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Datasheet for the decision of 21 November 2006

Case Number: T 0869/05 - 3.3.08

Application Number: 93913744.4

Publication Number: 0651793

IPC: C12N 9/20

Language of the proceedings: EN

Title of invention:
DNA sequences used in the production of recombinant human BSSL/CEL in transgenic non-human mammals, and the produced BSSL/CEL used in infant formulas

Applicant:
Arexis AB

Opponent:
-

Headword:
BSSL/CEL lipase/AREXIS

Relevant legal provisions:
EPC Art. 123(2), 83, 84, 54, 56

Keyword:
"Main request - added subject-matter (no), clarity (yes), sufficiency of disclosure (yes), novelty (yes), inventive step (yes)"
"Remittal to first instance - further prosecution (yes)"

Decisions cited:
T 0315/03

Catchword:
-

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Case Number: T 0869/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 21 November 2006

Appellant: Arexis AB
Arvid Wallgrens Backe
S-413 46 Göteborg (SE)

Representative: Pohlman, Sandra M.
df-mp
Dörries, Frank-Molnia & Pohlman
Triftstrasse 13
D-80538 München (DE)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 27 January 2005 refusing European application No. 93913744.4 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent application number 93 913 744.4 (published as WO 93/25669, to be referred to in the present decision as "the application as filed") was refused by the examining division pursuant to Article 97(1) EPC. The application was filed on 9 June 1993 and claimed priority from applications US 9201809-2 (11 June 1992), US 9201826-6 (12 June 1992), US 9202088-2 (3 July 1992) and US 9300902-5 (19 March 1993).

II. The reasons given for the refusal was that the main request and the first, second and third auxiliary requests did not fulfil the requirements of Article 56 EPC. The third auxiliary request was also considered to contravene Article 84 EPC.

III. The applicant (appellant) lodged an appeal against the decision of the examining division and, in the statement setting out the grounds of appeal, a main request and a first and second auxiliary requests were filed. The third auxiliary request before the examining division was also maintained.

IV. The examining division did not rectify its decision and, pursuant to Article 109(2) EPC, remitted the appeal to the Boards of Appeal.

V. By letter of 7 July 2006, the appellant was summoned to oral proceedings. In a communication under Article 11(1) of the Rules of Procedure of the Boards of Appeal ("RPBA") sent with the summons, the board expressed its provisional opinion on the issues of priority rights

(Articles 87 to 89 EPC), clarity (Article 84 EPC) and inventive step (Article 56 EPC). The board further drew the attention of the appellant to Articles 83 and 53a EPC as well as to Rule 23d(d) EPC.

VI. In a letter dated 20 October 2006, the appellant replied to the board's communication and filed therewith a new main request and a new first, second and third auxiliary requests.

VII. At the oral proceedings, which took place on 21 November 2006, the appellant withdrew all previous requests and filed a new main request.

VIII. Claims 1 to 3 of the **main request** read as follows:

"1. A DNA molecule encoding human Bile Salt-Stimulated Lipase/Carboxyl Ester Lipase (BSSL/CEL) containing 11 exons interrupted by 10 introns, comprising the *SphI* fragment of plasmid pS309 (DSM 7101), the *SacI* fragment of plasmid pS310 (DSM 7102), and the *BamHI* fragment of plasmid pS311."

"2. A DNA molecule encoding human Bile Salt-Stimulated Lipase/Carboxyl Ester Lipase (BSSL/CEL) containing 11 exons interrupted by 10 introns, which hybridizes under stringent hybridization conditions with the DNA molecule according to claim 1."

"3. A hybrid gene which is expressible in the mammary gland of an adult female of a non-human mammal harbouring said hybrid gene, said hybrid gene comprising a DNA molecule as defined in claim 1 or claim 2 and further comprising a DNA molecule encoding

a milk protein of a non-human mammal, wherein the DNA molecule encoding human BSSL/CEL is inserted into said milk protein gene so that human BSSL/CEL is produced when the hybrid gene is expressed."

Claims 4 to 5 further defined the milk protein genes of claim 3. Claims 6 to 7 concerned replicable expression vectors and claim 8 related to a mammalian expression vector system. Claims 9 to 11 were directed to mammalian cells and to non-human mammalian embryo cells. Claims 12 and 13 concerned processes for producing transgenic non-human mammals capable of expressing human BSSL/CEL. Claims 14 to 17 related to transgenic non-human mammals and the progeny thereof and claims 18 and 19 to the use of these transgenic non-human mammals.

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

A: U. Lidberg et al., Genomics, Vol. 13, pages 630 to 640, July 1992 (cited as expert opinion in the statement setting out the grounds of appeal),

P2: SE 9201826-6 (filing date 12 June 1992) (second priority document of the application),

D2: WO-A1-91/15234 (publication date 17 October 1991),

D4: A.K. Taylor et al., Genomics, Vol. 10, pages 425 to 431, 1991,

D6: B.V. Kumar et al., Biochemistry, Vol. 31, pages 6077 to 6081, 7 July 1992.

X. The appellant's arguments insofar as they are relevant to the present decision may be summarized as follows:

Articles 123(2), 83 and 84 EPC

The application as filed concerned the cloning and sequencing of the human BSSL/CEL gene and explicitly referred to a DNA molecule encoding the human BSSL/CEL and containing intron sequences. The elucidation of the structure of the human BSSL/CEL gene (11 exons interrupted by 10 introns) was the actual contribution to the prior art and it was clearly indicated throughout the application as filed, such as on pages 10, 24 (Example 1) and in Figure 8. The plasmids pS309, pS310 and pS311 were disclosed in Example 2 and they were characterized by the presence, in each plasmid, of a restriction fragment containing a different part of the human BSSL/CEL gene as illustrated in Figure 1. The application further provided the deposit information (in accordance with the Budapest Treaty) for the first two plasmids. The structural features characterizing the restriction fragment of the third plasmid, which (as mentioned in document P2) had also been deposited, were defined in Example 2 of the application and shown in Figure 1. The subject-matter of claim 1 was defined by a combination of a functional feature (bile salt-stimulated lipase activity) with several structural features (number of exons and introns, presence of restriction fragments) disclosed in the application and further supported by deposited material.

The application also referred to DNA molecules (and analogues thereof) which hybridized under stringent hybridization conditions with the DNA molecule encoding

the disclosed human BSSL/CEL gene or a specific part thereof. In particular, claim 3 as originally filed explicitly related to this subject-matter. Formal basis for the subject-matter of all other claims could also be found in the claims as filed and in the description of the application as filed.

Articles 87 to 89 EPC and Article 54 EPC

The three plasmids pS309, pS310 and pS311, were disclosed in Example 2 of the second priority document P2. The restriction fragments of these plasmids (comprising the different parts of the human BSSL/CEL gene) were also characterized in Example 2 and further shown in Figure 2. Reference was explicitly made to the deposit on 12 June 1992 of these three plasmids (in accordance with the Budapest Treaty) at the Deutsche Sammlung von Mikroorganismen (referred to below as the DSM). Document P2 referred to DNA molecules (and analogues thereof) which hybridized with the disclosed human BSSL/CEL gene, or a specific part thereof, under stringent hybridization conditions and claim 3 of document P2 was concerned with this specific subject-matter. Formal basis for all the other claims was also found in the description and/or in the claims of document P2. Thus, the main request was entitled to the filing date of the earlier priority document P2 and, since there was no prior art disclosing the claimed subject-matter earlier than the filing date of document P2, the claimed subject-matter was novel.

Article 56 EPC

The subject-matter of claims 1 and 2 concerned only the human BSSL/CEL gene and structurally related DNA molecules which hybridized thereto under stringent hybridization conditions. Combinations of the known BSSL/CEL cDNA with an arbitrary number and/or length of the BSSL/CEL introns or else with heterologous (non-BSSL/CEL) introns were not embraced by these claims.

Document D2 referred to the production of BSSL/CEL in transgenic animals using the human BSSL/CEL cDNA - explicitly disclosed in this document - or the human BSSL/CEL gene and appropriate complementing elements. However, for the human BSSL/CEL gene this disclosure was only on a theoretical level since the human BSSL/CEL gene was not disclosed and no guidance was provided for overcoming the problems encountered in isolating and characterizing that gene. Document D4 disclosed that the BSSL/CEL locus exhibited a high degree of polymorphism and contained or was closely associated with a hypervariable region of the insertion/deletion variety. The document referred to the isolation of the human BSSL/CEL gene and to the determination of the sequence of this hypervariable region for the purpose of developing rapid PCR polymorphism typing. However, no evidence was provided to show that they had succeeded in their effort.

Starting from this prior art, the objective technical problem was considered to be the cloning and sequencing of the human BSSL/CEL gene in order to produce genomic

DNA for the preparation of transgenic animals. The solution was provided by the claimed subject-matter.

Although it was obvious to follow the indications of this prior art and try, therefore, to isolate the human BSSL/CEL gene, the skilled person would have encountered important technical difficulties which required inventive skill to overcome. In particular, it was required to develop a very specific cloning method for isolating the human BSSL/CEL gene and for discriminating this gene from the closely structurally related human BSSL/CEL-like (CELL) (pseudo)gene (use of two different human genomic phage libraries, design of a specific PCR amplification assay with selection of part of intron 10). It was not at all trivial to distinguish between the human BSSL/CEL gene and the human BSSL/CELL (pseudo)gene and to determine which one was the functional gene. Post-published evidence demonstrated that, starting from the very same prior art, those technical problems had led to a failure to clone and sequence the BSSL/CEL gene.

XI. The appellant (applicant) requested that the decision under appeal be set aside and a patent be granted on the basis of the Main Request filed during the oral proceedings on 21 November 2006.

Reasons for the Decision

Article 123(2) EPC

1. The application as filed relates to DNA molecules encoding human Bile Salt-Stimulated Lipase/Carboxyl

Ester Lipase (BSSL/CEL) and containing intron sequences, in particular 11 exons interrupted by 10 introns (cf. *inter alia* page 1, lines 5 to 9, page 8, lines 21 to 27, page 24, lines 22 to 24, claims 1 to 3 as filed). Example 2 refers to the use of plasmids pS309, pS310 and pS311 for the construction of expression vectors for production of recombinant BSSL/CEL in milk of transgenic animals. These plasmids are described as containing different parts of the human BSSL/CEL gene, namely a *SphI* fragment (pS309), a *SacI* fragment (pS310) and a *BamHI* fragment (pS311), and Figure 1 shows these fragments to cover the BSSL/CEL gene from its 5' untranscribed region to the rest of the intron/exon structure, i.e. 11 exons interrupted by 10 introns (cf. page 26, lines 13 to 33). The deposit numbers of plasmids pS309 and pS310 are also explicitly disclosed in the application (cf. page 29, lines 26 to 32), which further refers to analogues which hybridize with the disclosed (BSSL/CEL) DNA molecules, "*or a specific part thereof*", under stringent hybridization conditions (cf. page 8, lines 29 to 34 and claim 3). Claims 1 and 2 have, therefore, formal support in the application as filed.

2. All other claims are similar or identical to those before the examining division, which, in the decision under appeal, did not raise any objection of added subject-matter. Nor does the board see any reason to raise such an objection itself. Thus, the main request is considered to fulfil the requirements of Article 123(2) EPC.

Articles 83 and 84 EPC

3. The subject-matter of claim 1, directed to a DNA molecule encoding BSSL/CEL, comprises a combination of functional (lipase) and structural (11 exons interrupted by 10 introns) features and further requires the DNA molecule to comprise three restriction fragments each from a different plasmid. Whereas two of these plasmids have been deposited in accordance with the Budapest Treaty at the DSM, namely plasmid pS309 (DSM 7101) and plasmid pS310 (DSM 702) (cf. page 29, lines 26 to 32), there is no deposit information for the third plasmid (pS311).

4. The plasmid pS311 is described in the application as filed as containing a *BamHI* restriction fragment which is structurally characterized by: i) the location and orientation of two members of the Alu class of repetitive DNA elements (cf. page 25, lines 1 to 4 and Figure 1), ii) the specific restriction map, iii) the location within the human BSSL/CEL gene (Figure 1) and iv) the exons and introns of the "*variant of the CEL gene*" covered by this *BamHI* fragment (cf. page 26, lines 27 to 30). The structure of this BSSL/CEL variant is also disclosed in the application, namely "*from a major part of the fifth intron*" (and thus, overlapping the *SacI* fragment of plasmid pS310 which extends "*to a part of the sixth intron*") to "*the rest of the intron/exon structure*", wherein the repetitive sequence of exon 11 encodes "*a truncated variant having 9 repeats*" instead of the known 16 repeats of the BSSL/CEL protein (cf. page 26, lines 30 to 33). In the light of all these structural features, the *BamHI*

fragment of plasmid pS311 is considered to be clearly characterized.

5. In addition to the structural characterization of the *BamHI* fragment of plasmid pS311, the application provides guidance for the construction of this plasmid pS311 as well as the other two plasmids, pS309 and pS310, which are available, respectively, from deposits DSM 7101 and DSM 7102 (cf. Examples 1 and 2). Moreover, since the *SacI* fragment of plasmid pS310 overlaps with the *BamHI* fragment of plasmid pS311 (cf. Figure 1), the former fragment - or an appropriate (*BamHI-SacI*) subfragment(s) thereof - might well be used as a suitable probe(s) for isolating the *BamHI* fragment of the human BSSL/CEL gene (which contains 16 repeats in exon 11 and thus, comprises the 9 repeats of the BSSL/CEL variant of plasmid pS311).

6. It is also worth noting that such a probe - comprising the fifth and sixth exon of the human BSSL/CEL gene - would discriminate the human BSSL/CEL gene (or the *BamHI* fragment thereof) from the closely structurally related human BSSL/CEL-like (BSSL/CELL) (pseudo)gene (or the *BamHI* fragment thereof), since the latter (pseudo)gene contains only five of the exons encoded by the human BSSL/CEL gene (exon 1' and exons 8' to 11') and lacks exons 2 to 7 (cf. page 632, right-hand column, full paragraph and Figure 1 of document A). Moreover, both genes - or BamHI fragments thereof - would be readily discriminated based on the number of the members of the Alu class of repetitive DNA elements and on the differences in their exons 10 and 11 (the latter non-coding in the human BSSL/CELL pseudogene) (cf. paragraph bridging pages 632 and 633 of document A and

Figure 1). Thus, in the light of the whole disclosure of the application, the board considers that it would not require an undue burden for the skilled person to reproduce the subject-matter of claim 1.

7. No objections were raised under Articles 83 and 84 EPC in the decision under appeal for the subject-matter of the other claims. Nor does the board, at present (cf. point 15 *infra*), see any reason to raise such an objection itself.

Articles 87 to 89 EPC and Article 54 EPC

8. The subject-matter of claim 1 enjoys the filing date of the second priority document P2. This document refers to general DNA molecules encoding human BSSL/CEL and containing introns, in particular 11 exons interrupted by 10 introns (cf. *inter alia* page 1, lines 6 to 9, page 8, lines 7 to 12, page 24, lines 4 to 6, claims 1 to 3). Example 2 discloses the plasmids pS309, pS310 and pS311, with the restriction fragments containing the different parts of the human BSSL/CEL gene (cf. page 26, lines 1 to 10 and Figure 1). Document P2 further refers to these three plasmids as being deposited at the DSM on 12 June 1992 (second priority date) (cf. page 28, lines 16 to 18). References are also found to analogues which hybridize with the disclosed BSSL/CEL DNA molecules, "*or a specific part thereof*" under stringent hybridization conditions (cf. page 8, lines 14 to 17 and claim 3). The subject-matter of all other claims is also disclosed in document P2 and therefore, the main request is considered to enjoy the filing date of the second priority document P2 (12 June 1992). Thus, the disclosure of document A,

published on July 1992, is not relevant for the purpose of Article 54 EPC.

9. Since there is no other prior art on file anticipating the claimed subject-matter, the board concludes that, on the evidence on file, the main request fulfils the requirements of Article 54 EPC.

Article 56 EPC

10. Document D2 is considered to be the closest prior art since, in accordance with the criteria established by the case law of the Boards of Appeal (cf. "Case Law of the Boards of Appeal of the EPO", 4th edition 2001, I.D.3.1, 102), this document discloses subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and it has the most relevant technical features in common.

11. Document D2 discloses the complete structure of the human BSSL/CEL cDNA (restriction map, nucleotide and amino acid sequences, etc.) (cf. Figures 1 and 2) and refers to the use of this cDNA for cloning the human BSSL/CEL gene by a typical cloning route, namely screening of a human (lambda bacteriophage) library with a probe derived from an appropriate region of the BSSL/CEL cDNA, selection of positive (lambda) clones and purification by (secondary and tertiary) plating, determination of the restriction map and DNA sequence of the human BSSL/CEL gene (cf. page 16, line 9 to page 17, line 4). Document D2 further refers to the expression of the human BSSL/CEL gene or cDNA in transgenic animals using "*promoters of secretory mammary gland (milk) proteins (such as casein or milk*

lysozyme) ... and appropriate complementing elements" in order to alter the composition of the milk of these transgenic animals, in particular cow and sheep which do not produce BSSL/CEL in their milk (cf. page 22, lines 3 to 16). The use of the recombinant BSSL/CEL protein for supplementation of non-human (infant) milk formulas is also explicitly mentioned in document D2 (cf. *inter alia* page 5, first and second full paragraphs and page 22, last paragraph). However, the human BSSL/CEL gene is not disclosed in the document.

12. Starting from this closest prior art, the technical problem to be solved is seen as the provision of the human BSSL/CEL gene for producing recombinant BSSL/CEL protein in non-human transgenic mammals. This technical problem is solved by the claimed subject-matter.

13. Although it would be obvious to follow the indications given in document D2 for cloning the human BSSL/CEL gene and, as stated by the appellant, some information on the chromosomal (highly polymorphic) locus of the BSSL/CEL gene and on its isolation was already known in the prior art (cf. document D4), the skilled person would have encountered unexpected technical problems and difficulties due to the unknown presence of a closely structurally related human gene, i.e. the human BSSL/CELL (pseudo)gene, in the same chromosomal (9q34-qter) region but in a different locus (cf. page 634, right-hand column to page 635, left-hand column, first paragraph of document A). These difficulties could not have been solved without undue burden as demonstrated by post-published evidence on file (cf. documents A and D6) and acknowledged also in the decision under appeal (cf. page 4, lines 1 to 4 of

the second full paragraph of the decision under appeal). Thus, in accordance with the established case law of the Boards of Appeal (cf. "Case Law", *supra*, I.D.6.2, 117), inventive step is to be acknowledged.

14. The requirements of Article 56 EPC are therefore considered to be fulfilled.

Remittal to the first instance for further prosecution

15. The board notes that the subject-matter of claims 14 to 17 relates to non-human transgenic mammals and the progeny thereof and that there is no reference in the decision under appeal to Article 53a EPC and Rules 23c(b) and 23d(d) EPC. In the light of the established case law (cf. T 315/03, OJ EPO, 2006, pages 15 to 82), a substantial examination of this subject-matter might be required. The introduction of possible amendments might also give rise to other considerations under Articles 84 and 83 EPC. Thus, the board, exercising its power under Article 111(1) EPC, decides to remit the case to the first instance to ensure that this issue will be fully assessed.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance for further prosecution on the basis of the Main Request filed during the oral proceedings.

The Registrar: The Chairman:

A. Wolinski L. Galligani