6 and Blimp-1 expression product "compared to a memory B cell or a naive B cell". However, there was no basis in the patent application for such a comparison with cells other than with memory B cell or a naïve B cell.

3. The board notes that claim 1 relates to a method for increasing the replicative life span of an antibody producing B cell, comprising enhancing Blimp-1 expression and increasing or maintaining the amount of BCL6 expression product as compared to a memory B cell or a naïve B cell within said antibody producing cell ... wherein the "an antibody producing cell" is defined as a B cell (see patent application as filed, page 5, lines 10 to 19, especially line 14 and 15 and lines 27 to 30).

Cells at different differentiation stages of the B cell lineage were also disclosed (see patent application, page 6, lines 16 to 18; page 7, lines 12 to 20; page 18, lines 7 to 13; page 19, line 26 to page 20, line 2).

On page 6, lines 16 to 18 describe that

> "[i]n the human body, differentiation of plasma cells from activated naïve or memory B cells involves downregulation of BCL6 and upregulation of Blimp-1. In germinal center cells BCL6 expression is high and Blimp-1 expression is low. In resting memory cells expression of BCL6 and Blimp-1 are low. Signals that trigger differentiation cause an upregulation of Blimp-1, and this Blimp-1 counteracts the expression of BCL6. The stage where both BCL6 and Blimp-1 are expressed is short-lived and is called a plasmablast."

and on page 7, lines 12 to 15 disclose that

> "[w]ith a method of the invention it has amongst other things become possible to convert a naïve B cell or a memory B cell into a plasmablast-like cell and to stabilize said cell, so that rapid differentiation into a plasma cell does not occur."

On page 19, line 20 to page 20, line 2, an embodiment is described which "provides a method for producing an antibody producing cell which is stable for at least one week, preferably for at least one month, more preferably for at least three months, more preferably for at least six months, the method comprising [the steps of]:

- providing a memory B cell or a naïve B cell;
- increasing an expression level of Blimp-1 in said cell; and
- increasing and/or maintaining a BCL6 expression level in said cell.

[...]. Said BCL6 and Blimp-1 expression levels are preferably brought to, and/or maintained at, essentially the same level, or at a higher level, as compared to a plasmablast."

Finally, "With a method of the invention it has become possible to regulate the replicative life span of an antibody producing cell. A replicative life span of an antibody producing cell is defined herein as the time span wherein a B cell and its progeny cells are capable of replicating while maintaining their capability of producing antibody and/or developing into a cell that produces antibody" (see patent application as filed, page 4, lines 9 to 13).

3.1 The decision under appeal established that the subject matter of claim 1 was directly and unambiguously

derived from the combination of claim 1 with dependent claims 2, 4, 5, 8 and 10 of the patent application, and from the teaching on page 3, line 19 to page 5, line 9, and page 10, line 15 to page 12, line 7, especially on page 4, lines 9 to 13 and lines 27 to 30 of the patent application. Appellant II did not contest this finding.

3.2 The board observes that with a method of the invention it actually becomes possible to convert a naïve B cell or a memory B cell into a plasmablast-like cell and to stabilize said cell, so that rapid differentiation into a plasma cell does not occur (see patent application as filed, page 4, line 27 to 30 and page 7, lines 12 to 15). This stabilization increases the replicative life span of said antibody producing cell (see dependent claim 2, page 3, lines 25 to page 4 line 1). Hence, a conversion and stabilization into a plasmablast-like cell with highly favourable proliferating and antibody-producing characteristics is provided by the present invention (see page 7, lines 8 to 11).

3.2.1 The expression levels of BCL6 and Blimp-1 during B cell differentiation from naïve or memory B cell to plasma cells is established (see Figure 4; on page 6, lines 16 to 24). However, the conversion from a naïve or memory B cell into a stabilised plasmablast-like cell requires an adjustment of the expression levels of BCL6 and Blimp-1 (see Fig. 4, normal and BLC6-transformed cells). The stabilised plasmablast-like cell, having an increased replicative life span, is achieved in that the BCL6 and Blimp-1 expression levels compared to memory B cell or naïve B cells, known to have a similar BCL6 and Blimp-1 expression level, is adjusted accordingly, by increasing and/or maintaining a BCL6 expression level and increasing an expression level of Blimp-1 in a memory B cell or a naïve B cell. In this

context, no minimum duration is required for said stabilisation. A minimum duration of the replicative life span and the fact that the method is an ex-vivo method is only specified in the embodiment described on page 19, line 20 to page 20, line 2.

3.2.2 Hence, from the passages cited above and the claims of the patent application as filed, the board finds a basis for a method for increasing the replicative life span of an antibody producing B cell in which no minimum duration of the replicative life span is required, in which the steps of enhancing Blimp-1 expression and increasing or maintaining the amount of BCL6 expression product as defined in claim 1 is compared to a memory B cell or a naïve B cell level, and wherein said method need not be limited to an ex-vivo method. Thus, claim 1 complies with the requirements of Article 123(2) EPC.

Claim 11

3.3 The method of claim 11 uses any antibody producing B cell instead of a memory B cell or a naïve B cell and comprises a step of increasing Blimp-1 expression in an antibody producing B cell "as compared to a memory B cell or a naïve B cell". Since the method of claim 11 relates to the same contested features as claim 1, appellant II argued that it infringes Article 123(2) EPC for the same reasons as claim 1.

3.3.1 In view of the teaching discussed in point 3 above, the board considered that the subject matter of claim 1 has a basis in the patent application (see item 3.2). Hence the board concludes that the subject-matter of claim 11 also complies with the requirements of Article 123(2) EPC.

Claim 27

3.4 Appellant II's objection against claim 27 of the preceding requests was that it was not limited to antibody producing B cells. Since the method of claim 27 is now limited to antibody producing B cells, this objection is moot.

3.5 Thus, auxiliary request 4 complies with the requirement of Article 123(2) EPC.

Article 83 EPC

4. It is established case law that an objection of lack of sufficient disclosure presupposes that there are serious doubts, substantiated by verifiable facts, and that in order to establish insufficiency, the burden of proof rests generally on the opponent (see Case Law of the Boards of Appeal, 9th edition, 2019, II.C.9).

4.1 The prevailing opinion among the boards is that the definition of the "forbidden area" of a claim should not be considered as a matter related to Articles 83 and 100(b) EPC (see Case Law of the Boards of Appeal, 9th edition, 2019, II.C.8.2).
If the question of insufficiency arises from a lack of clarity, because undefined parameters are used in the claims, "[...] it is not sufficient to establish a lack of clarity of the claims for establishing lack of compliance with Article 83 EPC 1973; it is necessary to show that the lack of clarity affects the patent as a whole (i.e. not only the claims) and that it is such that the skilled person - who can avail himself of the description and his common general knowledge - is hindered from carrying out the invention" (see decision

T 1811/13 of 8 November 2016, Reasons 5.1). In other words, it will normally be necessary to show that the skilled person was deprived of the promise of the invention due to this ambiguity (see decision T 608/07 of 27 April 2009, item 2.5.2).

4.2 Appellant II argued that, in the present case, it was impossible for the skilled person to determine whether he was working within the "forbidden area of the claims" because the use of different methods or standards to determine the functional features of the claims could result in the determination of different relative expression levels or replicative life span.

4.3 In the board's view, appellant II's objection that the skilled person could not determine the scope of protection due to missing parameters in claims 1 and 11, amounts to an objection under Article 84 EPC. Since claims 1 and 11 were unamended in this respect, they are not open to an objection under Article 84 EPC (see decision G 3/14, OJ 2015, A102, catchword).

4.3.1 The crucial issue in decision T 464/05 of 14 May 2007 was whether the lack of indications in the patent in suit in respect of how to measure a claimed parameter amounted to an undue burden for the skilled person trying to reproduce the invention. Since the technical effect of the present invention does not rely on any specific value and it has not been substantiated that the use of a plurality of test methods provided substantially different results, the conclusion of decision T 464/05 is not applicable to the present case.

4.3.2 Although the claims do not specify which type of Blimp-1 and BCL6 expression product needs to be

increased or increased and maintained, respectively, in an antibody producing B cell as compared to a memory B cell or a naïve B cell, no evidence was submitted that an increased expression product, such as mRNA or phosphorylated or non-phosphorylated BCL6 could not be determined and could not be compared to the corresponding expression product level measured in a memory B cell or a naïve B cell. In this context, the board notes that a phosphorylated BCL6 form is not an expression product but a post-translationally modified expression product.

4.4 Appellant II argued that IL-21 had to be supplied to B cells in a specific and controlled manner to result in an antibody producing B cell having an increased replicative life span. Documents D23 and D24 showed that the time point of the addition of IL-21 to B cells was important. IL-21 was apoptotic in certain situations while it strongly induced terminal cell differentiation in others. Reference was made to paragraph [0095] of the patent.

4.5 The board considers that the steps of the claimed invention rely on standard established prior art methods sufficiently disclosed to enable the skilled person to put the claimed method into practice. Despite the view provided in paragraph [0095] of Example 3 of the patent, which uses caSTAT5b-ER-IRES-NGFR transduced human B cell cultures and not a B cell transduced with a nucleic acid sequence encoding BCL6, there was no need to withdraw IL-21 from such cultures. Indeed, Examples 1 and 4 of the patent clearly show that BCL6-transduced B cells strongly proliferate in the presence of IL-21.

4.6 Since no verifiable facts were provided, the board considers that the finding with regard to Article 83 EPC in the decision under appeal is correct.

4.7 Thus, auxiliary request 4 complies with the requirement of Article 83 EPC.

Article 54 EPC

5. Appellant II argued that the method of claims 25 to 29 referring to claim 21, referring itself back to any method according to claims 1 to 20 represented a method which used a cell obtainable by a method according to claims 1 to 21, i.e. any cell characterized by the features resulting from said methods. Since claim 21 was not a method of increasing the lifespan of a cell or a method of producing a cell with increased replicative lifespan, document D26 anticipated the method of claims 25 to 29.

5.1 The board agrees with the findings of the opposition division in item 7.4 of decision under appeal. The method for producing a B cell line comprising an active step of "obtaining" an antibody producing B cell with a method according to any one of the claims 1 to 21, can only be understood as meaning that the step of "obtaining" an antibody producing B cell occurs by applying the method steps of any one of the methods according to claims 1 to 20 - in its broadest possible form according to the method of claims 1 and 11 - thereby including these steps by reference into the method of claim 25.

6. Appellant II contended that documents D1, D2, D12 and D13 anticipated the subject-matter of claims 1, 11, 12, 18 to 20 and 26.

6.1 In the board's view, none of the documents D1, D2, D12 and D13 discloses a method comprising the steps of claims 1 and 11.

The essential features of claim 1 are providing, i.e. transducing, an antibody producing B cell with a nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof and culturing these cells in the presence of IL-21 and achieving the effects mentioned in the claims

The essential features of claim 11 are increasing the expression level of Blimp-1 in a B cell by providing said B cell with a nucleic acid sequence encoding STAT3 or a functional part or a functional derivative thereof and/or culturing said B cell in the presence of IL-21, and transducing said B cell with a nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof and achieving the effects mentioned in the claims.

Documents D1, D2, D12 and D13 describe antibody producing B cells transduced with a nucleic acid sequence encoding BCL6 but not the culturing of said cells in the presence of IL-21. These documents neither describe antibody producing B cells transduced with a nucleic acid sequence encoding BCL6 and a nucleic acid sequence encoding STAT3 or a functional part or a functional derivative thereof capable of upregulating Blimp-1 expression.

6.2 In light of these considerations, the board concludes that the subject-matter of claims 1, 11, 12, 18 to 20 and 22, respectively, is novel over the disclosure of documents D1, D2, D12, and D13.

Inventive step
Closest prior art

7. Appellant II selected document D13, or alternatively document D1, D2, or D12, as closest prior art in combination with documents D4, D23 or D24.
Appellants I selected document D13.

7.1 Appellant II argued that the subject-matter of claims 1 and 11 covered B cells producing antibodies as well as B cells that may turn into cells capable of expressing such antibodies (see paragraph [0011] of the patent). An antibody producing B cell need not be a cell that produces antibodies, it merely had to be "capable" of being turned into one. Thus, antibody expression was not a technical effect of the claimed method.

7.1.1 The board is not convinced by appellant II's argument, because the method of claim 1 needs to enhance Blimp-1 expression and increase or maintain the amount of BCL6 expression product as compared to a memory B cell or a naïve B cell within said antibody producing B cell by means of its characterizing active steps.
First, an antibody producing B cell is provided with a nucleic acid sequence encoding BCL6 and its expression product is increased or maintained as compared to a memory B cell or a naïve B cell. The resulting antibody producing B cells demonstrate an increased replicative life span.
Second, said antibody producing B cells are then cultured in the presence of IL-21, increasing the expression of Blimp-1, so that said cells differentiate into plasmablasts, known to express antibodies (see Figure 4, especially second row). A plasmablast shows higher proliferation and higher antibody secretion

levels compared to a memory B cell, whereas a plasma cell, corresponding to the next developmental stage, secretes high antibody levels but is not capable of proliferating (see paragraph [0009], especially page 4, lines 3 to 4 of the patent).
Thus, although the term "antibody-producing cells", based solely on the definition in paragraph [0011] of the patent, does not require that the cells express antibodies, the methods of claims 1 and 11 do.
Both the replicative life span and the antibody production are technical effects achieved by the method of claims 1 and 11.

7.2 Appellant II submitted that the long term culture in the presence of IL-21 resulted in a loss of replicative properties over time, eventually necessitating its replacement with a combination of IL-2 and IL-4 (see [0095] and [0121] of the patent). Thus, a method lacking a replacement step of IL-21 would not achieve the technical effect and lack an inventive step.

7.2.1 The board is not convinced by appellant II's argument.
The antibody producing B cells according to the method of claim 1 are described in Example 1 of the patent. BCL6 was introduced into memory B cells. It greatly extended the cells' lifespan over normal B cells in culture (10 months vs. ~3 weeks). Culture of these cells on CD40L-L cells in the presence of IL-21 provided them a plasmablast-like cell surface phenotype ($CD38^{hi}CD20^{+}$, Figure 2) and a significant growth advantage (Figure 1). These transduced cells cultured with IL-21 secreted 300% more lgG compared to cells cultured with IL-2 and IL-4.
Paragraphs [0095] and [0121] of the patent, referred to by appellant II, relate to human B cell cultures transduced with a caSTAT5b-ER-IRES-NGFR construct. The

expression of caSTAT5b is activated by 4-Hydroxytamoxifen (4-HT). These paragraphs do not relate to BCL6 transduced B cells as specified in claim 1.

7.3 The board considers that the methods of claims 1 and 11 differ from the method described in document D13 in that antibody producing B cells are provided with

(i) a nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof, and

(ii) cultured in the presence of IL-21;

or alternatively in that antibody producing B cells are provided with

(i) a nucleic acid sequence encoding BCL6 or a functional part or functional derivative thereof, and

(ii) a nucleic acid sequence encoding STAT3 or a functional part or a functional derivative thereof and/or cultured in the presence of IL-21;

so that the amount of BCL6 expression product is increased or maintained, whereas the Blimp-1 expression is increased as compared to a memory B cell or a naïve B cell.

7.4 The effect associated with these distinguishing features is that the claimed method provides an antibody producing B cell wherein both the replicative life span and the antibody production of an antibody producing B cell are improved.

7.5 The technical problem is thus defined as the provision of an improved method for producing an antibody producing B cell capable of both proliferating and producing antibody (see patent, paragraphs [0014], [0015]).

7.6 In view of the experimental data disclosed in Example 1, paragraph [0086] and Figures 1 and 2 of the patent, the board is satisfied that the methods according to claims 1 and 11 solve the technical problem identified above.

Obviousness

It remains to be assessed whether the skilled person faced with the technical problem identified above and starting from the closest prior art method would have arrived at the claimed method in an obvious manner.

7.7 Document D13 discloses that the overexpression of STAT5b increased the expression of BCL-6, and the proliferation of B cells (see abstract). Bcl-6 extends the lifespan of normal human B cells. The BCL-6 transgene was introduced into primary tonsillar B cells and cultured with IL-2 and IL-4 for 77 days.
There are no pointers how the method of extending the life span of an antibody producing B cell could be improved so that they are capable of proliferating and producing antibodies, let alone by culturing the antibody producing B cell in the presence of IL-21 and/or by providing said B cell with a nucleic acid sequence encoding STAT3 or a functional part or a functional derivative thereof.

7.8 Appellant II submitted that the use of IL-21 was known in the prior art as an inducer of antibody expression from at least documents D4, D23 and D24 (see example 4, page 52, line 27 and paragraph bridging page 53 and 54).

7.9 Document D23 describes that IL-21 is a pivotal cytokine in the cell-dependent B cell activation, differentiation and Ig secretion, when highly purified naïve B cells were stimulated with anti-CD40, or anti-IgM and anti-CD40. The board notes that the addition of IL-21 results not only in a dramatic increase in proliferation but also in plasma cell differentiation as well as robust immunoglobulin secretion, as compared to cultures that contained no cytokine or the combination of IL-2 and IL-10.

7.9.1 Document D24 describes a method for inducing differentiation of a B cell progenitor into a memory B cell and/or a plasma cell (see example 4) and reports that in line with the effect of IL-21 on antibody production (Fig 5C), IL-21 induces expression of syndecan-1 (CD138), a plasma cell marker, and surface IgGl (Fig.6C) in B cells stimulated with anti-IgM with or without IL-4. Overall, IL-21 has pro-apoptotic effects for mature follicular B cells, induces an increase in immature B cells, alters the B cell phenotype, and is a potent inducer of B cell maturation to memory B/post-switch cells and plasma cells (page 53, lines 2 to 5; FIG. 8). Documents D4 and D5 confirm this view (see abstract, lines 3-8 and Title, respectively; items 5.11 and 6.10 of appellant II's statement of grounds of the appeal and of its reply to appellants I's statement of grounds of appeal respectively).

7.10 Thus, neither documents D4, D23 nor D24 disclose or suggest that human B cells transduced with Bcl-6-IRES-GFP expressing BCL6 should be cultured in the presence of IL-21, instead of IL-2 and IL-4.

7.11 Thus, the methods of claims 1 an 11 involve an inventive step in the light of the combined teachings of documents D13 and D4, D23 or D24.

8. Starting with any one of the alternative closest prior art documents D1, D2 or D12, the difference between the subject matter of claims 1 and 11 and this prior art remains the same as with document D13.

8.1.1 Document D1 discloses that "The present invention concerns materials and methods relating the production and maintenance of antibody producing human B cells" (see page 1, lines 7 to 9). The "[E]ctopic expression of BCL-6 into human peripheral blood B cells results in extension of the replicative life span of the cells and maintenance of cell surface immunoglobulin" (see page 46, lines 11 to 14). The B cells expressing a BCL-6 transgene were cultured in the presence of IL-2 and IL-4, while IL-2 is a Blimp-1 inducer according to the patent.

8.1.2 Document D2 discloses a method similar to that described in document D1 (see document D2, abstract and bridging paragraph on page 683, column 2, to page 684, column 1).

8.1.3 Document D12 discloses a method similar to that of document D1. BCL1 cells transduced with BCL-6 retroviral vectors maintained their growth (see page 1844, top of left column; Figure 1) and can suppress terminal differentiation by overexpression of the BCL-6 gene, even in the presence of IL-2 and IL-5 which normally serve to cause terminal differentiation (see bridging sentence page 1843, col.2 to page 1844 col.1; page 1844, col.1, lines 52 to 53).

8.2 Based on the technical effect underlying the differences identified above, the technical problem is the same as when starting from document D13.

8.3 The board is satisfied that the method according to claim 1 solves this problem.

8.4 Since there is no hint or pointer in documents D1, D2 and D12 based on which the skilled person, in order to solve the technical problem identified above, would have turned to document D4, D23 or D24, an inventive step is acknowledged for the subject-matter of claims 1 and 11.

8.5 This finding applies, mutatis mutandis, to the subject-matter of dependent claims 2 to 10 and 21 and to the method of claims 25, 26, 27 incorporating the method of claims 1 to 21 and claims dependent thereon.

8.6 The board concludes that auxiliary request 4 meets the requirements of Article 56 EPC.

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 on the basis of claims 1 to 29 of auxiliary request 4 filed with the statement of grounds of appeal, and to adapt the description accordingly.

The Registrar: The Chair:

L. Malécot-Grob B. Stolz

Decision electronically authenticated