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Datasheet for the decision of 4 July 2022

Case Number: T 3255/19 - 3.3.07

Application Number: 10730170.7

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Language of the proceedings: EN

Title of invention:
PEGYLATED L-ASPARAGINASE

Patent Proprietor:
Jazz Pharmaceuticals II SAS

Opponent:
Mathys & Squire LLP

Headword:
Asparaginase conjugates / JAZZ

Relevant legal provisions:
EPC Art. 83, 54, 56

Keyword:
Sufficiency of disclosure - (yes)
Novelty - (yes)
Inventive step - non-obvious alternative

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Case Number: T 3255/19 - 3.3.07

D E C I S I O N
of Technical Board of Appeal 3.3.07
of 4 July 2022

Appellant: (Opponent)	Mathys & Squire LLP The Shard 32 London Bridge Street London SE1 9SG (GB)
Representative:	Titmus, Craig Edward Mathys & Squire The Shard 32 London Bridge Street London SE1 9SG (GB)
Respondent: (Patent Proprietor)	Jazz Pharmaceuticals II SAS 84 Quai Charles de Gaulle Cité Internationale 69006 Lyon (FR)
Representative:	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 18 October 2019 concerning maintenance of the European Patent No. 2451486 in amended form.**

Composition of the Board:

Chairman	E. Duval
Members:	M. Steendijk
	Y. Podbielski

Summary of Facts and Submissions

I. European patent 2 451 486 ("the patent") was granted on the basis of fifteen claims.

Independent claim 1 as granted related to:

"A conjugate for use in medicine, the conjugate comprising an L-asparaginase from *Erwinia chrysanthemi* having at least 90% identity to the amino acid sequence of SEQ ID NO:1 and polyethylene glycol (PEG), wherein the PEG has a molecular weight less than or equal to about 5000 Da."

Claim 13 defined the conjugate for use according to any one of claims 1 to 10 by treating a patient for a disease treatable by L-asparagine depletion.

Dependent claim 15 as granted related to:

"The conjugate for use according to claim 13, wherein said patient has had:
(a) a previous hypersensitivity to an *E. coli* L-asparaginase or PEGylated form thereof or a previous hypersensitivity to an *Erwinia* L-asparaginase, wherein optionally said hypersensitivity is selected from the group consisting of allergic reaction, anaphylactic shock, and silent hypersensitivity; or
(b) a disease relapse, optionally wherein said disease relapse occurs after treatment with an *E. coli* L-asparaginase or PEGylated form thereof."

II. The grant of the patent was opposed on the grounds that its subject-matter lacked novelty and inventive step,

that the claimed invention was not sufficiently disclosed and that the patent comprised subject-matter extending beyond the content of the application as filed.

The appeal was filed by the opponent (appellant) against the interlocutory decision of the opposition division that the patent as amended in accordance with auxiliary request 1 was found to meet the requirements of the EPC.

III. The decision under appeal was based on the main request relating to the patent as granted and on auxiliary request 1 filed during the oral proceedings held before the opposition division on 9 September 2019.

The claims of this auxiliary request 1 differed from the claims as granted in that claim 1 defined "A conjugate for use in therapy..." instead of the conjugate for use in medicine and claim 15 was limited to embodiment (b) of claim 15 as granted by deletion of embodiment (a).

In its decision the opposition division cited *inter alia* the following documents:

D1: Gene 46, 25-35 (1986)
D4: Ann . N. Y. Acad. Sci. 1329, 81-92 (2014)
D5: Memorandum by Dr Wade (1985)
D7: Applied Biochemistry and Biotechnology 31, 213-222 (1991)
D8: Journal of Controlled Release 40, 199-209 (1996)
D10: Clin. Pharmacokinet. 44 (4), 367-393 (2005)
D11: Advanced Drug Delivery Reviews 54, 459-476 (2002)
D12: Biodrugs 22, 315~329 (2008)

D13: Critical Reviews in Oncolog1y/Hematology 28, 97-113 (1998)
D15: Journal of Pharmacology and Pharmacotherapeutics 7, 62-71 (2016)
D16: Centre for Applied Microbiology and Research Annual Report and Accounts (1997/1998), 15-17
D17: Report Director Dr H E Wade: "Public Health Laboratory Service (PHLS) Centre for Applied Microbiology and Research. Therapeutic Products Laboratory"
D18: "Health Protection Agency Corporate Plan (2003-2008)"
D23: Declaration by Dr Scawen
D24: Declaration by Dr Beer
D25: FEBS Journal 275, 4306-4316 (2008)
D26: Declaration by Dr Gervais
D27: Extracts from Dr Beer's laboratory notebook
D29: Declaration by Prof. Silman
D30: Annals New York Academy of Sciences 613, 95-108 (1990)
D33: Cancer Chemother. Pharmacol. 73:875-83 (2014)
D35: Centre for Applied Microbiology & Research - Annual Review 1994/95
D36: Pediatric Blood Cancer 65: e26873 (2018)
D37: ClinicalTrials.gov Identifier: NCT02257684
D38: AdisInsight Drug Profile for Pegcrisantaspase
D39: EP 0 211 639 B1

The opposition division arrived at the following conclusions:

(a) The subject-matter of claim 1 as granted included subject-matter extending beyond the content of the application as originally filed.

The subject-matter of claim 1 of auxiliary request 1 complied with the provision of Article 123(2) EPC.

(b) The invention defined in claim 1 of auxiliary request 1 was sufficiently disclosed.

The presented examples related to conjugates with a pegylation degree of 40% and 100% prepared from PEG with molecular weights of 2000 Da and 5000 Da. In view of the reported efficacy of these examples no further definition of a minimum pegylation degree, a pegylation pattern or a minimum molecular weight of the PEG was required for the claimed invention to comply with the requirement of sufficiency.

Taking account of the data provided in the patent concerning the reduced immunogenicity of the conjugate with respect to the unmodified protein reported in example 10, the suitability of the conjugate for therapeutic use as defined in claims 1 and 13 of auxiliary request 1 had been sufficiently disclosed. In view of document D36 it was merely not plausible that a subgroup of patients, which was defined in embodiment (a) of claim 15 as granted and deleted from auxiliary request 1, could be successfully treated.

(c) Document D7 did not disclose any use in therapy for the described PEGylated aspariginase.

Document D8 described the therapeutic utility of a PEG conjugate of asparaginase from *Erwinia carotovora*. However, it had not been proven that the asparaginase as described in document D8 had at

least 90% identity to SEQ:ID 1 as defined in claim 1 of auxiliary request 1.

The subject-matter defined in accordance with auxiliary request 1 was therefore new over the cited prior art.

(d) Document D8 represented the closest prior art, as it related to the same purpose as described in the patent for the claimed subject-matter, namely the therapeutic use of a PEGylated asparaginase enzyme from *Erwinia*. The difference between the claimed subject-matter and this prior art concerned the definition of the type of asparaginase.

It was not obvious to use asparaginase of the defined SEQ:ID 1 for providing an alternative PEGylated asparaginase for therapeutic use taking account of the teaching in document D25 that the asparaginase from *Erwinia carotovora* represented a more promising choice than the asparaginase from *Erwinia chrisantemi*. Even if the skilled person would consider the asparaginase from *Erwinia chrisantemi*, the teaching in the prior art would not suggest to use the asparaginase with at least 90% identity to the SEQ:ID 1 as defined in claim 1 of auxiliary request 1.

The subject-matter of auxiliary request 1 therefore also involved an inventive step.

IV. With the statement setting out the grounds of appeal the appellant contested the findings in the decision under appeal concerning the requirements of sufficiency of disclosure, novelty and inventive step and filed *inter alia* the following documents:

D42: Cossar et al., Biosensors & Bioelectronics 5 (1990) 273-289
D43: ClinicalTrials.gov record for trial NCT01551524

V. With the reply to the appeal the patent proprietor (respondent) upheld the request found to be allowable in the decision under appeal as its main request and filed auxiliary requests 1-8.

VI. With the summons of 12 July 2021 the Board invited the parties to attend oral proceedings on 11 July 2022.

In the communication pursuant to Article 15(1) RPBA of 14 October 2021 the Board expressed its preliminary opinion that the main request fulfilled the requirement of sufficiency of disclosure and summarized the parties' arguments with respect to novelty in view of D7 an D8, and inventive step in view of document D8, which were to be discussed during the oral proceedings.

With the letter of 13 May 2022 the appellant announced not to attend the oral proceedings.

The oral proceedings were cancelled with the communication of 31 May 2022.

VII. The arguments of the appellant relevant to the present decision can be summarized as follows:

(a) Admittance of documents D42 and D43

Documents D42 and D43 were filed as a justified response to the reasons presented in the decision under appeal.

(b) Sufficiency of disclosure

The termination of the clinical trial reported in documents D36-D38 following hypersensitivity reactions and rapid clearance of asparaginase activity in three of the four participating patients showed that the conjugate of claim 1 was not safe and not efficacious for use with patients with previous hypersensitivity to PEGylated *E. coli* asparaginase. The treatment of such hypersensitive patients evidently remained, as recognized in the patent (see paragraph [0011]), at the core of the claimed invention and the reason for the failure was inherent to the defined conjugation with PEG. The mention of favourable toxicity and efficacy in treatment of adult patients in document D36 was based on the clinical trial described in document D43, in which patients with previous hypersensitivity to PEGylated *E. coli* asparaginase were deliberately excluded. The reported trial involving adult patients was therefore immaterial to the lack of sufficient disclosure of the claimed invention demonstrated by the failure of the trial with hypersensitive patients reported in document D36.

The claimed therapeutic effects could further not be achieved over the whole scope of claim 1 due to the lacking definition of a lower limit for the degree of pegylation and of the lower limit for the PEG molecular weight as well as the lacking definition of a pegylation pattern.

(c) Novelty

Document D7 not only described a relevant PEG conjugate with *Erwinia* asparaginase, but also its use in therapy and therefore deprived the claimed subject-matter of novelty.

Document D8 described the therapeutic utility of a PEG conjugate with an *Erwinia* asparaginase. The skilled person was aware that references to the clinical use of *Erwinia* asparaginase concerned Porton Down's Erwinase(R), because this product was the only *Erwinia* asparaginase approved for therapeutic treatment (see documents D4, D8, D10, D13, D15-D18) and because Porton Down was the sole manufacturer of Erwinase(R) (see documents D13 and D35). The skilled reader therefore immediately understood that the *Erwinia* asparaginase used for the conjugates described in document D8 was Erwinase(R) from Porton Down. As evidenced by documents D1, D4, D5, D29 and D42 as well as documents D23, D24, D26 and D27 this asparaginase had always been produced from NCPPB 1066. The asparaginase from NCPPB 1066 was identical to SEQ ID NO:1, which was evident from documents D1 and D39 and recognized in the patent. Accordingly, document D8 described the therapeutic utility of the same PEGylated *Erwinia* asparaginase as defined in the claims of the patent.

(d) Inventive step

In as far as document D7 was not considered to disclose a therapeutic use of the described conjugate, such therapeutic use would have been obvious to the skilled person. It was common

general knowledge that the described asparaginase was approved for use in therapy. Moreover, pegylation was a well-known strategy for improving the pharmacokinetic properties of biopharmaceuticals, which was evident from document D12 and reflected in documents D7, D10, D11 and D30.

In as far as with respect to document D8 the claims were considered to define a conjugate with a different asparaginase, the replacement of the asparaginase in document D8 with the *Erwinia* asparaginase as defined in the patent would have been quite obvious to the skilled person, as the defined asparaginase corresponds to the only *Erwinia* asparaginase that was at the relevant time actually in clinical use, namely Erwinase(R) as obtained from NCPPB 1066. No comparison showed any improved activity for the claimed conjugate. Document D25 confirmed the known clinical use of asparaginase from *Erwinia chrysanthemi* and referred to asparaginase from *Erwinia carotovora* as having only limited potential. Moreover, taking account of the enhancement of asparaginase efficacy by glutamine deamination described in document D10, the glutaminase activity of *Erwinia chrysanthemi* mentioned in document D25 could be expected to contribute to the efficacy in therapy.

VIII. The arguments of the respondent relevant to the present decision can be summarized as follows:

(a) Admittance of documents D42 and D43

Documents D42 and D43 lacked relevance.

(b) Sufficiency of disclosure

Having regard to the well known therapeutic use of L-asparaginase from *Erwinia chrysantemi* (Erwinase(R)) and the enhanced potency as well as the reduced immunogenicity resulting from pegylation as demonstrated in examples 7-10, the patent plausibly disclosed the suitability of the claimed conjugates for use in therapy. The skilled person, who was aware that few pharmaceuticals are effective in all patients, was not confronted with any undue burden in carrying out the claimed invention when facing treatment failure as reported in document D36. This failure only concerned a small number of patients with pre-existing anti-PEG IgG antibodies (three out of four) belonging to a specific difficult-to-treat paediatric population. Document D36 itself actually confirmed the therapeutic utility in asparaginase naive adult patients.

With the examples in the patent at hand the skilled person was furthermore well able to prepare the therapeutically useful conjugates within the whole scope of the claims.

(c) Novelty

Document D7 described the pegylation of various proteins, including an *Erwinia chrysanthemi* aspariginase. Reference was made to the effects of pegylation on the biological properties of proteins in general, but no therapeutic utility of the described PEGylated aspariginase was disclosed.

Document D8 referred to asparaginase from *Erwinia carotovora*, which did not correspond to asparaginase from *Erwinia chrysanthemi* having at least 90% identity to SEQ ID NO:1. In line with the declaration in document D5 and as demonstrated by *inter alia* document D1 the taxonomic definition of *Erwinia carotovora* was at the time of publication of document D8 not used to indicate any *Erwinia chrysanthemi* strain as defined in the patent. It could further not be derived from document D8, which mentioned neither Erwinase(R) nor NCPPB 1066, that the asparaginase of the conjugate described in document D8 corresponded to the asparaginase as defined in the patent.

(d) Inventive step

Document D8 represented more pertinent prior art than document D7, which failed to provide any pointer towards the therapeutic use of the described enzymes.

The claimed invention differed from the teaching in document D8 in that the asparaginase of the conjugate of document D8 showed less than 90% identity with SEQ ID NO:1. This difference was associated with a higher glutaminase activity. In line with the post-published document D33 this higher glutaminase activity should contribute to the improved anti-tumor activity of the claimed conjugates. The prior art provided no suggestion towards the claimed conjugate as a solution to the problem of providing an alternative therapeutic agent, let alone as a solution of providing an improved agent. Document D25 would actually favour asparaginase from *Erwinia carotovora* over

asparaginase from *Erwinia chrysanthemi* for use in therapy. Moreover, in view of the difference in sequence between the asparaginases from *Erwinia carotovora* and *Erwinia chrysanthemi*, in particular the positions of the lysine residues that may be PEGylated, and taking account of the uncertain effect of differences in the pegylation pattern as mentioned in document D11 it could not be expected that the therapeutic utility of the conjugate of document D8 was preserved in the conjugate of the patent.

IX. The appellant requested that the decision under appeal be set aside and that the patent be revoked in its entirety. The appellant further requested admission of documents D42 and D43 into the proceedings.

X. The respondent requested that the appeal be dismissed.

Subsidiarily the respondent requested that the patent be maintained on the basis of any of auxiliary requests 1-8 as filed with the reply to the appeal.

The respondent further requested that documents D42 and D43 not be admitted into the appeal proceedings.

Reasons for the Decision

1. Admittance of documents D42 and D43

1.1 Document D42 refers (see page 276, under "Enzymes") to asparaginase enzyme assays using Erwinase(R) from the Division of Biotechnology (Porter Down) as well as a whole cell assay for asparaginase activity in *Erwinia*

chrysanthemi (NCPPB 1066). This document was submitted by the appellant with the statement of grounds of appeal to support the argument that, contrary to the finding in the decision under appeal, the commercial Erwinase(R) product was known to be identical in sequence with NCPPB 1066.

1.2 Document D43 provides details regarding the clinical trial NCT01551524, which concerned treatment of adult patients with hematological malignancy with PEGylated *Erwinia chrysanthemi* asparaginase. The document indicates that the trial excluded patients who had experienced grade 3 allergic reaction against *E. coli* asparaginase or Erwinase(R) (see D43, "Exclusion criteria"). The appellant submitted document D43 with the statement of grounds of appeal to support the argument that the reference to positive results from a clinical trial in document D36 cited in the decision under appeal does not affect the lack of sufficient disclosure for achieving effective treatment across the scope of the claims.

1.3 Having regard to Article 12(4) RPBA 2020 the Board has admitted documents D42 and D43 into the appeal procedure.

Main request

2. Sufficiency of disclosure

2.1 The patent presents in examples 7-10 experimental results demonstrating that pegylation of Erwiniase(R) with 2000 Da or 5000 Da PEG at a degree of 40% or 100% of the available sites allows for increased potency, prolonged half-life and reduced immunogenicity (see Figures 4-11 and Tables 3-5). These experimental

results have as such not been challenged by the appellant.

2.2 In view of the experimental results presented in the patent for the PEG conjugated Erwinia asparaginase defined in the claims and taking account of the undisputed known clinical use of the corresponding unconjugated Erwinia asparaginase, the Board is satisfied that the patent sufficiently discloses the suitability of the defined conjugate for the claimed use in therapy, in particular in treatment of a disease treatable by L-asparagine depletion. As noted in the decision under appeal (see pages 8-9, section 7.2), the suitability of a conjugate as defined in the patent for such treatment has in fact been confirmed in the post-published document D36 (page 2, right column), be it that in view of document D43 the effective treatment reported in document D36 will have excluded patients who had experienced grade 3 allergic reaction against *E. coli* asparaginase or Erwinase(R) (see D43, "Exclusion criteria").

The failure of treatment using a conjugate as claimed in 3 out of 4 patients with pre-existing hypersensitivity to pegaspargase (PEGylated asparaginase from *E. coli*) due to hypersensitive responses reported in document D36 (see abstract and page 5, left column, under "Discussion") does not imply that the skilled person is confronted with an undue burden when carrying out the claimed invention aimed at effective treatment.

As pointed out by the respondent, the skilled person is well aware that a therapeutic treatment which is generally effective, may still fail in individual cases. Reports of occasional failure do therefore not

necessarily call the validity of an otherwise generally effective method of therapeutic treatment into question, in particular in case such failure is reported in a subgroup of patients which would have been recognized by the skilled person as particularly difficult to treat. The failure of treatment in 3 out of 4 patients reported in document D36 concerned patients which may well be recognized as a difficult-to-treat subgroup of pediatric patients in view of their pre-existing hypersensitivity to pegaspargase.

As further pointed out by the respondent, the patent describes in paragraph [0072] a variety of conditions which are treatable by depletion of asparagine, including first line treatment as well as second line treatment. The Board finds therefore no basis for the appellant's contention that the treatment of patients with hypersensitivity to pegaspargase represented the core of the invention, in view of which the failure reported in document D36 should not be regarded as merely incidental.

The Board therefore considers that the failed treatment in 3 out of 4 of the particular group of pediatric patients reported in document D36 does not cast serious doubt on the utility of the conjugates in therapy, in particular in the treatment of diseases responsive to asparagine depletion as defined in the claims.

2.3 In view of the variation in the degree of pegylation (40% and 100%) and in the size of the PEG (2000 Da and 5000 Da) in the mentioned examples of the patent, the Board is further satisfied that the person skilled in the art finds in the patent sufficient guidance to prepare the defined therapeutically active conjugates

within the whole scope of the claims without undue burden.

In this context the Board observes that, whilst document D11 (see page 462, left column) cautions that the pattern of pegylation may influence the activity or binding characteristics of PEG modified proteins, the patent shows in the mentioned examples that the defined conjugates retain activity even at 100% pegylation. Without evidence to the contrary it is therefore reasonable to assume that for defined conjugates to retain activity no particular pattern of pegylation is required.

2.4 Accordingly, the Board concludes that the patent sufficiently discloses the claimed invention.

3. Novelty

3.1 Document D7

3.1.1 Document D7 reports on investigations concerning polyethylene glycol modified proteins, including *Erwinia chrysanthemi* asparaginase from PHLS Centre Porton Down modified with MPEG 5000 (see D7, page 215, lines 6-7 and 14; see also page 218, Table 1).

The appellant maintained that the skilled person derives the therapeutic utility of the modified proteins directly and unambiguously from the following passages in document D7:

"The covalent binding of monomethoxy(polyethylene glycol) (MPEG) to protein surfaces is a methodology that is acquiring increased application in biotechnology. This is because MPEG confers to the

modified proteins or enzymes new physicochemical properties that can be exploited in fields from biocatalysis to pharmacology. [...] MPEG-modified proteins present quite different biological properties in vivo with respect to the native enzyme: longer half-life time in blood, resistance against proteolytic enzymes, as well as reduced immunogenicity and antigenicity" (see D7, page 214, lines 5-16) and

"Using proteins SOD and RNase as model, we already found no difference in blood residence time between the protein modified with norleucine arm or with MPEG directly bound to protein prepared with standard methods" (see D7, page 221, lines 10-13).

3.1.2 The Board observes, however, that the first cited passage of document D7 only refers to the potential use in pharmacology and the in vivo biological properties of PEGylated proteins in general and that the second cited passage only reports on the blood residence times of modified proteins as determined using SOD and RNase as a model without specific disclosure of any therapeutic utility of the described asparaginase conjugate. The Board therefore considers that the opposition division correctly concluded in the decision under appeal (see pages 9-10 section 8.1) that document D7 does not describe the therapeutic utility of a PEG conjugate with *Erwinia* asparaginase as defined in claim 1 of the main request.

3.2 Document D8

3.2.1 Document D8 describes *inter alia* the preparation of a conjugate of asparaginase with 5000 Da PEG (see D8, abstract). The used asparaginase is described as asparaginase from *Erwinia carotovora* supplied by the

Center for Applied Microbiology and Research (Porton, Salisbury, UK) (see D8, page 200, right column, section 2, "Materials and methods").

Document D8 further observes in section 1 "Introduction" (see page 199, right column, lines 5-10):

"The spectrum of asparaginase dependent human tumours has turned out to be relatively narrow, and at the present asparaginases isolated from the microbial sources *Escherichia coli* and *Erwinia carotovora* are clinically used only for the treatment of acute lymphoblastic leukaemia".

3.2.2 As explained in document D5 the "ERWINASE" producing organism, NCPPB 1066 was originally classified as *Erwinia carotovora*, but was reclassified as *Erwinia chrysanthemi* early in the 1970s. Document D8 was published well after this re-classification (1996). The skilled person had therefore good reason to derive from document D8 that the asparaginase used for the preparation of the described conjugates was indeed obtained from *Erwinia carotovora* and not from *Erwinia chrysanthemi*. According to the patent (see paragraph [0031], table 1) asparaginases from *Erwinia carotovora* show only 75-77% sequence identity with the sequence of SEQ ID NO:1 of the patent. The conjugates described in document D8 are therefore not considered to correspond to the conjugate defined in claim 1 of the patent.

3.2.3 The appellant relied on the argument that by the identification of the supplier of the used asparaginase and the reference to the clinical use of asparaginase from *Erwinia carotovora* in document D8 it was evident to the skilled reader that the asparaginase used in

document D8 was actually the product Erwinase(R) produced by Porter Down from NCPPB 1066, which corresponded to SEQ ID NO:1 defined in the patent. The appellant relied in this context on evidence that Porter Down was the sole manufacture of Erwinase(R) (see documents D13 and D35), that Erwinase(R) was the only *Erwinia* asparaginase approved for clinical use (see documents D4, D8, D10, D13, D15-D18), and that Erwinase(R) has consistently been produced from NCPPB 1066 (see documents D1, D4, D5, D23, D24, D26, D27, D29 and D42).

3.2.4 The Board considers this argument not convincing.

The circumstance that the supplier of the *Erwinia carotovora* asparaginase used in document D8 to prepare the conjugate was also known as the sole manufacturer of the commercially available Erwinase, does not imply that this manufacturer could not have supplied any other *Erwinia* asparaginase. The mentioned circumstance does therefore not allow the skilled reader to conclude that the asparaginase used for the conjugates in document D8 must have been Erwinase(R).

As argued by the appellant the skilled person may in view of the presented evidence (see item 3.2.3 above) further have realized that the reference to a clinically used asparaginase isolated from *Erwinia carotovora* in the introduction of document D8 concerned the product Erwinase(R) produced from NCPPB 1066. As explained in document D5, NCPPB 1066 was indeed originally classified as *Erwinia carotovora*. However, the Board considers that following the established re-classification of NCPPB 1066 as *Erwinia chrysanthemi* well before the publication of document D8 it cannot be concluded that document D8 referred in the subsequent

section "Materials and methods" with asparaginase from *Erwinia carotovora* also to the clinically used Erwinase(R) produced from NCPPB 1066.

3.2.5 The skilled reader could therefore not directly and unambiguously derive from document D8 that the asparaginase from *Erwinia carotovora* used for the preparation of the described conjugates corresponded to an asparaginase from *Erwinia chrysanthemi* having 90% identity to SEQ. ID NO:1 as defined in the claims of the patent.

3.3 Accordingly, the Board concludes that the claimed subject-matter is new over documents D7 and D8.

4. Inventive step

4.1 Closest prior art

The patent is directed to stable PEGylated asparaginase conjugates with enhanced pharmacokinetics and reduced immunogenicity (see paragraphs [0015] to [0020], see also section 2.1 above).

Document D8 reports on the preparation, activity, stability, pharmacokinetics and immunological properties of PEGylated *Erwinia* asparaginase conjugates (see abstract, see page 200, right column under Materials and methods).

Document D7 reports on a method for the evaluation of the polymer content in polyethylene glycol modified proteins, including chrysanthemi asparaginase (see page 213, abstract). This document only mentions the potential use in pharmacology and in vivo biological properties of PEGylated proteins in general and fails

to specifically disclose any therapeutic utility of the described asparaginase conjugate (see section 3.1 above).

Document D8 thus relates to the same purpose as the claimed invention and therefore represents a more promising starting point than document D7, which is concerned with an entirely different purpose.

Accordingly, document D8 is considered to represent the closest prior art.

4.2 Problem to be solved

The difference between the claimed subject-matter and the teaching of document D8 concerns the definition of the L-asparaginase from *Erwinia chrysanthemi* having at least 90% identity to the amino acid sequence of SEQ ID NO:1 instead of the asparaginase from *Erwinia carotovora* (see section 3.2 above).

The patent demonstrates in its examples that the pegylation of the defined aspariginase allows for increased potency, prolonged half-life and reduced immunogenicity with respect to the unmodified aspariginase (see section 2.1 above). In the absence of comparative data with respect to the PEGylated asparaginase of document D8 the problem solved is seen in the provision of an alternative PEGylated asparaginase with therapeutic utility.

4.3 Assessment of the solution

The pegylation described in document D8 involves the conjugation of activated PEG to the amino groups of the asparaginase (see D8, page 203, left column, section

3.1). Document D11, which reviews the chemistry of pegylation of biological marcromolecules, indicates that the most common route for PEG conjugation of proteins is via the amino group of lysine residues (see pages 461-462, bridging paragraph). Document D11 notes that when multiple positions for conjugation are available, the pegylation typically results in heterogeneous mixtures due to differences in the degree and the positions of conjugation. In this context document D11 specifically cautions that the positional isomers are likely to influence whether or not the conjugate is active (see page 462, left column, lines 28-31).

As explained in the patent (see paragraph [0031]), asparaginase from *Erwinia carotovora* shows only 75-77% identity with asparaginase from *Erwinia chrysanthemi*. Moreover, as pointed out by the respondent in the reply to the statement of grounds of appeal (see pages 7-8, sections 3.9), alignment of the reference SEQ ID NO:1 presented in the patent for *Erwinia chrysanthemi* asparaginase with the sequence of the aspraraginase from *Erwinia carotovora* (GENBANK ACCESSION NO. AAP92666) shows that the lysine residues are not well conserved. Of the 20 lysine residues in the asparaginase in *Erwinia carotovora* 7 residues are not conserved in *Erwinia chrysanthemi*, whilst 5 lysine residues in *Erwinia chrysanthemi* are not matched in *Erwinia carotovora*. Moreover, document D9 indicates that *Erwinia chyrsanthemi* asparaginase includes a lysine residue in its active site (see D9, page 198, line 1 and page 200, lines 2-6).

In view of the significant differences between the asparaginases from *Erwinia carotovora* and *Erwinia chrysanthemi*, including the positions of multiple sites

for pegylation, and the unpredictability of the effects of these differences on the activity of the enzyme after pegylation, the person skilled in the art had no reasonable expectation of success that replacement of the asparaginase of document D8 by an asparaginase from *Erwinia chrysanthemi* having 90% identity to the amino acid sequence of SEQ ID NO:1 would yield a therapeutically active conjugate.

The appellant's argument that in view of documents D25 and D10 the skilled person would consider asparaginase from *Erwinia chrysanthemi* better suited for therapeutic treatment than asparaginase from *Erwinia carotovora* and accordingly exchange the asparaginase in the conjugate of document D8 is not considered convincing, because this argument does not take account of the unpredictability of the activity of the enzyme after pegylation.

The subject-matter of claim 1 would therefore not be obvious to the skilled person as solution to the problem of providing an alternative therapeutically active PEGylated asparaginase conjugate.

4.4 Accordingly, the Board concludes that the claimed subject-matter also involves an inventive step.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

B. Atienza Vivancos E. Duval

Decision electronically authenticated