

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
4 August 2005 (04.08.2005)

PCT

(10) International Publication Number
WO 2005/070451 A1

(51) International Patent Classification⁷: **A61K 38/18**,
C07K 14/505

(21) International Application Number:
PCT/SE2005/000068

(22) International Filing Date: 21 January 2005 (21.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/538,730 22 January 2004 (22.01.2004) US

(71) Applicant (for all designated States except US): **ZAFENA
AKTIEBOLAG** [SE/SE]; Sjöbacka Fallhagen 1, S-590 76
Vreta Kloster (SE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **RÅNBYS, Mats**
[SE/SE]; Sjöbacka Fallhagen 1, S-590 76 Vreta Kloster
(SE).

(74) Agents: **NILSSON, Brita** et al.; Zacco Sweden AB, Box
23101, S-104 35 Stockholm (SE).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING NON-GLYCOSYLATED ERYTHROPOIETIN

(57) Abstract: Pharmaceutical compositions for topical administration, comprising non-glycosylated erythropoietin (EPO), such as bacterially produced recombinant EPO, are described. The compositions are suitable for use in treatment of a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin, e.g. a disease or disorder selected from a conjunctivitis, a wound, a bedsore, a burn, an inflammation of the skin, mucous membranes, airways or lungs, an eczema or a skin disorder accompanied by necrosis, by dermatitis, by psoriasis or by diabetes mellitus. The pharmaceutical composition may be in the form of an ointment, a cream, a powder, an emulsion, a gel, a glycerogelatin, a paste, a plaster, a sprayable composition or a lotion.



WO 2005/070451 A1

Pharmaceutical composition comprising non-glycosylated erythropoietin

The present invention relates to a pharmaceutical composition for topical administration comprising non-glycosylated erythropoietin (EPO) and to the use of the composition, especially in the treatment of a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin.

Background of the invention

In the hematopoietic system of mammals, red blood cells are continuously produced. The production of these red blood cells is stimulated by the polypeptide erythropoietin (EPO). Naturally-occurring EPO is a glycoprotein. Thus, the EPO synthesized in mammalian organisms is glycosylated in that carbohydrate moieties are attached to one or more of the amino acid residue of the polypeptide backbone of the protein. If there are no carbohydrate moieties attached the EPO is non-glycosylated.

It is previously known to use pharmaceutical compositions comprising glycosylated recombinant human EPO for systemic administration as a therapeutic agent to treat anaemia. Systemically administered non-glycosylated EPO is rapidly removed from the bloodstream, cleared, and no appreciable effect of the administered EPO is obtained.

Description of the invention

The present invention provides a pharmaceutical composition which comprises erythropoietin (EPO) and a pharmaceutically acceptable diluent, adjuvant and/or carrier. The pharmaceutical composition is to be applied superficially, meaning topically.

The EPO according to the invention may have an amino acid sequence that is identical to the amino acid sequence of naturally occurring mammalian EPO or it may be a fragment or derivative thereof, e.g. with one or several point mutations, deletions, insertions or truncations with the respect to any naturally occurring mammalian EPO. For example the EPO according to the invention can contain a stretch of 5 or more amino acid residues that displays at least 80% identity to human EPO. The amino acid sequence of several human erythropoietin (hEPO) polypeptides can be found, for example, in GENBank Accession Nos. AAC78791, AAF23134, AAF23132, AAF17572 and AAF23123. Sequence identity can be calculated using any of a number of computer programs. For example, BLAST, DNA Star, or GCG can be used to determine the percent sequence identity between two nucleotide or amino acid sequences.

The present invention is based on the discovery that EPO can not only be administered systemically but also topically to provide a local therapeutic effect. The reason for this therapeutic effect may be an accelerating effect of EPO on all differentiation and proliferation processes of a large number of different progenitor cells. It has been found that these progenitor cells have receptors for EPO. EPO cannot only stimulate endothelial

progenitor cells but also mesenchymal progenitor cells such as human dermal stem cells and human hair follicle progenitor cells. The EPO used in the present invention is non-glycosylated and therefore a smaller molecule than the naturally-occurring glycosylated EPO.

The advantage of the topical application is that the EPO will have no appreciable systemic effect and therefore side effects associated with the systemic application can be avoided. Since the EPO is only to have a local effect the dosage with respect to the total body mass may be low, if desired, or high if that is of advantage in some applications of the invention.

The pharmaceutical composition may be topically applied for the treatment of a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin. The disease or disorder may be a disease or disorder of the skin, mucous membranes, airways or lungs, or of the eye. Surprisingly it was found that the topical, i.e. local application of EPO results in an accelerated healing of such a disease or disorder and of surgical lesions.

Thus, one aspect of the invention is directed to pharmaceutical composition for topical administration, comprising non-glycosylated erythropoietin (EPO) and a pharmaceutically acceptable diluent, adjuvant and/or carrier.

Another aspect of the invention is directed to use of non-glycosylated erythropoietin (EPO) for the manufacture of a pharmaceutical composition for topical treatment of a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin.

Yet another aspect of the invention is directed to a method of treating a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin comprising topical administration of a pharmaceutical composition comprising non-glycosylated erythropoietin (EPO) and a pharmaceutically acceptable diluent, adjuvant and/or carrier.

In one embodiment of each aspect of the invention the non-glycosylated EPO is a bacterially produced recombinant EPO.

In another embodiment the pharmaceutical composition of the invention is for use in treatment of a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin, such as a disease or disorder of the skin or the eye of a human being or an animal.

In yet another embodiment the EPO is a human EPO.

Pharmaceutical composition according to the invention is preferably selected from the group consisting of ointments, creams, powders, emulsions, gels, glycerogelatiness, pastes, plasters, sprayable compositions, and lotions, but any other form of composition that is suitable for topical administration of EPO to a human or animal may be used.

Typically the disease or disorder that can be treated by topical administration of a pharmaceutical composition according to the invention is selected from the group consisting of a conjunctivitis, a wound, e.g. an open leg wound, a bedsore, a burn, an inflammation of the skin, mucous membranes, airways or lungs, an eczema or a skin disorder accompanied by necrosis, by dermatitis, by psoriasis or by diabetes mellitus. An example of the disorder of the skin that can be treated is lack of growth or colouring of hair.

The EPO that is used in the present invention is in a form that is more rapidly cleared from the circulation of a human being or an animal than naturally-occurring EPO. The advantage of this feature is that it allows topical application of EPO for local treatment, including treatment at high dosages of EPO, with minimal risk of systemic side effects. The treatment may affect only tissues with a common lymph drain, but may allow effective EPO activities to be reached also at some depth below the point or area of the topical application. Thus, application according to the invention of low dosages of EPO will further reduce the already low risk of systemic side effects. For those skilled in the art it follows, that any form of non-glycosylated EPO that is quickly removed from circulation and/or inactivated, regardless of the biological mechanism, will function in the above described embodiments of the invention.

Surprisingly it was found that the non-glycosylated EPO causes an accelerated healing of the diseases, disorders or surgical lesions. As already mentioned, if non-glycosylated EPO is applied systemically it is rapidly removed, i.e. cleared, from the circulation, most likely by uptake and degradation by the liver.

When topically applying such a rapidly cleared form of the EPO according to the invention, it will predominantly have an effect only locally. As the EPO diffuses away from the point of application it will, e.g. via the lymph, reach the bloodstream and rapidly be cleared. The biological activity of the EPO is thereby lost and with that its ability to cause systemic effects, including systemic side effects.

An additional advantage of the fact that no glycosylation or other modification of amino acid residues is necessary is that the EPO may be cost-effectively produced in large amounts by gene technology in bacteria and without need of ex-vivo chemical modification. The bacteria may be *E. coli* as in the example of production presented below.

The pharmaceutically acceptable diluents, adjuvants and/or carriers that form part of the pharmaceutical composition of the invention are those commonly used in the prior art, and guidance for selecting these in a particular case can be found in the US or European Pharmacopoeia.

As mentioned, the pharmaceutical composition of the invention may be an ointment, a cream, a powder, an emulsion, a gel, a glycerogelatine, a paste, a plaster, a sprayable composition, or a lotion. In addition to a diluent, adjuvant and/or carrier, the

pharmaceutical composition of the invention may comprise further components that contribute to the healing process or are suitable for the administration form of choice.

The amount of non-glycosylated EPO in a pharmaceutical composition of the invention is decided by the manufacturer and/or the physician in accordance with the topical administration form produced, the desired low or high EPO content of the product, the disease or disorder to be treated, the condition of the patient etc., and the amount may e.g. vary from 0.01 microgram to 100 mg per 1000 mg of the composition.

Examples of preparation of non-glycosylated EPO

Recombinant human erythropoietin (hEPO) is obtainable as a non-glycosylated, non-soluble or soluble protein from cultured bacteria using standard techniques as described in "Molecular cloning: a laboratory manual, Joseph Sambrook, David W. Russell, 3rd ed., 2001, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York". The DNA sequence coding for hEPO (SEQ ID NO:1) may be fused with other DNA sequences. These DNA sequences may resemble sequences of protease cleavage sites and sequences of polypeptides that may facilitate the purification of the protein, e.g. affinity tags. The hEPO can be isolated from so-called "inclusion bodies" (IBs), which may be formed during expression in bacteria.

The hEPO is expressed in *E. coli* as a fusion protein comprising the sequence of hEPO (SEQ ID NO:2), optionally preceded at its N-terminus by the sequences of an enterokinase cleavage site and at least one affinity tag of which at least one may be a Strep-Tag® (IBA GmbH, Göttingen, Germany) as affinity tag. During the expression IBs are formed. The expressing bacteria are pelleted and disintegrated. The fusion protein is isolated from IBs by treatment with BugBuster® Protein Extraction Reagent solution (Novagen, Madison, USA) at a concentration of 5 ml BugBuster® Protein Extraction Reagent solution per 1 g wet bacterial cell pellet (= starting volume) for 30 minutes at room temperature according to manufacturer's instructions. The resulting suspension is pelleted by centrifugation at 20000 x g for 20 minutes at 4°C. The pellet is resuspended in 1-fold starting volume of undiluted BugBuster® Protein Extraction Reagent solution.

The resulting slurry is further diluted in 6-fold starting volume of 1:10 diluted BugBuster® Protein Extraction Reagent solution. Proteases may be inhibited by the addition of the general protease inhibitor Complete® (Boehringer Mannheim, Mannheim, Germany). Genomic DNA is degraded by the addition of Benzonase (Purity Grade > 99%, Merck KGaA, Darmstadt, Germany) as DNA-degrading enzyme. IBs are obtained by a subsequent centrifugation at 5000 x g for 15 minutes at 4°C. The IBs that are contained in the resulting pellet are further purified by at least one washing step by resuspending the pellet using 0.5-

fold starting volume of 1:10 diluted BugBuster® Protein Extraction Reagent solution and subsequent centrifugation at 5000 x g for 15 minutes at 4°C.

Purified IBs are obtained by a final washing step comprising the resuspension of the pellet in 0.5-fold starting volume of 1:10 diluted BugBuster® Protein Extraction Reagent solution and subsequent centrifugation at 16000 x g for 15 minutes at 4°C. The purified IBs is a preparation of insoluble non-glycosylated EPO from which preparations of greater purity and pharmaceutical acceptability may be produced by further successive washes in pharmaceutically acceptable diluents or carriers, such as 150 mM NaCl water solution, water ethanol and glycerin or mixtures thereof. The insoluble non-glycosylated EPO is resuspended in wash liquids and collected by sedimentation, filtration or centrifugation. The insoluble non-glycosylated EPO may be incorporated into the pharmaceutical composition as a slurry or moist paste, or it may be dried and incorporated as a powder. Preparations of insoluble non-glycosylated EPO are preferentially from bacterial clones that produce human hEPO without added, purification facilitating, amino acid sequences at their N-terminus.

The purified IBs are solubilized by resuspending them thoroughly at least one time with 0.5-fold starting volume of the following urea and detergent containing sodium-phosphate buffer: 1.6 mmol/l NaH_2PO_4 , 8.4 mmol/l Na_2HPO_4 , 6 mmol/l DTT, 2 mol/l urea, 0.002% Brij®58 P (Fluka, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), pH 8.0. Afterwards an insoluble portion of the IBs is spun down at least 30000 x g for 15 minutes at 4°C. The supernatant contains the solubilized fusion protein.

The fusion protein is separated from contaminating protein fractions by an affinity purification using a Strep-tag® column (IBA GmbH, Göttingen, Germany) on which the fusion protein is bound. Binding to the column is performed in presence of the above sodium-phosphate buffer. Bound fusion protein is eluted using the following elution buffer: one part of 1 mol/l Tris-Cl, pH 8.0, 1.5 mol/l NaCl, 10 mmol/l EDTA, 25 mmol/l desthiobiotin and nine parts of the above sodium-phosphate buffer, pH 8.0. The fusion protein may be cleaved at the enterokinase cleavage site by digestion with enterokinase preferentially directly on the column or after the above-mentioned elution. The resulting protein is dialyzed against a physiologically tolerated buffer, preferably PBS (137 mmol/l NaCl, 2.7 mmol/l KCl, 4.3 mmol/l Na_2HPO_4 , 1.47 mmol/l KH_2PO_4 , pH 7.4) containing 2 mol/l urea to obtain a preparation of non-glycosylated EPO that can be incorporated into a pharmaceutical composition, *vidi infra*. Alternatively a buffer without urea can be used.

Alternatively to the expression of EPO as Strep-Tag® fusion protein hEPO can also be expressed in E.coli as glutathione S-transferase fusion protein as described in Bill et al., 1995, Biochem Biophys Acta 1261, pages 35-43. For this purpose the vector pGEX (Amersham Biosciences, Freiburg, Germany) can be used.

Examples of pharmaceutical compositions.

The pharmaceutical composition according to the invention can be prepared by incorporating the produced non-glycosylated EPO into a standard cream formulation containing no or up to 12 % urea. As standard cream formulation "Cremaba Plus HT" (Spinnrad®, Certus Handels GmbH, 22848 Norderstedt, Germany) can be used. "Cremaba Plus HT" has the following ingredients: Aqua, Caprylic/Capric Triglyceride, Pentylene Glycol, Hydrogenated Lecithin, Butyrospermum Parkii, Glycerin, Squalane, Ceramide 3.

An alternative to the use of soluble non-glycosylated EPO is the use of water based or non-water based suspensions or pastes of insoluble non-glycosylated EPO. Preparations of soluble or insoluble non-glycosylated EPO, either moist or dry, may be used as such or combined with adjuvants and/or form ointments, creams, powders, emulsions, lotions, gels, pastes, plasters, glycerogelatiness or sprays for topical administration for topical treatment of disease, disorder or surgical lesion of a human being or an animal. This alternative allows direct use of preparations of insoluble non-glycosylated EPO from the inclusion bodies (IBs) of EPO producing bacteria.

Ointments, creams, powders, emulsions, lotions, gels, pastes, plasters, glycerogelatiness or sprays suited for topical application and treatment of disease, disorders or surgical lesions with non-glycosylated EPO, according to the invention, can be of any of the various kinds known in the prior art, e.g. as described in the monograph 'Pharmaceutical dosage forms and drug delivery systems' 1999, 7:th edition, copyright; Lippincott Williams and Wilkins, by Ansel HC, Allen LV Jr, Popovich NG.

As is known in the art, ointments are semisolid preparations intended for external application to the skin or mucous membranes. Ointments are prepared either by incorporation or by fusion, depending on the nature of the ingredients. Ointments can be with hydrocarbon bases, e.g. petrolatum, USP, white petrolatum, USP, yellow ointment, USP, or white ointment, USP, absorption bases, e.g. hydrophilic petrolatum, USP, or lanolin, USP, water-removable bases, e.g. hydrophilic ointment, USP, water-soluble bases, e.g. polyethylene glycol ointment, NF.

Creams are semisolid preparations containing one or more medicinal agent dissolved or dispensed in either an oil-in-water emulsion or in another type of water-washable base. Creams find use in topical skin products and products used rectally and vaginally. Creams are sometimes preferred to ointments because they are easier to spread and remove.

Gels are semisolid systems consisting of dispersions of large molecules in an aqueous liquid vehicle rendered jelly-like through the addition of a gelling agent. Examples of gelling agents are carbomer 934, cellulose derivatives as carboxymethylcellulose or

hydroxypropylmethyl-cellulose, and natural gums as tragacanth. Carbomers are high molecular weight water-soluble polymers of acrylic acid cross-linked with allyl ethers of sucrose and/or pentaerythritol.

5 Pastes are semisolid preparations for application to the skin. They generally contain a larger proportion of solid material than ointments and are therefore stiffer. Pastes may contain zinc oxide, starch and white petrolatum.

Plasters are solid or semi-solid adhesive masses spread upon a backing material of paper, fabric, moleskin or plastic. The adhesive material is a rubber base or synthetic resin. Plasters are applied to skin to provide prolonged contact at the site.

10 Glycerogelatiness are plastic masses containing gelatin, glycerol, water and a medicinal substance. They are applied to skin for long-term residence.

Sprays are aqueous or oleaginous solutions in the form of coarse droplets or as finely divided solids to be applied topically, most usually to the nasal-pharyngeal tract or to the skin. To achieve the breaking up of a solution into small particles so that it may be efficiently sprayed or to facilitate the spraying of a powder, any of several mechanical devices that have been developed for the purpose may be employed in practicing the invention.

It is also possible to prepare tablets or suppositories for direct administration to the mucous membrane of the mouth, intestinal tract, vagina and rectum, respectively.

20 Topical treatment of the airways and lung tissues can also be achieved without any appreciable side effects by using aerosols containing non-glycosylated EPO. Aerosols are pressurized dosage forms that emit a fine dispersion of liquid and/or solid material. Depending on the size of the particles emitted, the depth of the topical treatment into the airways can be predetermined. Particles in the range 6 to 10 microns will reach the bronchioles whereas particles smaller than about 2 microns are needed to reach the lung tissue, i.e. the alveolar ducts and the alveoli.

25

CLAIMS

1. Pharmaceutical composition for topical administration, comprising non-glycosylated erythropoietin (EPO) and a pharmaceutically acceptable diluent, adjuvant and/or carrier.
- 5 2. Pharmaceutical composition according to claim 1, wherein the non-glycosylated EPO is a bacterially produced recombinant EPO.
3. Pharmaceutical composition according to claim 1 or 2 for use in treatment of a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin.
- 10 4. Pharmaceutical composition according to claim 2, wherein the disease or disorder is a disease or disorder of the skin, mucous membranes, airways or lungs, or the eye of a human being or an animal.
5. Pharmaceutical composition according to any one of claims 1 – 4, wherein the EPO is a human EPO.
- 15 6. Pharmaceutical composition according to any one of the preceding claims, wherein the pharmaceutical composition is selected from the group consisting of ointments, creams, powders, emulsions, gels, glycerogelatiness, pastes, plasters, sprayable compositions, and lotions.
- 20 7. Pharmaceutical composition according to any one of the preceding claims, wherein the disease or disorder is selected from the group consisting of a conjunctivitis, a wound, a bedsore, a burn, an inflammation of the skin, mucous membranes, airways or lungs, an eczema or a skin disorder accompanied by necrosis, by dermatitis, by psoriasis or by diabetes mellitus.
8. Pharmaceutical composition according to any one of the preceding claims, wherein the disorder of the skin is lack of growth or colouring of hair.
- 25 9. Use of non-glycosylated erythropoietin (EPO) for the manufacture of a pharmaceutical composition for topical treatment of a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin.

10. Use according to claim 9, wherein the non-glycosylated EPO is a bacterially produced recombinant EPO.
11. Use according to claim 9 or 10, wherein the disease or disorder is a disease or disorder of the skin, mucous membranes, airways or lungs, or of the eye of a human being or an animal.
12. Use according to any one of claims 9 – 11, wherein the EPO is a human EPO.
13. Use according to any one of claims 9 -11, wherein the pharmaceutical composition is selected from the group consisting of ointments, creams, powders, emulsions, gels, glycerogelatiness, pastes, plasters, sprayable compositions and lotions.
14. Use according to any one of the claims 9 -13 , wherein the disease or disorder is selected from the group consisting of a conjunctivitis, a wound, a bedsore, a burn, an inflammation of the skin, mucous membranes, airways or lungs, an eczema or a skin disorder accompanied by necrosis, by a dermatitis, by psoriasis or by diabetes mellitus.
15. Use according to any one of the claims 9 - 14, whereby the disorder of the skin is a lack of growth or colouring of hair.
16. Method of treating a human being or an animal suffering from a disease, disorder or surgical lesion that is alleviated or cured by topical administration of erythropoietin comprising topical administration of a pharmaceutical composition comprising non-glycosylated erythropoietin (EPO) and a pharmaceutically acceptable diluent, adjuvant and/or carrier.
17. Method according to claim 16, wherein the non-glycosylated EPO is a bacterially produced recombinant EPO.
18. Method according to claim 16 or 17, wherein the disease or disorder is a disease or disorder of the skin or of the eye of a human being or an animal.
19. Method according to any one of claims 16 – 18, wherein the EPO is a human EPO.

10

20. Method according to any one of claims 16 -19, wherein the pharmaceutical composition is selected from the group consisting of ointments, creams, powders, emulsions, gels, glycerogelatiness, pastes, plasters, sprayable compositions and lotions.

5 21. Method according to any one of the claims 16 -20, wherein the disease or disorder is selected from the group consisting of a conjunctivitis, a wound, a bedsore, a burn, an inflammation of the skin, mucous membranes, airways or lungs, an eczema or a skin disorder accompanied by necrosis, by a dermatitis, by psoriasis or by diabetes mellitus.

22. Method according to any one of the claims 16 - 21, wherein the disorder of the skin is a lack of growth or colouring of hair.

10 -----

SEQUENCE LISTING

<110> ZAFENA AB

<120> Pharmaceutical composition comprising non-glycosylated erythropoietin

<130> 110126200

<150> US 60/538,730

<151> 2004-01-22

<160> 2

<170> PatentIn version 3.1

<210> 1

<211> 504

<212> DNA

<213> Homo sapiens

<400> 1
atggcccccac cagcctcat ctgtgacagc cgagtcctgg agaggtacct cttggaggcc 60
aaggaggccg agaatatcac gacgggctgt gctgaacact gcagcttgaa tgagaatata 120
actgtcccag acaccaaagt taatttctat gcctggaaga ggatggaggt cgggcagcag 180
gcogtagaag tctggcaggg cctggccctg ctgtcggaag ctgtcctgcg gggccaggcc 240
ctgttggtca actcttccca gccgtgggag ccctgcagc tgcattgtga taaagccgtc 300
agtggccttc gcagcctcac cactctgctt cgggctcttc gagcccagaa ggaagccatc 360
tccoctccag atgcggcctc agctgctcca ctccgaacaa tctactgctga cactttccgc 420
aaactcttcc gagtctactc caatttcctc cggggaaagc tgaagctgta cacaggggag 480

gcctgcagga caggggacag atga

504

<210> 2

<211> 167

<212> PRT

<213> Homo sapiens

<400> 2

Met Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr
1 5 10 15

Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu
20 25 30

His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn
35 40 45

Phe Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val
50 55 60

Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala
65 70 75 80

Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val
85 90 95

Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala
100 105 110

Leu Arg Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala
115 120 125

Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg
130 135 140

Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu
145 150 155 160

Ala Cys Arg Thr Gly Asp Arg
165

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000068

A. CLASSIFICATION OF SUBJECT MATTER		
IPC7: A61K 38/18, C07K 14/505 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC7: A61K, C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-INTERNAL, WPI DATA, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM. ABS. DATA		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 20030104988 A (MICHAEL BRINES ET AL), 5 June 2003 (05.06.2003), abstract, page 1, paragraph 0008; page 6, paragraph 0058; page 7, paragraph 0064; page 10, paragraph 0088 line 9; page 14; page 16 paragraph 0114; page 17 paragraph 0125; Example 4 --	1-22
A	Protein Engineering, volume 14, no. 2, 2001, Linda O.Narhi et al: "Asn to Lys mutations at three sites which are N-glycosylated in the mammalian protein decrease the aggregation of Escherichia coli-derived erythropoietin", pages 135-140 --	1-22
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
16 May 2005		18 -05- 2005
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86		Authorized officer Carolina Palmcrantz/Els Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000068

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6274158 B1 (VERONICA L. ZAHARIA CZEIZLER), 14 August 2001 (14.08.2001), column 7, line 34 - line 43 --	1-22
A	Pediatric research, volume 33, no. 4, part 2, 1993, Robin K. Obis et al: "A Model For Transdermal Erythropeitin Administration in Preterm Infants" -- -----	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000068

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16-22
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 16-22 relate to a method of treatment of the human or animal body by surgery or by therapy, as well as diagnostic

.../...
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000068

Box II.1

methods (Rule 39.1(iv)). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compound.

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/2005

International application No.

PCT/SE 2005/000068

US	20030104988	A	05/06/2003	CA	2432853	A	11/07/2002
				EE	200300267	A	15/10/2003
				EP	1406922	A	14/04/2004
				HU	0302549	A	28/11/2003
				IL	156399	D	00/00/0000
				JP	2005502584	T	27/01/2005
				NO	20032912	D	00/00/0000
				SK	9572003	A	04/11/2003
				US	20030072737	A	17/04/2003
				CN	1505638	A	16/06/2004
				CZ	20032059	A	17/12/2003
				WO	02053580	A	11/07/2002

US	6274158	B1	14/08/2001	US	6521245	B	18/02/2003
				US	20020169129	A	14/11/2002
				US	5951996	A	14/09/1999
				US	6284260	B	04/09/2001
				US	6124323	A	26/09/2000
