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Formulations antagonistes du VEGF

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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] Lyophilization (freeze drying under controlled conditions) is commonly used for long term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state (see, for example, U.S. 6,436,897).

[0004] The following documents are also referred to:

- WO 2005/000895 which refers to VEGF traps and therapeutic uses thereof;
- Fraser et al (2004) J. Clin Endocrinology & 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 2003/202972 which is concerned with a method of administering and using VEGF inhibitors for the treatment of cancer.

BRIEF SUMMARY OF THE INVENTION

[0005] 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 as defined in the claims.

[0006] The invention provides a stable liquid pharmaceutical formulation of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist, comprising a fusion protein consisting of amino acids 27-457 of SEQ ID NO:4 and glycosylated Asn residues at positions 62, 94, 149, 222 and 308, 5 mM phosphate buffer, 5 mM citrate buffer, 100 mM NaCl, 0.1 % polysorbate 20, 20% sucrose and 25 mg/ml of the fusion protein, at a pH of 6.0-6.1.

[0007] The invention further provides a pre-lyophilized formulation, comprising (i) 5-75 mg/ml of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist consisting of amino acids 27-457 of SEQ ID NO:4 and glycosylated Asn residues at positions 62, 94, 149, 222 and 308, (ii) a histidine buffer, comprising 5-50 mM histidine, (iii) 0.1-3.0% polyethylene glycol (PEG), (iv) 0.25-3.0% glycine, and (v) 0.5-6.0% sucrose, at a pH of 6.0- 6.5. In one embodiment, the pre-lyophilized formulation of the invention does not contain a preservative.

[0008] In any embodiment, the pre-lyophilized formulation may further comprise up to 0.05 mM citrate and/or 0.003-0.005% polysorbate. The polysorbate present may be, for example, polysorbate 20.

[0009] In a more specific embodiment, the pre-lyophilized formulation comprises about 10 mM histidine, about 1.5% PEG 3350, about 0.75% glycine, about 2.5% sucrose, and about 12.5 to 75 mg/ml VEGF-specific fusion protein, at a pH of about 6.25. In separate embodiments, the reconstituted formulation is 2 times the concentration of the pre-lyophilized formulation, e.g., a 20 mg fusion protein/ml pre-lyophilized formulation is reconstituted to a final formulation of 60 mg fusion protein/ml. Generally, the lyophilized formulation is reconstituted with sterile water suitable for injection. In one embodiment, the reconstitution liquid may be bacteriostatic water.

[0010] The invention further provides a method of producing a reconstituted lyophilized pharmaceutical formulation of a VEGF-specific fusion protein antagonist, comprising reconstituting the lyophilized formulation described with liquid, wherein a reconstituted lyophilized pharmaceutical formulation is generated. The lyophilized formulation may be lyophilized by any method known in the art for lyophilizing a liquid.

[0011] The invention further provides a method of producing a reconstituted lyophilized pharmaceutical formulation of a VEGF-specific fusion protein antagonist, comprising reconstituting the lyophilized formulation described with liquid, wherein a reconstituted lyophilized pharmaceutical formulation is generated. In one embodiment, the reconstituted

formulation is twice the concentration of the pre-lyophilized formulation, e.g., the method of the disclosure comprises: (a) producing a pre-lyophilized formulation of a VEGF-specific fusion protein antagonist, (b) subjecting the pre-lyophilized formulation of step (a) to lyophilization; and (c) reconstituting the lyophilized formulation of step (b).

[0012] In specific embodiments of the method of producing a reconstituted lyophilized formulation, a pre-lyophilized solution is present in a vial as a 25 mg VEGF-specific fusion protein antagonist per ml solution of pre-lyophilized formulation, which is lyophilized and reconstituted to an 50 mg/ml solution. In another embodiment, a 30 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 60 mg/ml solution. In another embodiment, a 40 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 80 mg/ml solution. In another embodiment, a 12.5 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 25 mg/ml solution. In another embodiment, a 50 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 100 mg/ml solution. In another embodiment, a 75 mg/ml pre-lyophilized solution is lyophilized and reconstituted to a 150 mg/ml solution. Preferably, the reconstituted lyophilized formulation does not contain a preservative.

DETAILED DESCRIPTION OF THE INVENTION

General Description

[0013] 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.

[0014] Chemical and physical stability can be promoted by removing water from the protein. Lyophilization (freeze-drying under controlled conditions) is commonly used for long-term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state. The lyophilized protein is normally reconstituted with water optionally containing a bacteriostatic preservative (e.g., benzyl alcohol) prior to administration.

Definitions

[0015] The term "lyophilized" or "freeze-dried" includes a state of a substance that has been subjected to a drying procedure such as lyophilization, where at least 50% of moisture has been removed.

[0016] The phrase "bulking agent" includes a compound that is pharmaceutically acceptable and that adds bulk to a lyo cake. Generally, acceptable bulking agents known to the art include, for example, carbohydrates, including simple sugars such as dextrose, ribose, fructose and the like, alcohol sugars such as mannitol, inositol and sorbitol, disaccharides including trehalose, sucrose and lactose, naturally occurring polymers such as starch, dextrans, chitosan, hyaluronate, proteins (e.g., gelatin and serum albumin), glycogen, and synthetic monomers and polymers. In the formulations of the disclosure, PEG 3350 is an organic co-solvent which is used to stabilize the fusion protein when agitated, mixed, or handled, and as a bulking agent to help produce an acceptable bulk.

[0017] The term "lyoprotectant" includes a substance that may be added to a freeze-dried or lyophilized formulation to help maintain protein structure when freeze-dried or lyophilized.

[0018] A "preservative" includes a bacteriostatic, bacteriocidal, fungistatic or fungicidal compound that is generally added to formulations to retard or eliminate growth of bacteria or other contaminating microorganisms in the formulations. Preservatives include, for example, benzyl alcohol, phenol, benzalkonium chloride, m-cresol, thimerosol, chlorobutanol, methylparaben, propylparaben and the like. Other examples of pharmaceutically acceptable preservatives can be found in the USP.

VEGF Antagonists

[0019] An 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. The fusion protein used in the formulation of the invention comprises amino acids 27-457 of SEQ ID NO:4 and is glycosylated at Asn residues 62, 94, 149, 222 and 308. In specific embodiments, the VEGF antagonist is expressed in a mammalian cell line such as a CHO cell and is modified post-translationally.

[0020] 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 result of the purification process, for example, low or trace amounts of citrate and/or polysorbate.

Lyophilization and Lyophilized Formulations

[0021] Lyophilized formulations can be reconstituted into solutions, suspensions, emulsions, or any other suitable form for administration or use. Lyophilized formulations are typically first prepared as liquids, then frozen and lyophilized. The total liquid volume before lyophilization can be less, equal to, or more than, the final reconstituted volume of the lyophilized formulation. The lyophilization process is well known to those of ordinary skill in the art, and typically includes sublimation of water from a frozen formulation under controlled conditions.

[0022] Lyophilized formulations can be stored at a wide range of temperatures. Lyophilized formulations may be stored below 25°C, for example, refrigerated at 4°C, or at room temperature (e.g., approximately 25°C). Preferably, lyophilized formulations are stored below about 25°C, more preferably, at about 4-20°C; below about 4°C; below about -20°C; about - 40°C; about -70°C, or about -80°C.

[0023] Lyophilized formulations are typically reconstituted for use by addition of an aqueous solution to dissolve the lyophilized formulation. A wide variety of aqueous solutions can be used to reconstitute a lyophilized formulation. Preferably, lyophilized formulations are reconstituted using water. Lyophilized formulations are preferably reconstituted with a solution consisting essentially of water (e.g., USP WFI, or water for injection) or bacteriostatic water (e.g., USP WFI with 0.9% benzyl alcohol). However, solutions comprising buffers and/or excipients and/or one or more pharmaceutically acceptable carries can also be used.

[0024] Freeze-dried or lyophilized formulations are typically prepared from liquids, that is, from solutions, suspensions, emulsions, and the like. Thus, the liquid that is to undergo freeze-drying or lyophilization preferably comprises all components desired in a final reconstituted liquid formulation. As a result, when reconstituted, the freeze-dried or lyophilized formulation will render a desired liquid formulation upon reconstitution. Freeze-dried or lyophilized formulations comprise histidine, since histidine, in comparison to phosphate, is more effective at stabilizing the fusion protein when the fusion protein is lyophilized. Organic co-solvents, such as PEG 3350, are used to stabilize the fusion protein when agitated, mixed, or handled. A lyoprotectant is preferably used in freeze-dried or lyophilized formulations. Lyoprotectants help to maintain the secondary structure of proteins when freeze-dried or lyophilized. Two preferred example lyoprotectants are glycine and sucrose, which are preferably used together.

Stable Liquid Formulations

[0025] The invention provides a stable liquid pharmaceutical formulation as defined above.

[0026] A combination of NaCl and sucrose has been established to stabilize the fusion protein more effectively than either individual stabilizer alone.

[0027] 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.

[0028] Formulations, whether liquid or freeze-dried and lyophilized, 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

[0029] 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
24	Pass	0.00	6.2	93	98.3

[0030] 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

[0031] 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

[0032] 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
15	Pass	0.00	6.3	98	94.8

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

[0033] 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

[0034] 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. Further Embodiments of Stable VEGF Trap Formulations

[0035] In one embodiment, the disclosure provides a stable liquid VEGF-binding fusion protein (VEGF trap) formulations

comprising 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 0.1% Polysorbate 20, 20% sucrose, 25 mg/ml VEGF trap protein, pH 6.0. This formulation can either be delivered subcutaneously or diluted and delivered by intravenous infusion. Due to the high osmolality of this formulation, it is diluted 3-fold to achieve an iso-osmolar solution for intravenous administration. Stability studies showed less than about 1 % degradation was detected after 3 years of storage at 2-8°C.

[0036] Also referred to is a lyophilized formulation which is preferably concentrated two-fold from the pre-lyophilized to the post-lyophilized formulation, e.g., 50 to 100 mg/ml; 75 to 150 mg/ml, or 100 to 200 mg/ml VEGF trap protein. In one specific embodiment, the pre-lyophilized formulation comprises 10 mM histidine, 1.5% PEG 3350, 0.75% glycine, 2.5% sucrose, 50 mg/ml VEGF trap protein, pH 6.3, and is reconstituted to a formulation comprising 20 mM histidine, 3% PEG 3350, 1.5% glycine, 5% sucrose, 100 mg/ml VEGF trap protein, pH 6.3. Stability studied showed no degradation of the VEGF trap was detected after 6 months of storage at 2-8 °C.

[0037] Also referred to is a formulation that 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
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

Example 5. Stability and Activity of Lyophilized and Liquid

[0038] The stability of a reconstituted lyophilized formulation was determined over a 6 month period. The pre-lyophilized formulation contained 10 mM histidine, 1.5% PEG 3350, 2.5% sucrose, 0.75% glycine and 50 mg/ml VEGF trap protein. After lyophilization, the reconstituted formulation contained 20 mM histidine, 3% PEG 3350, 5% sucrose, 1.5% glycine, and 100 mg/ml VEGF trap protein (SEQ ID NO:4). The results shown in Table 8. Activity was determined in a cell based bioassay which directly measures the ability of the VEGF trap to inhibit the biological effects of human VEGF on a mouse Baf/3 VEGFR1/EpoR cell line. Therefore, this bioassay directly measures the biological activity of the protein. The results are expresses as percent relative potency (test sample IC_{50}/ reference VEGF IC_{50} standard x 100). The binding affinity of VEGF to the VEGF trap is measured using a sensitive ELISA that specifically measures free VEGF in equilibrated mixtures containing VEGF and various concentrations of the VEGF trap. Results are expressed as percent relative binding (IC_{50} of test sample/IC_{50} of reference x 100). Measured pH ranged between 6.3 - 6.5. All solutions where visually clear. The concentration of VEGF trap recovered was determined with a UV spectrophotometer as mg/ml at A_{280} nm. The percent VEGF trap recovered in the native configuration (main peak purity) was determined with SE-HPLC.

Table 8. Stability of VEGF Trap Lyophilized Formulation Stored at 5 °C (VGT-RS475)

Months	Bioassay	Binding Assay	% Recovered	% Native Configuration
0	120	126	97.9	98.7
1	117	74	97.9	98.6
1 + 24 hr	126	72	99.0	98.5
1+ 4 hr	94	81	101.5	98.2
3	101	98	98.1	98.6
3 + 24 hr	65	94	98.1	98.2

(continued)

Months	Bioassay	Binding Assay	% Recovered	% Native Configuration
6 + 4 hr			96.9	98.7
6 + 24 hr			98.8	98.6

[0039] A formulation containing about 5 mM phosphate, 5 mM citrate, 100 mM NaCl, 0.1% polysorbate 20, 20% sucrose, and 25 mg/ml VEGF trap protein was tested for stability and activity over 36 months when stored at 5 °C. The results are shown in Table 9. All samples were clear and colorless as determined by visual inspection. pH ranged from 6.0-6.1. *Binding assay results for two measurements (1 and 2 months) are expressed directly and not as a percent of the standard.

Table 9. Stability and Activity of Liquid Formulation (VGT-FS405)

Months	% Native Configuration	Bioassay	Binding Assay	Protein Content mg/ml
0	99.7	106	72	25.0
1	99.9	119	4.4 pM*	25.2
2	99.6	102	5.4 pM*	25.1
3	99.6	97	88	25.1
6	99.6	101	106	25.0
9	99.4	89	126	25.4
12	99.5	85	95	25.2
18	99.4	99	81	25.5
24	99.3	75	95	25.6
36	98.8	109	79	25.6

SEQUENCE LISTING

[0040]

<110> Regeneron Pharmaceuticals, Inc.

<120> VEGF Antagonist Formulations

<130> 4030A-WO

<140> To be assigned
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<150> 60/665,125
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Claims

1. A stable liquid pharmaceutical formulation of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist, comprising a fusion protein consisting of amino acids 27-457 of SEQ ID NO:4 and glycosylated Asn residues at positions 62, 94, 149, 222 and 308, 5 mM phosphate buffer, 5 mM citrate buffer, 100 mM NaCl, 0.1 % polysorbate 20, 20% sucrose and 25 mg/ml of the fusion protein, at a pH of 6.0-6.1.

2. A pre-lyophilized formulation, comprising (i) 5-75 mg/ml of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist consisting of amino acids 27-457 of SEQ ID NO:4 and glycosylated Asn residues at positions 62, 94, 149, 222 and 308, (ii) a histidine buffer, comprising 5-50 mM histidine, (iii) 0.1-3.0% polyethylene glycol (PEG), (iv) 0.25-3.0% glycine, and (v) 0.5-6.0% sucrose, at a pH of 6.0- 6.5.

3. The pre-lyophilized formulation of claim 2, comprising 10 mM histidine, 1.5% PEG 3350, 0.75% glycine, 2.5% sucrose, and 12.5 to 75 mg/ml of the fusion protein, at a pH of 6.25.

4. The pre-lyophilized formulation of claim 3, comprising 10 mM histidine, 1.5% PEG 3350, 0.75% glycine, 2.5% sucrose, and 50 mg/ml of the fusion protein.

5. A method of producing a lyophilized pharmaceutical formulation of a VEGF-specific fusion protein antagonist, com-

prising subjecting the pre-lyophilized formulation of any one of claims 2 to 4 to lyophilization to generate a lyophilized pharmaceutical formulation.

6. A method of producing a reconstituted lyophilized pharmaceutical formulation of a VEGF-specific fusion protein antagonist, comprising reconstituting the lyophilized formulation of claim 5 with liquid, wherein a reconstituted lyophilized pharmaceutical formulation is generated.

7. The method of claim 6, wherein the liquid is sterile water or bacteriostatic water.

Patentansprüche

1. Stabile flüssige pharmazeutische Formulierung eines vaskulären Endothel-Wachstumsfaktor (VEGF)-spezifischem Fusionsproteinantagonisten, umfassend ein Fusionsprotein, bestehend aus den Aminosäuren 27 - 457 von SEQ ID NO:4 und glykosylierten Asn-Resten an den Positionen 62, 94, 149, 222 und 308, 5 mM Zitratpuffer, 100 mM NaCl, 0,1 % Polysorbat 20, 20 % Sucrose und 25 mg/ml des Fusionsproteins bei einem pH-Wert von 6,0 - 6,1.

2. Vorlyophilisierte Formulierung, umfassend (i) 5 - 75 mg/ml eines vaskulären Endothel-Wachstumsfaktor (VEGF)-spezifischen Fusionsproteinantagonisten, bestehend aus den Aminosäuren 27 - 457 von SEQ ID NO:4 und glykosylierten Asn-Resten an den Positionen 62, 94, 149, 222 und 308, (ii) einen Histidinpuffer, umfassend 5 - 50 mM Histidin, (iii) 0,1 - 3,0 % Polyethylenglycol (PEG), (iv) 0,25 - 3,0 % Glycin und (v) 0,5 - 6,0 % Sucrose bei einem pH-Wert von 6,0 - 6,5.

3. Vorlyophilisierte Formulierung nach Anspruch 2, umfassend 10 mM Histidin, 1,5 % PEG 3350, 0,75 % Glycin, 2,5 % Sucrose und 12,5 bis 75 mg/ml des Fusionsproteins bei einem pH-Wert von 6,25.

4. Vorlyophilisierte Formulierung nach Anspruch 3, umfassend 10 mM Histidin, 1,5 % PEG 3350, 0,75 % Glycin, 2,5 % Sucrose und 50 mg/ml des Fusionsproteins.

5. Verfahren der Herstellung einer lyophilisierten Formulierung eines VEGF-spezifischen Fusionsproteinantagonisten, umfassend das Unterziehen der vorlyophilisierten Formulierung nach einem der Ansprüche 2 bis 4 einer Lyophilisierung, um eine lyophilisierte pharmazeutische Formulierung zu erzeugen.

6. Verfahren der Herstellung einer rekonstituierten lyophilisierten pharmazeutischen Formulierung eines VEGF-spezifischen Fusionsproteinantagonisten, umfassend das Rekonstituieren der lyophilisierten Formulierung nach Anspruch 5 mit einer Flüssigkeit, wobei eine rekonstituierte lyophilisierte pharmazeutische Formulierung erzeugt wird.

7. Verfahren nach Anspruch 6, wobei die Flüssigkeit steriles Wasser oder bakteriostatisches Wasser ist.

Revendications

1. Formulation pharmaceutique liquide stable d'un antagoniste de protéine de fusion spécifique du facteur de croissance endothéliale vasculaire (VEGF), comprenant une protéine de fusion constituée des acides aminés 27 à 457 de la SEQ ID n° : 4 et de résidus Asn glycosylés aux positions 62, 94, 149, 222 et 308, d'un tampon de phosphate 5 mM, d'un tampon de citrate 5 mM, de NaCl 100 mM, de polysorbate 20 à 0,1 %, de saccharose à 20 % et de 25 mg/ml de la protéine de fusion, à un pH de 6,0 à 6,1.

2. Formulation pré-lyophilisée, comprenant (i) de 5 à 75 mg/ml d'un antagoniste de protéine de fusion spécifique du facteur de croissance endothéliale vasculaire (VEGF), constituée des acides aminés 27 à 457 de la SEQ ID n° : 4 et de résidus Asn glycosylés aux positions 62, 94, 149, 222 et 308, (ii) un tampon d'histidine, comprenant de l'histidine 5 à 50 mM, (iii) du polyéthylène glycol (PEG) à 0,1 à 3,0 %, (iv) de la glycine à 0,25 à 3,0 % et (v) du saccharose à 0,5 à 6,0 %, à un pH de 6,0 à 6,5.

3. Formulation pré-lyophilisée selon la revendication 2, comprenant de l'histidine 10 mM, PEG 3350 à 1,5 %, de la glycine à 0,75 %, du saccharose à 2,5 % et de 12,5 à 75 mg/ml de la protéine de fusion, à un pH de 6,25.

4. Formulation pré-lyophilisée selon la revendication 3, comprenant de l'histidine 10 mM, du PEG 3350 à 1,5 %, de

la glycine à 0,75 %, du saccharose à 2,5 % et 50 mg/ml de la protéine de fusion.

5. Procédé de production d'une formulation pharmaceutique lyophilisée d'un antagoniste de protéine de fusion spécifique du VEGF, comprenant la soumission de la formulation pré-lyophilisée selon l'une quelconque des revendications 2 à 4 à une lyophilisation pour générer une formulation pharmaceutique lyophilisée.

6. Procédé de production d'une formulation pharmaceutique lyophilisée reconstituée d'un antagoniste de protéine de fusion spécifique du VEGF, comprenant la reconstitution de la formulation lyophilisée selon la revendication 5 avec du liquide, une formulation pharmaceutique lyophilisée reconstituée étant générée.

7. Procédé selon la revendication 6, dans lequel le liquide est de l'eau stérile ou de l'eau bactériostatique.

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.

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- WO 02060489 A **[0004]**
- US 2003202972 A **[0004]**
- WO 60665125 A **[0040]**

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- **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 Endocrinology & Metabolism,* 2004, vol. 90 (2), 1114-1122 **[0004]**

VEGF antagonista formulációk

Szabadalmi igénypontok

1. Stabil folyékony vaszkuláris endotél növekedési faktor (VEGF)-specifikus fúziós protein antagonista gyógyászati formuláció, mely egy 4. számú szekvencia 27-457. számú aminosavjaiból és a 62, 94, 149, 222 és 308. helyzetekben glikozilezett Asn gyökökből álló fúziós proteint, 5 mM foszfátpuffert, 5 mM citrátpuffert, 100 mM NaCl-t, 0.1 % poliszorbát 20-t, 20% szacharózt és 25 mg/ml koncentrációban a fúziós proteint tartalmazza, 6.0-6.1 pH-értéken.

2. Előre liofilizált formuláció, mely (i) 5-75 mg/ml 4. számú szekvencia 27-457. számú aminosavjaiból és a 62, 94, 149, 222 és 308. helyzetekben glikozilezett Asn gyökökből álló vaszkuláris endotél növekedési faktor (VEGF)-specifikus fúziós proteint, (ii) egy 5-50 mM hisztidint tartalmazó hisztidin puffert, (iii) 0.1-3.0% polietilénglikolt (PEG), (iv) 0.25-3.0% glicint, és (v) 0.5-6.0% szacharózt tartalmaz, 6.0-6.5 pH-értéken.

3. A 2. igénypont szerinti előre liofilizált formuláció, mely 10 mM hisztidint, 1.5% PEG 3350-t, 0.75% glicint, 2.5% szacharózt, és 12.5-75 mg/ml fúziós proteint tartalmaz, 6.25 pH-értéken.

4. A 3. igénypont szerinti előre liofilizált formuláció, mely 10 mM hisztidint, 1.5% PEG 3350-t, 0.75% glicint, 2.5% szacharózt, és 50 mg/ml fúziós proteint tartalmaz.

5. Eljárás egy VEGF-specifikus protein antagonista liofilizált gyógyászati formulációjának előállítására, melynek során a 2-4. igénypontok bármelyike szerinti előre liofilizált formulációt liofilizálásnak vetjük alá, egy liofilizált gyógyászati formuláció előállítására.

6. Eljárás egy VEGF-specifikus fúziós protein antagonista rekonstruált liofilizált gyógyászati formuláció előállítására, melynek során az 5. igénypont szerinti liofilizált formulációt egy folyadékkal rekonstruáljuk, ahol egy rekonstruált gyógyászati formuláció jön létre.

7. A 6. igénypont szerinti eljárás, ahol a folyadék steril víz vagy bakteriosztatikus víz.