(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 364 691 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
14.09.2011 Bulletin 2011/37

(21) Application number: **11157965.2**

(22) Date of filing: **14.06.2007**

(51) Int Cl.:
A61K 9/00 *(2006.01)* ***A61K 9/19*** *(2006.01)*
A61K 38/00 *(2006.01)*

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

(30) Priority: **16.06.2006 US 814484 P**

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
07809593.2 / 2 029 103

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Remarks:
This application was filed on 11-03-2011 as a divisional application to the application mentioned under INID code 62.

(54) VEGF antagonist formulations suitable for intravitreal administration

(57) Ophthalmic formulations of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist are provided suitable for intravitreal administration to the eye. The ophthalmic formulations include a stable liquid formulation and a lyophilizable formulation. Preferably, the protein antagonist has the amino acid sequence showing in SEQ ID No: 4 or amino acids 27-457 of SEQ ID No: 4.

EP 2 364 691 A1

Description

BACKGROUND OF INVENTION

Field of the Invention

[0001] The present invention is directed to pharmaceutical formulations suitable for intravitreal administration comprising agents capable of inhibiting vascular endothelial growth factor (VEGF), and to methods for making and using such formulations. The invention includes liquid pharmaceutical formulations having increased stability, as well as formulations that may be lyophilize and reconstituted for intravitreal administration.

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] Ophthalmic formulations are known, see for example, U.S. 7,033,604 and 6,777,429, An ophthalmic formulation of a VEGF antibody is described in US 6,676,941.

[0004] 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).

BRIEF SUMMARY OF THE INVENTION

[0005] Stable formulations of a VEGF-specific fusion protein antagonist are provided. Pharmaceutically acceptable formulations are provided that comprise a VEGF "trap" antagonist with a pharmaceutically acceptable carrier. In specific embodiments, liquid and lyophilized formulations are provided.

[0006] In a first aspect, a stable liquid ophthalmic formulation of a VEGF-specific fusion protein antagonist is provided, comprising a fusion protein that comprises a receptor component consisting essentially of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and I_g domain 3 of a second VEGF receptor, and a multimerizing component (also termed a "VEGF trap"). In a specific embodiment of the VEGF-specific fusion protein antagonist, the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1 or Flt4. In a more specific embodiment the fusion protein has the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Preferably, the VEGF antagonist is a dimer comprising two fusion proteins of SEQ ID NO:4.

[0007] In one aspect, a stable liquid ophthalmic formulation is provided that comprises 1-100 mg/ml VEGF-specific fusion protein antagonist, 0.01-5% of one or more organic co-solvent(s), 30-150 mM of one or more tonicity agent(s), 5-40 mM of a buffering agent, and optionally, 1.0-7.5% of a stabilizing agent, pH between about 5.8-7.0.

[0008] In one or more specific embodiments, the organic co-solvent may be polysorbate, for example, polysorbate 20 or polysorbate 80, polyethylene glycol (PEG), for example, PEG 3350, or propylene glycol, or a combination thereof; the tonicity agent may be, for example, sodium chloride or potassium chloride; the stabilizing agent may be sucrose, sorbitol, glycerol, trehalose, or mannitol; and the buffering agent may be, for example, phosphate buffer. In a specific embodiment, the phosphate buffer is a sodium phosphate buffer.

[0009] In various embodiments, the organic co-solvent is polysorbate and/or PEG, the stabilizing agent is sucrose, the buffering agent is phosphate buffer, and the tonicity agent is sodium chloride.

[0010] More specifically, the stable liquid ophthalmic formulation comprises about 40-50 mg/ml of the VEGF antagonist (SEQ ID NO:4), about 10 mM phosphate buffer, 0.01-3% polysorbate and/or PEG, 40-135 mM sodium chloride, and optionally 5.0% sucrose, pH about 6.2-6.3.

[0011] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 50 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 50 mM sodium chloride, 0.1 % polysorbate, and 5% sucrose, pH about 6.2-6.3.

[0012] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 50 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 50 mM sodium chloride, 3% PEG, and 5% sucrose, pH about 6.2-6.3.

[0013] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 40 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 40 mM sodium chloride, 0.03% polysorbate, and

5% sucrose, pH about 6.2-6.3.

[0014] In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 40 mg/ml of the VEGF antagonist (SEQ ID NO,4), 10 mM sodium phosphate buffer, 135 mM sodium chloride, and 0.03% polysorbate, pH about 6.2-6.3.

[0015] In another aspect, a stable liquid ophthalmic formulation is provided that comprises 1-100 mg/ml VEGF-specific fusion protein antagonist; 0.01-5% of one or more organic co-solvent(s); 5-40 mM of a buffering agent; and optionally 30-150 mM of one or more tonicity agent(s) and/or 1.0-7.5% of a stabilizing agent; having a pH between about 5.8-7.0.

[0016] In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10 to about 80 mg/ml. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10, about 20, about 30, about 40, about 50, about 60, about 70, or about 80 mg/ml. In a preferred embodiment, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 40 mg/ml.

[0017] In another embodiment, the stabilizing agent is selected from one or more of sucrose, sorbitol, glycerol, trehalose, and mannitol.

[0018] In another embodiment, the organic co-solvent is selected from one or more of polysorbate, for example, polysorbate 20 or polysorbate 80, polyethylene glycol (PEG), for example, PEG 3350, and propylene glycol.

[0019] In another embodiment, the buffer is a phosphate buffer, for example, sodium phosphate.

[0020] In another embodiment, the tonicity agent is a salt, for example, sodium chloride.

[0021] In one embodiment, the stable liquid ophthalmic formulation comprises 10 mM sodium phosphate buffer, about 0,03 to about 0.1% polysorbate and/or about 3% PEG or propylene glycol, about 40 mM sodium chloride, and about 5% sucrose. In a specific embodiment, the stable liquid ophthalmic formulation comprises 10 mM sodium phosphate buffer, about 0,03% polysorbate, about 40 mM sodium chloride, and about 5% sucrose. In another specific embodiment, the pH of the formulation is about 6.2 to about 6.3. In another specific embodiment, the pH is achieved by mixing mono- and dibasic sodium phosphate to the desired pH without acid/base titration.

[0022] In a specific embodiment, the stable liquid ophthalmic formulations consists essentially of a VEGF antagonist (SEQ ID NO:4) at 40 mg/ml, 10 mM sodium phosphate buffer, polysorbate at 0.03%, sodium chloride at 40 mM, and sucrose at 5%, pH 6.2-6.3.

[0023] In another aspect, a stable liquid ophthalmic formulation is provided that comprises about 10 to about 80 mg/ml VEGF antagonist, about 10 mM sodium phosphate buffer, about 0.03% polysorbate, and about 135 mM sodium chloride, pH of 6.2 to 6.3.

[0024] In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10 to about 80 mg/ml. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10, about 20, about 30, about 40, about 50, about 60, about 70, or about 80 mg/ml. In a specific embodiment, the VEGF antagonist (SEQ ID NO: 4) is present at a concentration of about 40 mg/ml.

[0025] In one embodiment, the stable liquid ophthalmic formulation comprises 40 mg/ml of VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 0.03% polysorbate, and 135 mM sodium chloride at pH 6.2-6.3. In a specific embodiment, the stable liquid ophthalmic formulation consists essentially of 40 mg/ml of VEGF antagonist (SEQ ID NO: 4), 10 mM sodium phosphate buffer, 0.03% polysorbate, and 135 mM sodium chloride at pH 6,2-6.3,

[0026] In another aspect, a lyophilizable formulation of a VEGF antagonist is provided, wherein upon lyophilization followed by reconstitution, a stable liquid ophthalmic formulation as described herein is obtained.

[0027] In another aspect, a lyophilizable formulation of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist is provided, comprising 5-50 mg/ml of the VEGF antagonist, 5-25 mM buffer, such as phosphate buffer, 0.01 to 0.15% of one or more of an organic co-solvent, such as polysorbate, propylene glycol and/or PEG, and optionally 1-10% of a stabilizing agent such as sucrose, sorbitol, trehalose, glycerol, or mannitol, pH about 5.8-7.0, In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at about 5, about 10, about 20, about 30, or about 40 mg/ml. In a specific embodiment, the lyophilizable ophthalmic formulation of the invention comprises 20 mg/ml of the VEGF antagonist, 10 mM sodium phosphate buffer, 0.03% polysorbate, 0.1 % PEG, and 2.5% sucrose, pH about 6.2-6.3. In further embodiments, the lyophilizable formulation further comprises sodium chloride. In a specific embodiment, the sodium chloride is present at a concentration of about 20 mM. In another specific embodiment, the sodium chloride is present at a concentration of about 67.5 mM.

[0028] In another specific embodiment, the lyophilizable ophthalmic formulation of the invention comprises 20 mg/ml of the VEGF antagonist, 5 mM sodium phosphate buffer, 0.01 % polysorbate, 20 mM sodium chloride, and 2.5% sucrose, pH about 6.2-6.3.

[0029] In another embodiment, the lyophilizable ophthalmic formulation comprises 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, at pH 6.2-6.3. In a specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, at pH 6.2-6.3.

[0030] In another specific embodiment, the lyophilizable ophthalmic formulation comprises 20 mg/ml of the VEGF

antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3. In a more specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 20 mg/ml of the VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0,015% polysorbate, and 67.5 mM sodium chloride, pH 6.2-6.3.

[0031] In another specific embodiment, the lyophilizable ophthalmic formulations comprises 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3. In a more specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3.

[0032] Generally, the reconstituted formulation is about 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 40 mg fusion protein/ml.

[0033] Generally, the lyophilized formulation is reconstituted with sterile water suitable for injection. In one embodiment, the reconstitution liquid is bacteriostatic water.

[0034] In another aspect, the invention features a method of producing a lyophilized formulation of a VEGF-specific fusion protein antagonist, comprising subjecting the lyophilizable formulation of the invention to lyophilization to generate a lyophilized formulation. The lyophilized formulation may be lyophilized by any method known in the art for lyophilizing a liquid,

[0035] In another related aspect, the invention features a method of producing a reconstituted lyophilized formulation of a VEGF antagonist, comprising reconstituting the lyophilized formulation of the invention to a reconstituted formulation. In one embodiment, the reconstituted formulation is twice the concentration of the pre-lyophilized formulation, e.g., the method of the invention 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).

[0036] The invention further features ophthalmic formulations provided in a pre-filled syringe or vial, particularly suitable for intravitreal administration.

[0037] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION OF THE INVENTION

[0038] The present invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting unless indicated, since the scope of the present invention will be limited only by the appended claims.

[0039] Unless stated otherwise, all technical and scientific terms and phrases used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

General Description

[0040] 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 and/or precipitation.

[0041] 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 may be reconstituted with water optionally containing a bacteriostatic preservative (e.g., benzyl alcohol) prior to administration.

Definitions

[0042] The term "carrier" includes a diluent, adjuvant, excipient, or vehicle with which a composition is administered, Carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like.

[0043] The term "excipient" includes a non-therapeutic agent added to a pharmaceutical composition to provide a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried

skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0044] 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 90% of moisture has been removed.

VEGF Antagonists

[0045] 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 is the fusion protein of SEQ ID NO:2 or 4: more preferably, SEQ ID NO:4. In specific embodiments, the VEGF antagonist is 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. Preferably, the VEGF antagonist is a dimer composed of two fusion proteins of SEQ ID NO:4.

[0046] The VEGF antagonist of the methods and formulations of the invention 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 pharmaceutical effective formulation. Unless stated otherwise, the phosphates employed are sodium phosphates and a desired buffering pH is achieved by mixing appropriate amounts of mono- and dibasic sodium phosphate,

Stable Liquid Ophthalmic Formulations

[0047] In one aspect, the invention provides a stable pharmaceutically acceptable formulation comprising a VEGF antagonist, wherein the formulation is a liquid formulation suitable for ophthalmic use. Preferably, the liquid formulation comprises a pharmaceutically effective amount of the VEGF antagonist. The formulation can also comprise one or more pharmaceutically acceptable carriers, buffers, tonicity agents, stabilizers, and/or excipients. An example of a pharmaceutically acceptable liquid formulation comprises a VEGF antagonist in a pharmaceutically effective amount, a buffer, an organic co-solvent such as polysorbate, a tonicity agent such as NaCl, and optionally, a stabilizer such as sucrose or trehalose.

[0048] 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, and purity is determined by, for example, 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 by the VEGF antagonist of the invention.

[0049] Liquid 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, nitrogen or argon. Liquid formulations are preferably stored at about 5°C.

Ophthalmic Lyophilized Formulations

[0050] In one aspect of the invention, an ophthalmically acceptable formulation comprising a VEGF antagonist is provided, wherein the formulations is a lyophilizable formulation. Lyophilizable formulations can be reconstituted into solutions, suspensions, emulsions, or any other suitable form for administration or use. Lyophilizable formulation are typically first prepared as liquid, 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.

[0051] Lyophilized formulations can be stored at a wide range of temperatures. Lyophilized formulations may be stored below 25°C, for example, refrigerated at 2-8°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. Stability of the lyophilized formulation may be determined in a number of ways known to the art, for example, by visual appearance of the cake and/or by moisture content.

[0052] 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.

[0053] 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.

EXAMPLES

[0054] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not Intended to be limiting, since the scope of the present invention will be limited only to the appended claims.

[0055] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Example 1. Stability of 50 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0056] An ophthalmic liquid formulations containing 50 mg/ml VEGF Trap (SEQ ID NO:4), 10 mM phosphate, 50 mM NaCl, 0.1% polysorbate 20, 5% sucrose, and pH 6.25, was stored at 5 °C in 3 ml glass vials and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability was determined by SE-HPLC. The results are shown in Table 1. Turbidity was measured at OD_{405} nm; and percent recovered protein and purity by size exclusion HPLC.

Table 1. Stability of 50 mg/ml VEGF Trap Protein (VGFT-SS065)

Months	Visual Appearance	Turbidity (OD_{405} nm)	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	98.8
3	Pass	0.00	6.2	101	98.7
6	Pass	0.01	6.3	100	98.3
9	Pass	0.01	6.3	101	98,3
12	Pass	0.01	6.3	104	98.4
18	Pass	0.01	6.3	96	98.1
24	Pass	0.01	6.3	105	98.1

Example 2. Stability of 50 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0057] A liquid formulation containing 50 mg/ml VEGF Trap (SEQ ID NO:4), 10 mM phosphate, 50 mM NaCl, 3% polyethylene glycol 3350, 5% sucrose, and pH 6.25, was stored at 5°C in 3 nil glass vials and samples tested at 3, 6, 9, 12, 18 and 24 months. Stability results are shown in Table 2. Turbidity, percent recovered protein and purity was determined as described above.

Table 2. Stability of 50 mg/ml VEGF Trap Protein (VGFT-SS065)

Months	Visual Appearance	Turbidity	PH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.2	100	98.9
3	Pass	0.00	6.1	104	98.5
6	Pass	0.01	6.3	99	98.3
9	Pass	0.00	6.3	102	97.6

(continued)

Months	Visual Appearance	Turbidity	PH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
12	Pass	0.01	6.3	103	98.0
18	Pass	0.00	6.3	113	97.7
24	Pass	0.00	6.2	106	97.6

Example 3. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0058] A liquid formulation containing 40 mg/ml VEGF Trap (SEQ ID NO:4), 10 mM phosphate, 40 mM NaCl, 0.03% polysorbate, 20, 5% sucrose, and pH 6.3, was stored at 5 °C in 3 ml glass vials and samples tested at 0.5, 1, 2, 3, and 4 months. stability results are shown in Table 3. Turbidity, percent recovered protein and purity was determined as described above.

Table 3. Stability of 40 mg/ml VEGF Trap Protein (VGFT.SS207)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.5
0.5	Pass	0.00	6.3	99	99.4
1	Pass	0.00	6.2	98	99.5
2	Pass	0.00	6.2	95	99.2
3	Pass	0.01	6.4		
4	Pass	0.01	6.3		

Example 4. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in Pre-filled Glass Syringe.

[0059] A liquid formulation containing 40 mg/ml VEGF trap (SEQ ID NO:4), 10 mM phosphate, 40 mM NaCl, 0.03% polysorbate 20, 5% sucrose, and pH 6.3, was stored at 5 °C in 1 ml prefilled luer glass syringe with 4023/50 FluroTec coated plunger and samples tested at 0.5, 1, 2, 3, and 4 months. Stability results are shown in Table 4. Turbidity, percent recovered protein and purity was determined as described above.

Table 4. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS207)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.4
0.5	Pass	0.00	6.3	100	99.3
1	Pass	0.00	6.3	100	99.4
2	Pass	0.00	6.3	97	99.1
3	Pass	0.01	6.4		
4	Pass	0.01	6.3		

Example 5. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 3 ml Glass Vials.

[0060] A liquid formulation containing 40 mg/ml VEGF trap (SEQ ID NO:4), 10 mM phosphate, 135 mM NaCl, 0.03% polysorbate, 20, and pH 6.3, was stored at 5°C in 3 ml glass vials and samples tested at 0.5, 1, 2, 3, and 4 months. Stability results are shown in Table 5. Turbidity, percent recovered protein and purity was determined as described above.

Table 5. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS203)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.3
0.5	Pass	0.00	6.2	87	99.2
1	Pass	0.00	6.2	88	99.1
2	Pass	0.00	6.3	103	99.2
3	Pass	0.00	6.3	88	99.0
4	Pass	0.00	6.2	85	98.9
5	Pass	0.00	6.3	84	99.0

Example 6. Stability of 40 mg/ml VEGF Trap Liquid Formulation Stored at 5°C in 1 ml Pre-filled Glass Syringe.

[0061] A liquid formulation containing 40 mg/ml VEGF trap (SEQ ID NO:4), 10 mM phosphate, 135 mM NaCl, 0.03% polysorbate 20, and pH 6.3, was stored at 5 °C in 1 ml prefilled glass luer syringe with 4023/50 FluroTec coated plunger and samples tested at 0.5, 1, 2, 3, 4, and 5 months. Stability results are shown in Table 6. Turbidity, percent recovered protein and purity was determined as described above.

Table 6. Stability of 40 mg/ml VEGF Trap Protein (VGFT-SS203)

Months	Visual Appearance	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Configuration
0	Pass	0.00	6.3	100	99.2
0.5	Pass	0.01	6.3	101	99.2
1	Pass	0.00	6.3	101	99.2
2	Pass	0.00	6.3	-	-
3	Pass	0.01	6.3	102	99.1
4	Pass	0.01	6.3	103	98.8
5	Pass	0.00	6.3	99	98.9

Example 7. Stability of Lyophilized 20 mg/ml VEGF Trap Formulation Stored at 5° C in 3 ml Glass Vials and Reconstituted to 40 mg/ml.

[0062] 0.8 ml of a liquid formulation containing 20 mg/ml VEGF trap (SEQ ID NO:4), 5 mM phosphate. 20 mM NaCl, 0.015% polysorbate 20, 2.5% sucrose, and pH 6.3, were lyophilized in 3 ml glass vials. Samples were stored at 5°C and tested at 1, and 2 months. VEGF trap was reconstituted to a final concentration of 40 mg/ml VEGF Trap (final volume of 0.4 ml). Stability results are shown in Table 7 (t = time in months; * = visual appearance; ** = reconstitution time). Turbidity, percent recovered protein and purity was determined as described above.

Table 7. Stability of Lyophilized 20 mg/ml VEGF Trap Protein (VGFT-SS216).

t	Vis. App.*	Recon. Time** (min)	Vis. App.* Reconst'd Liquid	Turbidity	pH	% VEGF Trap Recovered	%VEGFTrap Native Config.
0	Pass	0.6	Pass	0.00	6.3	100	99.5
1	Pass	0.6	Pass	0.01	6.3	106	99.4
2	Pass	0.4	Pass	0.01	6.2	103	99.3

Example 8. Stability of Lyophilized 20 mg/ml VEGF Trap Formulation Stored at 5°C In 3 ml Glass Vials.

[0063] 0.8 ml of a liquid formulation containing 20 mg/ml VEGF trap (SEQ ID NO:4), 5 mM phosphate, 67.5 mM NaCl, 0.015% polysorbate 20, and pH 6.3, were lyophilized in 3 ml glass vials. Samples were stored at 5°C and tested at 1, 2, and 3 months. VEGF trap was reconstituted to a final concentration of 40 mg/ml VEGF trap (final volume of 0.4 ml). Stability result are shown in Table 8 (t = time in months; * = visual appearance; ** = reconstitution time).

Table 8. Stability of Lyophilized 20 mg/ml VEGF Trap Protein (VGFT-SS216)

t	Vis. App.*	Recon. Time** (min)	Vis. App. Reconst'd Liquid	Turbidity	pH	% VEGF Trap Recovered	% VEGF Trap Native Config.
0	Pass	0.7	Pass	0.00	6.3	100	99.0
1	Pass	0.7	Pass	0.01	6.2	105	98.9
2	Pass	0.4	Pass	0.01	6.2	103	98.9

ASPECTS OF THE DISCLOSURE

[0064] The following are aspects of the disclosure.

(1) an ophthalmic formulation of a vascular endothelial growth factor (VEGF) antagonist, comprising

(a) 1-100 mg/ml a VEGF antagonist comprising the amino acid sequence of SEQ ID NO: 4;
(b) 0.01-5% of one or more organic co-solvent(s) which is one or more of polysorbate, polyethylene glycol (PEG), and propylene glycol;
(c) 30-150 mM of a tonicity agent selected from sodium chloride or potassium chloride; and,
(d) 5-40 mM of sodium phosphate buffer; and optionally further comprising 1.0-7.5% of a stabilizing agent is selected from the group consisting of sucrose, sorbitol, glycerol, trehalose, or mannitol, pH between about 5.8-7.0.

(2) An ophthalmic formulation according to (1), comprising about 1-100 mg/ml, preferably 10-80 mg/ml, of the VEGF antagonist, 10 mM sodium phosphate buffer, 40 mM NaCl, 0.03% polysorbate, and 5% sucrose, pH about 6.2-6.3.

(3) An ophthalmic formulation according to (2), comprising VEGF antagonist at a concentration selected from the group consisting of 10 mg/ml, 20 mg/ml, 40 mg/ml, and 80 mg/ml.

(4) An ophthalmic formulation according to any one of (1) to (3), comprising 10-80 mg/ml VEGF antagonist, 10 mM sodium phosphate, 0.03% polysorbate, and 135 mM sodium chloride, pH about 6.2-6.3.

(5) A lyophilizable formulation of a vascular endothelial growth factor (VEGF) antagonist, comprising

(a) 5-50 mg/ml of the VEGF antagonist, preferably 5 mg/ml, 10 mg/ml, 20 mg/ml or 40 mg/ml comprising the amino acid sequence of SEQ ID NO: 4;
(b) 5-25 mM of sodium phosphate buffer, pH about 5.8-7.0;
(c) 0.01-0.15% of an organic co-solvent, selected from the group consisting of polysorbate, polyethylene glycol (PEG), propylene glycol, and a combination thereof; and, optionally
(d) 1-10% of a stabilizing agent selected from the group consisting of sucrose, sorbitol, glycerol, trehalose, and mannitol; or 20-150 mM of a tonicity agent, preferably sodium chloride; or 1 -10% of the stabilizing agent and 20-150 mM of the tonicity agent.

(6) A lyophilizable formulation according to (5), comprising about 20 mg/ml of the VEGF antagonist, about 10 mM sodium phosphate buffer, about 0.03% polysorbate, about 0.1% PEG, and about 2.5% sucrose, pH about 6.2-6.3.

(7) A lyophilizable formulation according to (5), comprising about 20 mg/ml of the VEGF antagonist, about 5 mM sodium phosphate buffer, about 0.015% polysorbate, about 2.5% sucrose, and further comprising sodium chloride at about 20 mM, pH about 6.2-6.3.

(8) A lyophilizable formulation according to (6), comprising about 20 mg/ml of the VEGF antagonist, about 5 mM sodium phosphate buffer, about 0.015% polysorbate, and further comprising sodium chloride at about 67.5 mM, pH about 6.2-6.3.

(9) A method of producing a lyophilized formulation of a VEGF antagonist, comprising subjecting the pre-lyophilized formulation according to (5) to (8) to lyophilization to generate a lyophilized formulation.

(10) A pre-flled syringe suitable for intravitreal administration comprising the formulation of (1).

SEQUENCE LISTING

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<120> VEGF Antagonist Formulations Suitable For Intravitreal Administration

<130> 5050A-WO

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Claims

1. An ophthalmic formulation of a vascular endothelial growth factor (VEGF) antagonist, comprising:

   (a) 1-100 mg/ml of a VEGF antagonist, comprising (i) amino acids 27-457 of SEQ ID No: 4 or (ii) the amino acid sequence of SEQ ID No: 4;
   (b) 0.01-5% of one or more organic co-solvent(s) which is one or more of polysorbate, polyethylene glycol (PEG), and propylene glycol;
   (c) 30-150 mM of a tonicity agent selected from sodium chloride or potassium chloride; and
   (d) 5-40 mM of sodium phosphate buffer, pH between about 5.8-7.0.

2. An ophthalmic formulation according to claim 1, further comprising 1.0-7.5% of a stabilizing agent selected from the group consisting of sucrose, sorbitol, glycerol, trehalose, and mannitol.

3. An ophthalmic formulation according to claim 1 or 2, comprising about 1-100 mg/ml, preferably 10-80 mg/ml, of the

VEGF antagonist, 10 mM sodium phosphate buffer, 40 mM NaCl, 0.03% polysorbate, and 5% sucrose, pH about 6.2-6.3.

4. An ophthalmic formulation according to claim 3, comprising VEGF antagonist at a concentration selected from the group consisting of 10 mg/ml, 20 mg/ml, 40 mg/ml, and 80 mg/ml.

5. An ophthalmic formulation according to any one of the above claims, comprising 10-80 mg/ml VEGF antagonist, 10 mM sodium phosphate, 0.03% polysorbate, and 135 mM sodium chloride, pH about 6.2-6.3.

6. An ophthalmic formulation according to claim 1 comprising:

(a) 10 mg/ml or 40 mg/ml of a VEGF antagonist comprising amino acids 27-457 of SEQ ID No: 4;
(b) 0.03% of polysorbate 20;
(c) about 40 mM of sodium chloride;
(d) 10 mM of sodium phosphate buffer, pH 6.2-6.3; and
(e) 5% sucrose.

7. An ophthalmic formulation according to any one of the preceding claims, wherein the formulation is suitable for intravitreal administration.

8. A lyophilizable formulation of a vascular endothelial growth factor (VEGF) antagonist, comprising

(a) 5-50 mg/ml of the VEGF antagonist, preferably 5 mg/ml, 10 mg/ml, 20 mg/ml or 40 mg/ml, comprising (i) amino acids 27-457 of SEQ ID No: 4 or (ii) the amino acid sequence of SEQ ID No: 4;
(b) 5-25 mM of sodium phosphate buffer, pH about 5.8-7.0; and
(c) 0.01-0.15% of an organic co-solvent, selected from the group consisting of polysorbate, polyethylene glycol (PEG), propylene glycol, and a combination thereof.

9. A lyophilizable formulation according to claim 8, further comprising:

(d) 1-14% of a stabilizing agent selected from the group consisting of sucrose, sorbitol, glycerol, trehalose, and mannitol; or 20-150 mM of a tonicity agent, preferably sodium chloride; or 1-10% of the stabilizing agent and 20-150 mM of the tonicity agent.

10. A lyophilizable formulation according to claim 8 or 9, comprising about 20 mg/ml of the VEGF antagonist, about 10 mM sodium phosphate buffer, about 0.03% polysorbate, about 0.1 % PEG, and about 2.5% sucrose, pH about 6.2-6.3.

11. A lyophilizable formulation according to claim 8, comprising about 20 mg/ml of the VEGF antagonist, about 5 mM sodium phosphate buffer, about 0.015% polysorbate, about 2.5% sucrose, and further comprising sodium chloride at about 20 mM, pH about 6.2-6.3.

12. A lyophilizable formulation according to claim 10, comprising about 20 mg/ml of the VEGF antagonist, about 5 mM sodium phosphate buffer, about 0.015% polysorbate, and further comprising sodium chloride at about 67.5 mM, pH about 6.2-6.3.

13. A lyophilizable formulation according to any one of claims 8 to 12, wherein the formulation is suitable for intravitreal administration.

14. A method of producing a lyophilized formulation of a VEGF antagonist, comprising subjecting the pre-lyophilized formulation according to claim 8 to 12 to lyophilization to generate a lyophilized formulation.

15. A pre-filled syringe suitable for intravitreal administration comprising the formulation of any one of claims 1 to 7.

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 11 15 7965

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
Y	WO 2006/047325 A (GENENTECH INC [US]; SHAMS NAVEED [US]) 4 May 2006 (2006-05-04) * example 1 * * claims 1-21 * * paragraph [0018] - paragraph [0077] * -----	1-10	INV. A61K9/00 A61K9/19 A61K38/00
Y	WO 2005/000895 A (REGENERON PHARMA [US]; DALY THOMAS J [US]; FANDL JAMES P [US]; PAPADOP) 6 January 2005 (2005-01-06) * sequence 10 * * example 3 * * claims 1-14 * * paragraph [0031] - paragraph [0075] * -----	1-10	
X,P	WO 2006/104852 A (REGENERON PHARMA [US]; DIX DANIEL [US]; FRYE KELLY [US]; KAUTZ SUSAN [] 5 October 2006 (2006-10-05) * sequence 4 * * example 1 * * claims 1-9 * * paragraph [0018] - paragraph [0077] * -----	1-10	TECHNICAL FIELDS SEARCHED (IPC)
X,P	US 2006/217311 A1 (DIX DANIEL [US] ET AL) 28 September 2006 (2006-09-28) * sequence 4 * * examples 1-4 * * claims 1-19 * * paragraph [0019] - paragraph [0031] * -----	1-10	A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	4 August 2011	Schifferer, Hermann

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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