(19) DANMARK

(10) DK/EP 1861116 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 61 K 38/17 (2006.01)* *A 61 K 47/00 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2015-11-09**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2015-09-16**

(86) Europæisk ansøgning nr.: **06748594.6**

(86) Europæisk indleveringsdag: **2006-03-22**

(87) Den europæiske ansøgnings publiceringsdag: **2007-12-05**

(86) International ansøgning nr.: **US2006010600**

(87) Internationalt publikationsnr.: **WO2006104852**

(30) Prioritet: **2005-03-25 US 665125 P**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR**

(73) Patenthaver: **REGENERON PHARMACEUTICALS, INC., 777 Old Saw Mill River Road, Tarrytown, NY 10591, USA**

(72) Opfinder: **DIX, Daniel, 55 Memory Trail, LaGrangeville, New York 12540, USA**
FRYE, Kelly, 29 N. Ridge Road, Pomona, New York 10970, USA
KAUTZ, Susan, 3 Hall Place, Albany, New York 12210, USA

(74) Fuldmægtig i Danmark: **JESPER LEVIN A/S, Strandvejen 656, 2930 Klampenborg, Danmark**

(54) Benævnelse: **VEGF-antagonistformuleringer**

(56) Fremdragne publikationer:
WO-A-2005/000895
WO-A1-02/060489
US-A1- 2002 004 478
US-A1- 2003 202 972
US-A1- 2005 032 699
US-A1- 2005 276 808
GLADE-BENDER J ET AL: "VEGF BLOCKING THERAPY IN THE TREATMENT OF CANCER" EXPERT OPINION ON BIOLOGICAL THERAPY, ASHLEY, LONDON, GB, vol. 3, no. 2, April 2003 (2003-04), pages 263-276, XP009060849 ISSN: 1471-2598
KIM EUGENE S ET AL: "Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 99, no. 17, 20 August 2002 (2002-08-20), pages 11399-11404, XP002391853 ISSN: 0027-8424 cited in the application
HOLASH JOCELYN ET AL: "VEGF-Trap: A VEGF blocker with potent antitumor effects" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 99, no. 17, 20 August 2002 (2002-08-20), pages 11393-11398, XP002391854 ISSN: 0027-8424 cited in the application
FRASER HAMISH M ET AL: "Single injections of vascular endothelial growth factor trap block ovulation in the

Fortsættes ...

macaque and produce a prolonged, dose-related suppression of ovarian function", JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM, vol. 90, no. 2, February 2005 (2005-02), pages 1114-1122, ISSN: 0021-972X
A López ET AL: "Comparative enhancer effects of Span 20 with Tween 20 and Azone on the in vitro percutaneous penetration of compounds with different lipophilicities", International Journal of Pharmaceutics, vol. 202, no. 1-2, 1 July 2000 (2000-07-01), pages 133-140, XP055089180, ISSN: 0378-5173, DOI: 10.1016/S0378-5173(00)00427-0

DESCRIPTION

Field of the Invention

[0001] The present invention is directed to pharmaceutical formulations comprising agents capable of inhibiting vascular endothelial growth factor (VEGF). The invention includes pharmaceutical formulations having increased stability.

Statement of Related Art

[0002] Vascular endothelial growth factor (VEGF) expression is nearly ubiquitous in human cancer, consistent with its role as a key mediator of tumor neoangiogenesis. Blockade of VEGF function, by binding to the molecule or its VEGFR-2 receptor, inhibits growth of implanted tumor cells in multiple different xenograft models (see, for example, Gerber et al. (2000) Cancer Res. 60:6253-6258). A soluble VEGF-specific fusion protein antagonist, termed a "VEGF trap" has been described (Kim et al. (2002) Proc. Natl. Acad. Sci. USA 99:11399-404; Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8).

[0003] The following documents are also referred to:

- WO 2005/000895 which refers to VEGF traps and therapeutic uses thereof;
- Fraser et al (2005) J. Clin. Endocrinol. & Metabolism, 90(2): 1114-1122 which is concerned with single injections of vascular endothelial growth factor trap block ovulation;
- US 2005/032699 which is concerned with a composition of a VEGF antagonist and an anti-proliferative agent;
- WO 02/060489 which is concerned with a method of using a variant of VEGF receptor to treat psoriasis and to enhance wound healing; and
- US 2005/276808 which is concerned with a method of administering and using VEGF inhibitors for the treatment of cancer.

BRIEF SUMMARY OF THE INVENTION

[0004] Stable formulations of a VEGF-specific fusion protein antagonist are herein provided. The pharmaceutically acceptable formulations of the invention comprise the VEGF "trap" antagonist with a pharmaceutically acceptable carrier.

[0005] The invention provides a liquid formulation of a VEGF antagonist, comprising 10 mM histidine, 50 mM NaCl, 5-20% sucrose, and 50-100 mg/ml of a fusion protein having a receptor component consisting of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component, at a pH of 6.0-6.5, where the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1 or Flt4, further comprising 0.1 % polysorbate 20 or 3% PEG 3350.

[0006] In one embodiment, the fusion protein has the amino acid sequence of SEQ ID NO:4.

[0007] The fusion protein may comprise amino acids 27-457 of SEQ ID NO:4 and is glycosylated at Asn residues 62, 94, 149, 222 and 308.

DETAILED DESCRIPTION OF THE INVENTION

General Description

[0008] Safe handling and administration of formulations comprising proteins represent significant challenges to pharmaceutical formulators. Proteins possess unique chemical and physical properties that present stability problems: a variety of degradation pathways exist for proteins, implicating both chemical and physical instability. Chemical instability includes deamination, aggregation, clipping of the peptide backbone, and oxidation of methionine residues. Physical instability encompasses many

phenomena, including, for example, aggregation.

VEGF Antagonists

[0009] A VEGF antagonist is a compound capable of blocking or inhibiting the biological action of vascular endothelial growth factor (VEGF), and includes fusion proteins capable of trapping VEGF. In a preferred embodiment, the VEGF antagonist used in the formulation of the invention is the fusion protein of SEQ ID NO:4. The VEGF antagonist can be expressed in a mammalian cell line such as a CHO cell and may be modified post-translationally. In a specific embodiment, the fusion protein comprises amino acids 27-457 of SEQ ID NO:4 and is glycosylated at Asn residues 62, 94, 149, 222 and 308.

[0010] The VEGF antagonist can be prepared by any suitable method known in the art, or that comes to be known. The VEGF antagonist is preferably substantially free of protein contaminants at the time it is used to prepare the pharmaceutically acceptable formulation. By "substantially free of protein contaminants" is meant, preferably, that at least 90% of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein, more preferably at least 95%, most preferably at least 99%. The fusion protein is preferably substantially free of aggregates. "Substantially free of aggregates" means that at least 90% of the weight of fusion protein is not present in an aggregate at the time the fusion protein is used to prepare the pharmaceutically effective formulation. The fusion protein may contain low or trace amounts of compounds as a results of the purification process, for example, low or trace amounts of citrate and/or polysorbate.

Stable Liquid Formulations

[0011] The invention provides a stable liquid pharmaceutically acceptable formulation as defined above.

[0012] Stability is determined in a number of ways at specified time points, including determination of pH, visual inspection of color and appearance, determination of total protein content by methods known in the art, e.g., UV spectroscopy, SDS-PAGE, size-exclusion HPLC, bioassay determination of activity, isoelectric focusing, and isoaspartate quantification. In one example of a bioassay useful for determining VEGF antagonist activity, a BAF/3 VEGFR1/EPOR cell line is used to determine VEGF165 binding.

[0013] Formulations can be stored in an oxygen-deprived environment. Oxygen-deprived environments can be generated by storing the formulations under an inert gas such as, for example, argon, nitrogen, or helium.

EXAMPLES

Example 1. Stability of a 50 mg/ml Liquid Formulation of VEGF Trap

[0014] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 50 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability was determined by SE-HPLC. The results, shown in Table 1, show that 98.6% and 98.3% of VEGF trap protein remained intact (non-degraded) at 12 and 24 months, respectively. Turbidity was measured at OD_{405} nm; and percent recovered protein by size exclusion HPLC.

Table 1. Stability of 50 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS065)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	99.0
3	Pass	0.00	6.2	102	98.8
6	Pass	0.01	6.2	103	98.7
9	Pass	0.01	6.3	102	98.2
12	Pass	0.01	6.3	106	98.6
18	Pass	0.00	6.3	103	98.4

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
24	Pass	0.00	6.2	93	98.3

[0015] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 3% PEG 3350, 20% sucrose, and 50 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability results are shown in Table 2.

Table 2. Stability of 50 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS065)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	99.0
3	Pass	0.00	6.2	100	98.8
6	Pass	0.01	6.3	103	98.5
9	Pass	0.00	6.3	103	98.3
12	Pass	0.01	6.3	110	98.3
18	Pass	0.00	6.3	113	98.0
24	Pass	0.01	6.2	90	97.8

Example 2. Stability of a 75 mg/ml Liquid Formulation of VEGF Trap

[0016] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 75 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 3.

Table 3. Stability of 75 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	97.1
1	Pass	0.00	6.2	96	97.0
2.3	Pass	0.00	6.2	98	96.7
3	Pass	0.00	6.2	97	96.1
9	Pass	-0.01	6.0	101	96.0
12	Pass	0.00	6.3	110	94.5
15	Pass	0.00	6.3	92	95.6

[0017] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 3% PEG 3350, 20% sucrose, and 75 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 4.

Table 4. Stability of 75 mg/ml VEGF Trap Protein When Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	96.8
1	Pass	0.00	6.2	99	96.7
2.3	Pass	0.00	6.2	97	96.3
3	Pass	0.00	6.2	89	95.6
9	Pass	-0.01	6.2	98	95.4
12	Pass	-0.01	6.3	112	94.1

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
15	Pass	0.00	6.3	98	94.8

Example 3. Stability of a 100 mg/ml Liquid Formulation of VEGF Trap

[0018] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 100 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 5.

Table 5. Stability of 100 mg/ml VEGF Trap Protein Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	96.7
1	Pass	0.00	6.2	92	96.6
2.3	Pass	0.00	6.2	92	96.2
6	Pass	0.00	6.2	99	95.5
9	Pass	-0.01	6.2	92	95.5
12	Pass	-0.01	6.2	110	93.9
15	Pass	0.00	6.3	108	94.8

[0019] A liquid formulation containing 10 mM phosphate, 50 mM NaCl, 3% PEG 3350, 20% sucrose, and 100 mg/ml VEGF trap (SEQ ID NO:4), pH 6.25, was stored at 5 °C and samples tested at 0, 1, 2.3, 3, 9, 12 and 15 months. Stability results are shown in Table 6.

Table 6. Stability of 100 mg/ml VEGF Trap Protein Stored at 5 °C (VGFT-SS101)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	96.5
1	Pass	0.01	6.2	94	96.2
2.3	Pass	0.01	6.2	93	95.7
6	Pass	0.01	6.2	102	94.6
9	Pass	0.00	6.2	95	94.6
12	Pass	0.00	6.3	96	92.8
15	Pass	0.01	6.3	102	93.9

Example 4. Stable VEGF Trap Formulation

[0020] The formulation of the invention comprises 10 mM histidine, 50 mM NaCl, 5-20% sucrose, 50-100 mg/ml VEGF trap, and one of 0.1% polysorbate 20 or 3% PEG 3350. One advantage of this liquid formulation is that it provides a higher concentration of VEGF trap without requiring the manufacture of a lyophilized product. Thus, this formulation provides ease for subcutaneous delivery, for example, by allowing provision of a liquid pre-filled syringe at a concentration higher than that delivered by IV infusion. Also, this formulation could advantageously be used to provide lower infusion volumes and shorter infusion times. The amount of degradation determined by SE-HPLC following incubation at 5 °C for up to 15 or 24 months is summarized in Table 7.

Table 7. Stability of Liquid Formulation with 50-100 mg/ml VEGF Trap (VGFT-SS101)

Incubation (months)	VEGF Trap (mg/ml)	% Polysorbate 20	% PEG 3350	% Degradation
24	50	0.1	-	0.7

Incubation (months)	VEGF Trap (mg/ml)	% Polysorbate 20	% PEG 3350	% Degradation
24	50	-	3	1.3
15	75	0.1	-	1.5
15	75	-	3	2.0
15	100	0.1	-	1.9
15	100	-	3	2.6

SEQUENCE LISTING

[0021]

<110> Regeneron Pharmaceuticals, Inc.

<120> VEGF Antagonist Formulations

<130> 4030A-WO

<140> To be assigned
<141> 2006-03-22

<150> 60/665,125 <151> 2005-03-25

<160> 4

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 1453
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 1

```
aagcttgggc tgcaggtcga tcgactctag aggatcgatc cccgggcgag ctcgaattcg 60
caaccaccat ggtcagctac tgggacaccg gggtcctgct gtgcgcgctg ctcagctgtc 120
tgcttctcac aggatctagt tccggaggta gacctttcgt agagatgtac agtgaaatcc 180
ccgaaattat acacatgact gaaggaaggg agctcgtcat tccctgccgg gttacgtcac 240
ctaacatcac tgttacttta aaaaagtttc cacttgacac tttgatccct gatggaaaac 300
gcataatctg ggacagtaga aagggcttca tcatatcaaa tgcaacgtac aaagaaatag 360
ggcttctgac ctgtgaagca acagtcaatg ggcatttgta taagacaaac tatctcacac 420
atcgacaaac caatacaatc atagatgtgg ttctgagtcc gtctcatgga attgaactat 480
ctgttggaga aaagcttgtc ttaaattgta cagcaagaac tgaactaaat gtggggattg 540
acttcaactg ggaatacctt tcttcgaagc atcagcataa gaaacttgta aaccgagacc 600
taaaaaccca gtctgggagt gagatgaaga aatttttgag caccttaact atagatggtg 660
taacccggag tgaccaagga ttgtacacct gtgcagcatc cagtgggctg atgaccaaga 720
agaacagcac atttgtcagg gtccatgaaa agggcccggg cgacaaaact cacacatgcc 780
caccgtgccc agcacctgaa ctcctggggg gaccgtcagt cttcctcttc cccccaaaac 840
ccaaggacac cctcatgatc tcccggaccc ctgaggtcac atgcgtggtg gtggacgtga 900
gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag gtgcataatg 960
ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc agcgtcctca 1020
ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc tccaacaaag 1080
ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc cgagaaccac 1140
aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc agcctgacct 1200
gcctggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc aatgggcagc 1260
cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc ttcttcctct 1320
atagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg 1380
tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg tctccgggta 1440
aatgagcggc cgc                                                    1453
```

<210> 2
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 2

```
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1               5                  10                  15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu
            20                  25                  30
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
        35                  40                  45
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
    50                  55                  60
Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
65                  70                  75                  80
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu
                85                  90                  95
Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
            100                 105                 110
Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
        115                 120                 125
Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
    130                 135                 140
Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
145                 150                 155                 160
Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
                165                 170                 175
Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
            180                 185                 190
Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
        195                 200                 205
Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
    210                 215                 220
Val His Glu Lys Gly Pro Gly Asp Lys Thr His Thr Cys Pro Pro Cys
225                 230                 235                 240
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
                245                 250                 255
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
            260                 265                 270
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
        275                 280                 285
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
    290                 295                 300
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
305                 310                 315                 320
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
                325                 330                 335
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
            340                 345                 350
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
        355                 360                 365
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
    370                 375                 380
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
385                 390                 395                 400
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
                405                 410                 415
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
            420                 425                 430
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
        435                 440                 445
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
```

<210> 3

<211> 1377

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic

<400> 3

```
atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60
acaggatcta gttccggaag tgataccggt agacctttcg tagagatgta cagtgaaatc 120
cccgaaatta tacacatgac tgaaggaagg gagctcgtca ttccctgccg ggttacgtca 180
cctaacatca ctgttacttt aaaaaagttt ccacttgaca ctttgatccc tgatggaaaa 240
cgcataatct gggacagtag aaagggcttc atcatatcaa atgcaacgta caaagaaata 300
gggcttctga cctgtgaagc aacagtcaat gggcatttgt ataagacaaa ctatctcaca 360
catcgacaaa ccaatacaat catagatgtg gttctgagtc cgtctcatgg aattgaacta 420
tctgttggag aaaagcttgt cttaaattgt acagcaagaa ctgaactaaa tgtggggatt 480
gacttcaact gggaataccc ttcttcgaag catcagcata agaaacttgt aaaccgagac 540
ctaaaaaccc agtctgggag tgagatgaag aaatttttga gcaccttaac tatagatggt 600
gtaacccgga gtgaccaagg attgtacacc tgtgcagcat ccagtgggct gatgaccaag 660
aagaacagca catttgtcag ggtccatgaa aaggacaaaa ctcacacatg cccaccgtgc 720
ccagcacctg aactcctggg gggaccgtca gtcttcctct tccccccaaa acccaaggac 780
accctcatga tctcccggac ccctgaggtc acatgcgtgg tggtggacgt gagccacgaa 840
gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca 900
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 960
caccaggact ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca 1020
gcccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac 1080
accctgcccc catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctggtc 1140
aaaggcttct atcccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac 1200
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctacagcaag 1260
ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat 1320
gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctccggg taaatga    1377
```

<210> 4
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 4

```
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
 1               5                  10                  15
Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Asp Thr Gly Arg Pro
            20                  25                  30
Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu
        35                  40                  45
Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr
    50                  55                  60
Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
65                  70                  75                  80
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
                85                  90                  95
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His
            100                 105                 110
Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile
        115                 120                 125
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu

    130                 135                 140
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
145                 150                 155                 160
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
                165                 170                 175
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
            180                 185                 190
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
        195                 200                 205
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
    210                 215                 220
Phe Val Arg Val His Glu Lys Asp Lys Thr His Thr Cys Pro Pro Cys
225                 230                 235                 240
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
                245                 250                 255
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
            260                 265                 270
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
        275                 280                 285
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
    290                 295                 300
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
305                 310                 315                 320
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
                325                 330                 335
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
            340                 345                 350
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
        355                 360                 365
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
    370                 375                 380
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
385                 390                 395                 400
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
                405                 410                 415
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
            420                 425                 430
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
        435                 440                 445
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455
```

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO2005000895A [0003]
- US2005032699A [0003]
- WO02060489A [0003]
- US2005276808A [0003]
- US60665125B [0021]

Non-patent literature cited in the description

- **GERBER et al.**Cancer Res., 2000, vol. 60, 6253-6258 [0002]
- **KIM et al.**Proc. Natl. Acad. Sci. USA, 2002, vol. 99, 11399-404 [0002]
- **HOLASH et al.**Proc. Natl. Acad. Sci. USA, 2002, vol. 99, 11393-8 [0002]
- **FRASER et al.**J. Clin. Endocrinol. & Metabolism, 2005, vol. 90, 21114-1122 [0003]

Patentkrav

1. Flydende formulering af en VEGF-antagonist, omfattende 10 mM histidin, 50 mM NaCl, 5 - 20% sucrose og 50 - 100 mg/ml af et fusions-protein, der har en receptor-komponent bestående af et immunoglobulin-lignende (Ig) domæne 2 af en første VEGF-receptor, og et Ig-domæne 3 af en anden VEGF-receptor, og en multimeriserings-komponent, ved en pH på 6,0 - 6,5, hvor den første VEGF-receptor er Flt1, og den anden VEGF-receptor er Flk1 eller Flt4, yderligere omfattende 0,1 % polysorbat 20 eller 3% PEG 3350.

2. Flydende formulering ifølge krav 1, hvor fusionsproteinet har aminosyresekvensen SEQ ID NO:4.

3. Flydende formulering ifølge krav 1 eller 2, hvor fusions-proteinet omfatter aminosyrerne 27 - 457 af SEQ ID NO:4, og er glycosyleret ved Asn-resterne 62, 94, 149, 222 og 308.