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**Datasheet for the decision
of 25 September 2017**

Case Number: T 1822/13 - 3.3.04

Application Number: 07818168.2

Publication Number: 2068935

IPC: A61K47/48, C07K14/47,
C07K14/52, A61M25/00

Language of the proceedings: EN

Title of invention:

Polymer conjugates of Box-A of HMGB1 Box-A and Box-A variants
of HMGB1

Patent Proprietor:

Creabilis Therapeutics S.R.L.

Opponent:

Bio3 Research S.R.L.

Headword:

Polymer conjugates of HMGB1 Box-A/CREABILIS

Relevant legal provisions:

EPC Art. 56

Keyword:

Inventive step - (no)

Decisions cited:

T 0936/96

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

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Case Number: T 1822/13 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 25 September 2017

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Decision under appeal: **Decision of the Opposition Division of the
European Patent Office posted on 25 June 2013
rejecting the opposition filed against European
patent No. 2068935 pursuant to Article 101(2)
EPC.**

Composition of the Board:

Chairman G. Alt
Members: A. Chakravarty
P. de Heij

Summary of Facts and Submissions

- I. European patent No. 2 068 935, entitled "*Polymer Conjugates of Box-A of HMGB1 and Box-A Variants of HMGB1*" was opposed under Article 100(a) EPC in conjunction with Article 56 EPC on the ground that its subject-matter lacked an inventive step.
- II. The patent was granted with claims 1 and 2 as follows:

"1. Polymer conjugate of the human and/or non-human wild type HMGB1 high affinity binding domain Box-A (HMGB1 Box-A) or of a biologically active fragment of HMGB1 Box-A.

2. Polymer conjugate of a polypeptide variant of the human and/or non-human HMGB1 high affinity binding domain Box-A (HMGB1 Box-A) or of a biologically active fragment of HMGB1 Box-A, whereby the amino acid sequence of said polypeptide variant differs from the amino acid sequence of the wild type HMGB1 Box-A by the mutation of one or more single amino acid".
- III. The opposition division decided to reject the opposition. An appeal was filed by the opponent (appellant) against this decision. The patent proprietor is respondent to this appeal.
- IV. With the statement of grounds of appeal, the appellant submitted documents D11 and D12 (see below, section IX).
- V. The respondent replied to the appellant's statement of grounds of appeal and submitted six sets of claims as auxiliary requests I to VI.

Claim 1 of auxiliary requests I and II is identical to claim 1 of the main request (claims as granted, see section II above). Claim 1 of auxiliary request III is identical to claim 2 of the main request.

Claim 1 of auxiliary request IV reads:

"1. Polymer conjugate of a polypeptide variant of the human and/or non human HMGB1 high affinity binding domain Box-A (HMGB1 Box-A) or of a biologically active fragment of HMGB1 Box-A, whereby the amino acid sequence of said polypeptide variant differs from the amino acid sequence of the wild type HMGB1 Box-A by the mutation of one or more single amino acid, wherein the polypeptide variant is selected from the group consisting of SEQ ID NOs: 33, 35, 37-39, 42-45, 47-49, 52, 55, 57, 59, 62, 64, 67, 69, and 104".

In claim 1 of auxiliary request V "the polypeptide variant is selected from the group consisting of SEQ ID NOs: 45, 49, 52, 55, 59, 64, and 67", while claim 1 of auxiliary request VI specifies that "the polypeptide variant is selected from SEQ ID NO:64" (*sic*).

- VI. The board issued a summons to oral proceedings. In a letter replying to this summons, the appellant informed the board that it would not be represented at said oral proceedings.
- VII. The board issued a communication pursuant to Article 15(1) RPBA, setting out its preliminary appreciation of some of the substantive and legal matters concerning the appeal.

- VIII. Oral proceedings before the board were held in the absence of the appellant, in accordance with Rule 115(2) EPC and Article 15(3) RPBA on 25 September 2017.
- IX. At the oral proceedings, the respondent withdrew their objection that the appellant's opposition was inadmissible. They also withdrew their request to disregard documents D7, D11 and D12.
- X. At the end of the proceedings, the Chairman announced the decision of the board.
- XI. The following documents are referred to in this decision.

D1: EP 1 368 060

D2: WO 2002/060929

D3: WO 2004/056862

D4: Tsubery H *et al.*, Journal Of Biological Chemistry, vol. 279, no. 37, 10 September 2004, pages 38118-38124.

D5: Yang Z *et al.*, Cancer Research, vol. 64, 15 September 2004, pages 6673-6678.

D6 Roberts M J *et al.*, Advanced Drug Delivery Reviews, vol. 54, no. 4, 17 June 2002, pages 459-476.

D7 WO 2006/024547

D8 Pasut G *et al.*, Expert Opin. Ther. Patents, 2004, vol. 14(6), pages 859-894.

D11: Eliason J F, BioDrugs , 2001, vol. 15, no. 11, pages 705-711.

D12: Harris J M and Chess R B, Nature Reviews Drug Discovery, 2003, no. 2, 214-221.

XII. The appellant's arguments, made in writing, are summarised as follows:

Inventive step (Article 56 EPC)

Main request - claims 1 and 2

Closest prior art

Document D7 disclosed human and/or non-human wild type high affinity binding domain Box-A (HMGB1 Box-A) protein, as well as fragments and variants thereof. These proteins acted as an antagonist of the treatment of pathological conditions induced and/or sustained by HMGB1. The same pathologies were treated by the conjugates of the contested patent (see statement of grounds of appeal, point 4.1.2).

Document D7 and the claimed invention were directed to the same purpose and had the most relevant technical (structural) features in common and should therefore be considered as representing the closest prior art for the invention.

The objective technical problem

The difference between the molecules of claim 1 and those disclosed in document D7 was that the former were conjugated polymer conjugates of the latter, in particular they were PEGylated versions thereof. The

technical effect associated with this feature was an improved pharmacokinetic profile vis-à-vis the unconjugated proteins. The problem solved by the claimed invention was therefore the provision of a HMGB1 Box-A protein or fragment or a variant thereof with an improved pharmacokinetic profile.

Obviousness

Motivation for the skilled person to improve the pharmacological profile of any given drug had been recognised in the decision under appeal and was further supported by document D7 (see page 1, lines 8-14) which disclosed attempts to improve the pharmacological profile of HMGB1 Box-A proteins by mutation.

It was common general knowledge in the art that the distinguishing feature of the invention, conjugation of therapeutically active proteins to a polymer, in particular to polyethylene glycol (PEGylation) could improve their pharmacokinetic profile. This was illustrated, for instance in documents D6 (page 460 column 1, 2nd and 3rd paragraph), D11 (page 709, chapter 6) and D12 (pages 215, 217 and 219).

The person skilled in the art faced with the problem of improving the pharmacokinetic profile of HMGB1 Box-A protein or variants thereof, would have used his common general knowledge that PEGylation was a suitable strategy, to arrive at the claimed solution. The claimed polymer conjugates were therefore an obvious solution to the objective technical problem. Furthermore, the skilled person considering PEGylation of the known HMGB1 Box-A proteins as a solution to the technical problem had a reasonable expectation of success. The opposition division was mistaken in

holding that documents D6 and D8 disclosed that PEGylation also had undesirable effects. In fact, the reduced activity of PEGylated dimeric G-CSF disclosed in document D8 was not necessarily ascribable to PEGylation. Although there were cases in the art in which PEGylation was associated with loss of therapeutic activity or had no effect on the half life (of the PEGylated substance), the successful cases overwhelmed these few particular cases.

Auxiliary requests

The appellant did not submit any arguments in respect of the auxiliary requests.

- XIII. The respondent's arguments, made in writing and during the oral proceedings, are summarised as follows:

Inventive step (Article 56 EPC)

Main request - claims 1 and 2

Closest prior art

Document D7 could be taken to represent the closest prior art. It disclosed variants of the HMGB1 Box-A protein in which the sequence had been mutated from the wild type in order to make them more resistant to proteases (cf. document D7, page 1, paragraph 1). However, document D7 did not contain any experimental results about the actual pharmacokinetic profile of the variants disclosed. The particular HMGB1 Box-A variants disclosed in document D7 were, in fact, unconjugated versions of those exemplified in the patent in suit.

The objective technical problem

The technical effects of conjugation to polyethylene glycol (PEG), as noted in paragraph [0021] of the patent, were "*improving the pharmacologic and toxicologic properties*" of HMGB1 Box-A proteins, in particular "*[extended] circulation half life, [...] [increased...] protein solubility, stability and decrease[d...] protein immunogenicity*". In view of these technical effects, the technical problem underlying the invention was the provision of HMGB1 inhibitors with improved pharmacologic/pharmacokinetic properties.

Obviousness

The claimed HMGB1 Box-A/polymer conjugates solved this problem, as shown by Example 3 of the patent. However, the claimed solution would not have been obvious to the skilled person starting from document D7. There was no hint in document D7 or anywhere else in the prior art that the pharmacokinetic profile of inhibitors of HMGB1 could be improved at all, let alone via polymer conjugation.

Although PEGylation was a commonly known technique for achieving the technical effects mentioned in paragraph [0021] of the patent, it was not common general knowledge that PEGylation of proteins always improved their pharmacokinetic profiles. Documents D6, D11 and D12 disclosed specific examples of proteins where PEGylation had been more or less successfully tested, but this did not amount to a teaching that PEGylation could be successfully used to improve the pharmacokinetic profile of any given protein. Document D8 disclosed that PEGylation of IFN- β and IL2, both

cytokines binding to a cell surface receptor, did not result in any improvement of their pharmacokinetic profile or led to complete inactivation of the protein. The authors also regarded PEGylation as "*revolutionary*", illustrating that PEGylation was not a straightforward process for improving the pharmacological profile of proteins.

Documents D6 and D12 demonstrated that the person skilled in the art could have adopted various different technical strategies when seeking for an improvement of the pharmacokinetic and pharmacodynamic properties of biomolecules. Document D6 (page 460, left column, second paragraph) disclosed manipulation of the amino acid sequence, fusion or conjugation to immunoglobulins and serum proteins and incorporation in drug delivery vehicles for protection and conjugation to natural or synthetic polymers as possible strategies for improving the pharmacokinetic properties of drugs.

In the case at hand, the HMGB1 Box-A protein was both small (54 amino acids; see document D7, Figure 3a) and highly conserved among mammals (see document D7, page 2, paragraph 2). Thus, the skilled person would have realised that PEGylation was likely to have adverse effects on bioactivity. Moreover, the bulky size of PEG would have given the skilled person concerns about potential interference with the Box-B binding site on the target, when the PEGylated Box-A protein was bound to the Box-A binding site. The skilled person would therefore not have expected PEGylation to be the successful choice from amongst the different strategies available to improve its pharmacokinetic and pharmacodynamic profile.

The disclosure in document D7, that care was needed to preserve physico-chemical properties when carrying out mutagenesis (see page 11, paragraph 3), would also have made the skilled person sceptical about whether biological activity would be retained after PEGylation. This was particularly relevant for the HMGB1 Box-A protein which acted as a competitive inhibitor of the wild-type protein. The skilled person would have realised that any loss in target binding affinity due to PEGylation would lead to a loss of function as a competitive antagonist. The fact that the HMGB1 Box-A protein was lysine rich was a further problem faced by the skilled person, since lysine was the amino acid at which PEGylation commonly took place. The skilled person would have been concerned that PEGylation might interfere with the protein's biological activity.

In summary, the skilled person would not have considered that PEGylation offered a reasonable expectation of success for solving the problem of provision of HMGB1 inhibitors with improved pharmacologic/pharmacokinetic properties.

Auxiliary requests

Auxiliary requests III to VI related to HMGB1 Box-A variants. The arguments on inventive step given for the main request applied equally to these. Moreover, even in their unconjugated form, these variants exhibited better than expected activity in a chemotaxis assay (see paragraph [0082] and Figure 7.3 of the patent) and better than expected protease resistance (see Example 2, paragraph [0090] and Figure 10 of the patent). Moreover, Example 3 of the patent demonstrated a synergistic effect in the improvement of the

pharmacokinetic profile of the PEGylated Box-A polypeptides with Box-A variant number 64 as Example. These were reasons why an inventive step should be recognised for the subject-matter of auxiliary requests III to VI, which went beyond the reasons already given for the subject-matter of the main request.

- XIV. The appellant requested that the decision of the opposition division be set aside and that the patent be revoked.
- XV. The respondent requested that the appeal be dismissed or, alternatively, that the decision under appeal be set aside and that the patent be maintained on the basis of one of auxiliary requests I to VI.

Reasons for the Decision

1. The appeal complies with Articles 106 to 108 and Rule 99 EPC and is therefore admissible.
2. The appellant did not attend the oral proceedings and is treated as relying on the written case (Article 15(3) RPBA).

Introduction

3. High affinity binding domain (HMGB1) protein belongs to the family of high mobility group proteins which play a generalised "architectural" role in DNA bending, looping, folding and wrapping, since they either distort, bend or modify DNA structures and complexes with transcription factors or histones. The HMGB1 protein is usually a nuclear factor, in particular a transcriptional regulatory molecule causing DNA bending

and facilitating the binding of several transcriptional complexes (see paragraph [0002] of the patent). The HMGB1 molecule has a tripartite structure composed of three distinct domains: two DNA binding domains called HMG Box-A and Box-B, and an acid carboxyl terminus, making it bipolarly charged (see paragraph [0003] of the patent). The invention relates to polymer conjugates of the HMGB1 high affinity binding domain Box-A (HMGB1 Box-A) or of a biologically active fragment of HMGB1 Box-A and to polymer conjugates of polypeptide variants of HMGB1 Box-A or of a biologically active fragment of HMGB1 Box-A and their use as a medicament for the prevention, alleviation or treatment of HMGB1-associated pathologies or pathologies associated with the HMGB1 homologous proteins, particularly selected from pathological conditions mediated by activation of the inflammatory cytokine cascade (see claim 16 of the patent).

Inventive step (Article 56 EPC)

Main request - Claims 1 and 2

4. Claim 1 is for a polymer conjugate of the human and/or non-human wild type HMGB1 high affinity binding domain Box-A (HMGB1 Box-A) or of a biologically active fragment of HMGB1 Box-A. Claim 2 is as claim 1 but for variants of HMGB1 Box-A or of a biologically active fragment thereof in which of one or more single amino acids are mutated from the wild type.
5. The purpose of the claimed polymer conjugates is the diagnosis, prevention, alleviation and/or treatment of pathologies associated with extracellular HMGB1 and/or associated with an increased expression of RAGE (see paragraphs [0001] and [0055] to [0057] of the patent).

The claimed conjugates act as a selective antagonist and/or inhibitor of extracellular HMGB1 (see paragraph [0022] of the patent).

Closest prior art

6. Document D7 was accepted by both parties as representing the closest prior art for the claimed invention. It discloses the *"use of polypeptides obtained through systematic mutations of single amino acids of human and non-human Box-A of HMGB1 to prevent and/or antagonize pathologies induced by HMGB1"* (see title). A broad spectrum of therapeutic applications is suggested (see claim 25). Thus, the polypeptides disclosed in document D7 serve the same purpose as those presently claimed. Claim 1 of document D7 reads: *"Polypeptide variant of the human and/or non human HMGB1 high affinity binding domain Box-A (HMGB1 Box-A) or of a biologically active fragment of HMGB1 Box-A, characterized in that the amino acid sequence of said polypeptide variant differs from the amino acid sequence of the wild type HMGB1 Box-A by the mutation of one or more single amino acid"*. The variant HMGB1 Box-A proteins mentioned in the patent in suit are the same as those disclosed in document D7.

7. In view of the fact that document D7 relates to *"variants"* of the HMGB1 Box-A protein, it inherently also discloses the wild type HMGB1 Box-A protein.

The objective technical problem

8. The difference between the HMGB1 Box-A proteins disclosed in document D7 and those of claim 1 and 2 of the patent is the conjugation of the latter to a polymer.

9. The technical effects of this difference are improved pharmacological and toxicological properties, in particular extended circulation half-life, increased protein solubility, stability and decreased protein immunogenicity (see paragraph [0021], Example 3 and Table 2 of the patent).
10. Accordingly, and in agreement with both parties, the technical problem underlying the invention is the provision of HMGB1 inhibitors with improved pharmacological/pharmacokinetic properties.

Obviousness

11. The question to be answered by the board is therefore whether the skilled person, faced with the above formulated technical problem and starting from the HMGB1 Box A fragments disclosed in document D7, representing the closest prior art, would have considered it obvious to provide the claimed HMGB1 Box-A - polymer conjugates.
12. The board agrees with the appellant that at the priority date, PEGylation as a strategy for improving the pharmacological and toxicological properties of proteins, was common general knowledge in the art. This common knowledge is reflected in both documents D6 and D12, which relate to PEGylation of peptide and protein pharmaceuticals, and in paragraph [0021] of the patent.
13. Document D6 is a review entitled "*Chemistry for peptide and protein PEGylation*". Its main thrust is the chemistry of PEGylation. It contains some remarks about the purpose of protein PEGylation, as follows (page 460 left column): "*Several strategies have emerged as ways to improve the pharmacokinetic and pharmacodynamic*

properties of biopharmaceuticals, including [...] conjugation to [...] synthetic polymers. Those in the biomedical, biotechnical and pharmaceutical communities have become quite familiar with the improved pharmacological and biological properties that are associated with the covalent attachment of poly(ethylene glycol) or PEG to therapeutically useful polypeptides. For instance, PEG conjugation can shield antigenic epitopes of the polypeptide, thus reducing reticuloendothelial (RES) clearance and recognition by the immune system and also reducing degradation by proteolytic enzymes. PEG conjugation also increases the apparent size of the polypeptide, thus reducing renal filtration and altering biodistribution".

14. Document D12 is a review article entitled "*Effect of pegylation on pharmaceuticals*" from the year 2003 which contains the following relevant remark in the abstract: "*Protein and peptide drugs hold great promise as therapeutic agents. However, many are degraded by proteolytic enzymes, can be rapidly cleared by the kidneys, generate neutralizing antibodies and have a short circulating half-life. Pegylation, the process by which polyethylene glycol chains are attached to protein and peptide drugs, can overcome these and other shortcomings*". Furthermore, on page 215 it is disclosed that: "*[...] pegylation confers on drugs a number of properties that are likely to result in a number of clinical benefits, such as sustained blood levels that enhance effectiveness, fewer adverse reactions, longer shelf life and improved patient convenience. However, pegylation can produce a decrease in the in vitro activity of proteins, but generally this negative effect is offset in biological systems by an increased half-life. Pegylation can influence the binding affinity of therapeutic proteins to cellular receptors,*

which results in changes in the bioactivity of polypeptides."

15. The above can be summarised by the conclusion reached in document D12 that PEGylation of protein pharmaceuticals was *"now established as the method of choice for improving the pharmacokinetics and pharmacodynamics of protein pharmaceuticals"*. In view of this, the board concludes that the skilled person would have considered it obvious to provide PEGylated versions of the HMGB1 Box-A protein and variants thereof disclosed in document D7 as a solution to the technical problem.

16. The respondent was of the view that the particular circumstances of HMGB1 Box-A would have dissuaded the skilled person from going down this, otherwise obvious, path. Specifically, the respondent argued that the skilled person would not reasonably have expected PEGylated versions of the HMGB1 Box-A proteins disclosed in document D7 to represent a solution to the technical problem in view of certain features peculiar to them (i.e. sensitivity of their biological activity to structural change and the need to retain binding ability as a competitive antagonist, see section XIII., above).

17. The board takes from the respondent's arguments, that they consider that the prior art contained a teaching away from the invention, or at least a disincentive to go down the path of PEGylation in the case of HMGB1 Box-A protein, as a potential solution to the technical problem. However, the board has not been able to identify such a teaching in document D7 or in any other cited prior art document. In the board's view, in the absence of an unambiguous statement to that effect, the

skilled person would not consider the disclosure in document D7 that HMGB1 is highly conserved among mammals, to amount to a teaching that HMGB1 Box-A is unsuitable for PEGylation. Nor would the skilled person be dissuaded from PEGylation due to the fact that HMGB1 Box-A is lysine rich. In fact, document D12 at page 216, left column, final paragraph, addresses this latter problem stating "*Pegylating site-specifically can minimize the loss of biological activity and reduce immunogenicity. For instance, because there are far fewer cysteine residues than lysine groups on polypeptides, the thiol groups of cysteine are ideal for specific modifications. Moreover, cysteines can be added to polypeptides precisely where they are desired by genetic engineering*". It is therefore apparent that the skilled person would not have considered that PEGylating HMGB1 Box-A would inevitably lead to an unacceptable loss of bioactivity.

18. The potential loss of bioactivity associated with PEGylation is furthermore addressed by a passage in document D12 (see page 215, right column, paragraph 2) which reads "*[...] pegylation can produce a decrease in the in vitro activity of proteins, but generally this negative effect is offset in biological systems by an increased half-life*". Thus, the skilled person knew about a potential loss of *in vivo* bioactivity due to PEGylation but also knew that this was not a reason to disregard the strategy of PEGylation.

19. In summary, none of the documents cited by the respondent, either alone or in combination with common knowledge, contain a teaching that would have dissuaded the skilled person from following the otherwise obvious path of PEGylation, as a solution to the technical problem.

20. The board therefore concludes that the skilled person seeking to improve the pharmacological properties of the HMGB1 Box-A proteins disclosed in document D7 would, in the view of the common general knowledge in the art of the pharmacological benefits of PEGylation of therapeutic proteins (as reflected in documents D6 and D12), have provided PEGylated versions of wild type HMGB1 Box-A and variant HMGB1 Box-A proteins. Claim 1 and claim 2 of the main request therefore do not meet the requirements of Article 56 EPC.

Auxiliary requests I to III

21. Claim 1 of auxiliary requests I and II is identical to claim 1 of the main request. Claim 1 of auxiliary request III is identical to claim 2 of the main request. It follows that these requests also do not meet the requirements of Article 56 EPC.

Auxiliary requests IV to VI

22. Claim 1 of auxiliary requests IV to VI is for PEGylated versions of specific variants of HMGB1 Box-A. Each of these polypeptide variants was disclosed in document D7. Since the reasons set out above for the claims 1 and 2 were for all HMGB1 Box-A compounds disclosed in document D7, i.e. for all variants, as well as for the wild type, they are equally valid for the subject-matter of claim 1 auxiliary requests IV to VI. In line with established case law (see Case Law of the Boards of Appeal of the European Patent Office, 8th edition 2016, I.D.10.8), the board holds that any additional effects exhibited by the claimed conjugates, i.e. the alleged synergy between the improvement due to the choice of variant and that due to PEGylation, do not alter the outcome of the above analysis. The finding of

lack of an inventive step of the claimed polymer conjugated polypeptides is not altered by the fact that they inherently also solve a further technical problem. Thus, the subject-matter of claim 1 of auxiliary requests IV to VI lacks an inventive step.

23. No claim request 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 patent is revoked.

The Registrar:

The Chairman:



D. Hampe

G. Alt

Decision electronically authenticated