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	Docket Number	PAT055157-US-NP

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

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UTILITY PATENT APPLICATION TRANSMITTAL AND FEE SHEET

Transmitted herewith for filing under 37 CFR §1.53(b) is the utility patent application of

Applicant (or identifier): Sigg, Juergen et al.

Title: SYRINGE

Enclosed are:

1. ☒ Specification (Including Claims and Abstract) - 27 pages
2. ☒ Drawings - 1 sheets
3. ☐ Executed Declaration and Power of Attorney (original or copy)
4. ☐ Microfiche Computer Program (appendix)
5. Nucleotide and/or Amino Acid Sequence Submission
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13. ☒ Other: a) Submission of Sequence Listing Including Statement of Verification (1 Sheet)
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Respectfully submitted,

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Date: January 25, 2013

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SYRINGE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised. Of course, the syringe must also remain easy to use, in that the force required to depress the plunger to administer the medicament must not be too high.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber

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from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant. In one embodiment, despite the low silicone oil level, the stopper break loose and slide force is less than 20N.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) or plunger with the mark, thus expelling excess ophthalmic solution and any air bubbles from the

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syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes

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where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

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In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution. Although unapproved for ophthalmic indications, bevacizumab is used off-label in such ophthalmic indications at a concentration of 25mg/ml; typically at a dosage volume of 0.05ml (1.25mg). In one embodiment, the extractable volume from the syringe (that is the amount of product obtainable from the syringe following filling, taking into account loss due to dead space in the syringe and needle) is about 0.09ml.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

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As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. With multiple injections, the amount of silicone droplets can build up in the eye, causing potential adverse effects, including "floaters" and an increase in intra-ocular pressure. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel, though a survey of manufacturers reported that 500-1000µg was typically used in pre-filled syringes (Badkar *et al.* 2011, AAPS PharmaSciTech, 12(2):564-572). Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. If the syringe comprises a low level of silicone oil, this may be more than about 1µg, more than about 3µg, more than about 5µg, more than about 7µg or more than about 10µg silicone oil in the barrel. Thus, in one embodiment, the syringe may comprise about 1µg-about 500µg, about 3µg-about 200µg, about 5µg-about 100µg or about 10µg-about 50µg silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was surprisingly found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase (see e.g. Schoenknecht, AAPS National Biotechnology Conference 2007 – Abstract no. NBC07-000488, which indicates that while 400µg silicone oil is

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acceptable, usability improves when increased to 800μg). Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Smooth administration is particularly important in sensitive tissues such as the eye, where movement of the syringe during administration could cause local tissue damage. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100μg-about 800μg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the forces are measured with a 30G x 0.5 inch needle attached to the syringe. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100μg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line. A further way to reduce silicone oil and inorganic silica levels in a pre-filled syringe is to avoid the use of silicone tubing in filling lines, for example between storage tanks and pumps.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles ≥25μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution

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comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789 (United States Pharmacopoeia: Particulate Matter in Ophthalmic Solutions). In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

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SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG
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and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

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Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDT
LIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVL
TIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPQPGEKTHTCPLCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
ATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLKYGADVNAKDFQGW
TPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNEDLAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

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The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-

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antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

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Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide (EtO) or a hydrogen peroxide (H_2O_2) sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months, 24 months or longer. Thus, in one embodiment, a syringe according to the invention (whilst in its blister pack) may have a shelf life of up to 6 months, 9 months, 12 months, 15 months, 18 months, 24 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes ≤10% (preferably ≤5%, ≤3%, ≤1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen

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peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x\pm10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

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MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the

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backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper movement forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm. Stopper design 2 had a 45% increase in the distance between the front circumferential rib and rear circumferential rib.

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		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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Listing of Claims:

CLAIMS

1. A pre-filled syringe, the syringe comprising a glass body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist wherein:

(a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

(b) the syringe is filled a dosage volume of between about 0.03ml and about 0.05ml of said VEGF antagonist solution,

(c) the syringe barrel comprises less than about 500µg silicone oil, and

(d) the VEGF antagonist solution comprises no more than 2 particles ≥50µm in diameter per ml.

2. A pre-filled syringe according to claim 1, wherein the syringe is filled with between about 0.15ml and about 0.175ml of a VEGF antagonist solution.

3. A pre-filled syringe according to claim 1, wherein the syringe is filled with about 0.165ml of said VEGF antagonist solution.

4. A pre-filled syringe according to claim 1, wherein the syringe is filled with dosage volume of about 0.05ml of a VEGF antagonist solution.

5. A pre-filled syringe according to claim 1, in which the dosage volume is determined by the volume of the variable volume chamber when a predetermined part of the stopper is aligned with a priming mark on the syringe.

6. A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less, preferably 400nm or less, preferably 350nm or less, preferably 300nm or less, preferably 200nm or less, preferably 100nm or less, preferably 50nm or less, preferably 20nm or less.

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7. A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of less than about 500μg silicone oil, preferably less than about 100μg silicone oil, preferably less than about 50μg silicone oil, preferably less than about 25μg silicone oil, preferably less than about 10μg silicone oil.

8. A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of more than about 1μg, more than about 3μg, more than about 5μg, more than about 7μg or more than about 10μg silicone oil.

9. A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of about 1μg-about 500μg, about 3μg-about 200μg, about 5μg-about 100μg or about 10μg-about 50μg silicone oil.

10. A pre-filled syringe according to claim 1, wherein the silicone oil is DC365 emulsion.

11. A pre-filled syringe according to claims 1, wherein the syringe is silicone oil free.

12. A pre-filled syringe according to claim 1, wherein the VEGF antagonist solution further comprises one or more of (i) no more than 5 particles $\geq$25μm in diameter per ml, and (ii) no more than 50 particles $\geq$10μm in diameter per ml.

13. A pre-filled syringe according to claim 1, wherein the VEGF antagonist solution meets USP789.

14. A pre-filled syringe according to claim 1, wherein the VEGF antagonist is an anti-VEGF antibody.

15. A pre-filled syringe according to claim 14, wherein the anti-VEGF antibody is ranibizumab.

16. A pre-filled syringe according to claim 15, wherein the ranibizumab is at a concentration of 10mg/ml.

17. A pre-filled syringe according to claim 1 wherein the VEGF antagonist is a non-antibody VEGF antagonist.

18. A pre-filled syringe according to claim 17, wherein the non-antibody VEGF antagonist is aflibercept or conbercept.

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19. A pre-filled syringe according to claim 18, wherein the non-antibody VEGF antagonist is aflibercept at a concentration of 40mg/ml.

20. A pre-filled syringe according to claim 1, wherein the syringe has a stopper break loose force of less than about 11N.

21. A pre-filled syringe according to claim 20, wherein the syringe has a stopper break loose force of less than about 5N.

22. A pre-filled syringe according to claim 1, wherein the syringe has a stopper slide force of less than about 11N.

23. A pre-filled syringe according to claim 22, wherein the syringe has a stopper slide force of less than about 5N.

24. A pre-filled syringe according to claim 20, wherein the stopper break loose force or stopper slide force is measured using a filled syringe, at a stopper travelling speed of 190mm/min, with a 30G x 0.5 inch needle attached to the syringe.

25. A blister pack comprising a pre-filled syringe according to claim 1, wherein the syringe has been sterilised using H_2O_2 or EtO.

26. A blister pack comprising a pre-filled syringe according to claim 25, wherein the outer surface of the syringe has $\leq$1ppm EtO or H_2O_2 residue.

27. A blister pack comprising a pre-filled syringe according to claim 25, wherein the syringe has been sterilised using EtO or H_2O_2 and the total EtO or H_2O_2 residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

28. A blister pack comprising a pre-filled syringe according to claim 25, wherein $\leq$5% of the VEGF antagonist is alkylated.

29. A blister pack comprising a pre-filled syringe according to claim 25, wherein the syringe has been sterilised using EtO or H_2O_2 with a Sterility Assurance Level of at least 10^{-6}.

30. A method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME),

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diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe according to claim 1.

31. The method of claim 30, further comprising an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

32. A method according to claim 30, wherein the VEGF antagonist administered is a non-antibody VEGF antagonist and wherein the patient has previously received treatment with an antibody VEGF antagonist.

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ABSTRACT

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

Regeneron Exhibit 1002.0024

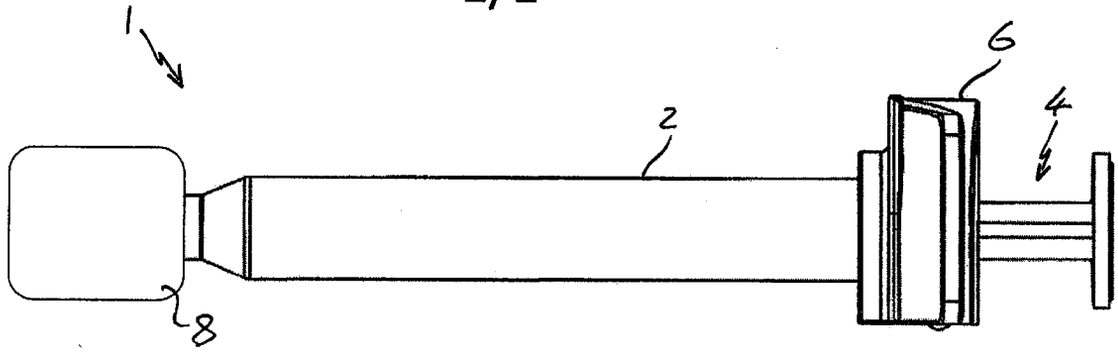
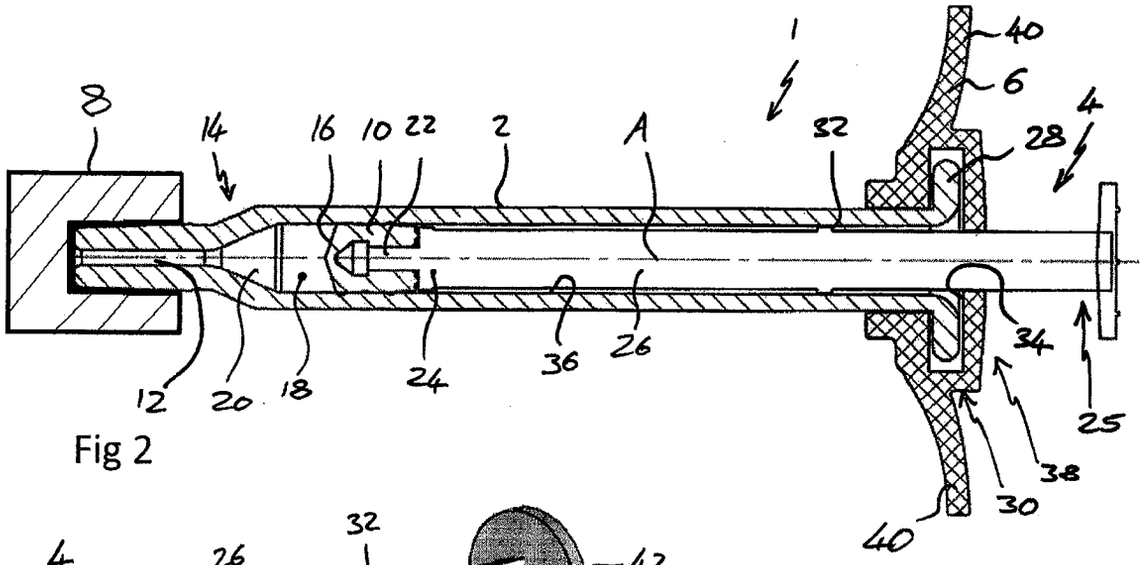


Fig 1



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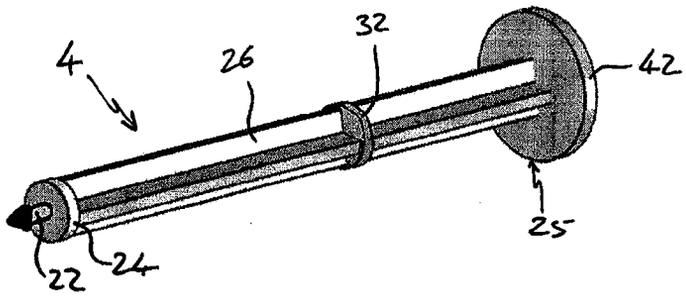

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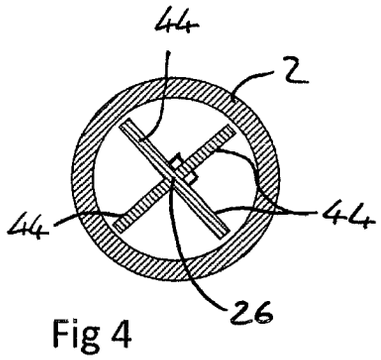

Fig 4

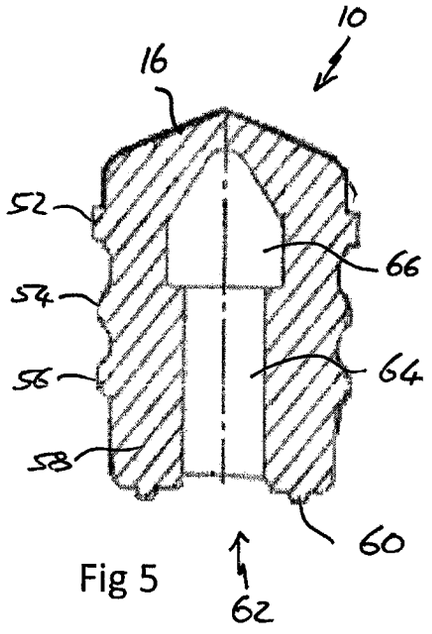

Fig 5

Regeneron Exhibit 1002.0025

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Regeneron Exhibit 1002.0026

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Regeneron Exhibit 1002.0027

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Regeneron Exhibit 1002.0028

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Regeneron Exhibit 1002.0029

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Regeneron Exhibit 1002.0031

Case PAT055157-US-NP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Sigg, Juergen et al.
APPLICATION NO: Not Yet Known
FILED: Herewith
FOR: SYRINGE

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ within three months of the filing date of the application. Therefore, no fees are required.

☒ before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required.

If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

/ Andrew Holmes /

Andrew Holmes
Agent for Applicant
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Date: January 25, 2013

PTO/AIA/01 (06-12)
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	SYRINGE

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☑ United States application or PCT international application number PCT/EP2013/051491 filed on 25 January 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

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LEGAL NAME OF INVENTOR

Inventor: Juergen Sigg Date (Optional): _____

Signature: _____

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	SYRINGE

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☑ United States application or PCT international application number PCT/EP2013/051491 filed on 25 January 2013

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Christophe Royer Date (Optional): _____

Signature: _____

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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LEGAL NAME OF INVENTOR

Inventor: Andrew Mark Bryant Date (Optional): _____

Signature: _____

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LEGAL NAME OF INVENTOR

Inventor: Heinrich Martin Buettgen Date (Optional): _____

Signature: _____

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

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LEGAL NAME OF INVENTOR

Inventor: Marie Picci Date (Optional): _____

Signature: _____

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Application Number	Not Yet Known
Filing Date	Herewith
First Named Inventor	Juergen Sigg
Title	SYRINGE
Art Unit	
Examiner Name	
Attorney Docket Number	55157-US-NP

SIGNATURE of Applicant or Patent Practitioner

Signature	/ Andrew Holmes /	Date	January 25, 2013
Name	Andrew Holmes	Telephone	862-778-5816
Registration Number	51,813		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications.

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I hereby revoke all previous powers of attorney given in the application identified in the attached transmittal letter.

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

01095

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☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

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I am the Applicant:

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☐ Legal Representative of a Deceased or Legally Incapacitated Inventor

☒ Assignee or Person to Whom the Inventor is Under an Obligation to Assign

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (*e.g.*, a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent

Signature	/Andrew Holmes/	Date	January 25, 2013
Name	Andrew Holmes	Telephone	862-778-5816
Title and Company	Authorized Signatory, Novartis AG		

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 June 2010 (03.06.2010)

PCT

(10) International Publication Number
WO 2010/060748 A1

(51) International Patent Classification:
C07K 14/47 (2006.01)

(21) International Application Number:
PCT/EP2009/064483

(22) International Filing Date:
3 November 2009 (03.11.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
08168166.0 3 November 2008 (03.11.2008) EP

(71) Applicant *(for all designated States except US)*: **MOLECULAR PARTNERS AG** [CH/CH]; Wagistrasse 14, CH-8952 Schlieren (CH).

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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: BINDING PROTEINS INHIBITING THE VEGF-A RECEPTOR INTERACTION

(57) **Abstract**: The present invention relates to binding proteins specific for VEGF-A, in particular to recombinant binding proteins comprising a binding domain, which inhibits VEGF-Axxx binding to VEGFR-2. Examples of such binding proteins are proteins which comprise an ankyrin repeat domain with the desired binding specificity. The binding proteins are useful in the treatment of cancer and other pathological conditions, e.g. eye diseases such as age-related macular degeneration.

WO 2010/060748 A1

Regeneron Exhibit 1002.0040

Binding Proteins inhibiting the VEGF-A receptor interaction

Field of the invention

The present invention relates to recombinant binding proteins specific for VEGF-A, as well as nucleic acids encoding such VEGF-A binding proteins, pharmaceutical compositions comprising such proteins, and the use of such proteins in the treatment of tumors and eye diseases.

Background of the invention

Angiogenesis, the growth of new blood vessels from pre-existing vasculature, is a key process in several pathological conditions, including tumor growth and eye diseases, in particular ocular neovascularization diseases such as age-related macular degeneration (AMD) or diabetic macular edema (DME) (Carmeliet, P., Nature *438*, 932–936, 2005). Vascular endothelial growth factors (VEGFs) stimulate angiogenesis and lymphangiogenesis by activating VEGF receptor (VEGFR) tyrosine kinases in endothelial cells (Ferrara, N., Gerber, H. P. and LeCouter, J., Nature Med. *9*, 669–676, 2003).

The mammalian VEGF family consists of five glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D (also known as FIGF) and placenta growth factor (PlGF, also known as PGF). VEGF-A has been shown to be an effective target for anti-angiogenic therapy (Ellis, L. M. and Hicklin, D. J., Nature Rev. Cancer *8*, 579-591, 2008). The VEGF-A ligands bind to and activate three structurally similar type III receptor tyrosine kinases, designated VEGFR-1 (also known as FLT1), VEGFR-2 (also known as KDR) and VEGFR-3 (also known as FLT4). The VEGF ligands have distinctive binding specificities for each of these tyrosine kinase receptors, which contribute to their diversity of function. In response to ligand binding, the VEGFR tyrosine kinases activate a network of distinct downstream signaling pathways. VEGFR-1 and VEGFR-2 are primarily found on the vascular endothelium whereas VEGFR-3 is mostly found on the lymphatic endothelium. These receptors all have an extracellular domain, a single transmembrane region and a consensus tyrosine kinase sequence interrupted by a kinase-insert domain. More recently neuropilin (NRP-1), originally identified as a receptor for the semaphorin / collapsin family of neuronal guidance mediators, was shown to act as an isoform specific receptor for VEGF-A.

Regeneron Exhibit 1002.0041

Various isoforms of VEGF-A are known that are generated by alternative splicing from eight exons within the *VEGF-A* gene. All isoforms contain exons 1–5 and the terminal exon, exon 8. Exons 6 and 7, which encode heparin-binding domains, can be included or excluded. This gives rise to a family of proteins termed according to their amino acid number: VEGF-A165, VEGF-A121, VEGF-A189, and so on. Exon 8, however, contains two 3' splice sites in the nucleotide sequences, which can be used by the cell to generate two families of isoforms with identical length, but differing C-terminal amino acid sequences (Varey, A.H.R. et al., British J. Cancer *98*, 1366-1379, 2008). VEGF-Axxx ("xxx" denotes the amino acid number of the mature protein), the pro-angiogenic family of isoforms, is generated by use of the most proximal sequence in exon 8 (resulting in the inclusion of exon 8a). The more recently described anti-angiogenic VEGF-Axxxb isoforms are generated by the use of a distal splice site, 66 bp further along the gene from the proximal splice site. This results in splicing out of exon 8a and the production of mRNA sequences that encode the VEGF-Axxxb family. VEGF-A165 is the predominant pro-angiogenic isoform and is commonly overexpressed in a variety of human solid tumors. VEGF-A165b was the first of the exon 8b-encoded isoforms identified and was shown to have anti-angiogenic effects (Varey et al., loc. cit.; Konopatskaya, O. et al., Molecular Vision *12*, 626-632, 2006). It is an endogenous inhibitory form of VEGF-A, which decreases VEGF-A induced proliferation and migration of endothelial cells. Although it can bind to VEGFR-2, VEGF-A165b binding does not result in receptor phosphorylation or activation of the downstream signaling pathways.

There are several approaches to inhibiting VEGF-A signaling, including neutralization of the ligand or receptor by antibodies, and blocking VEGF-A receptor activation and signaling with tyrosine kinase inhibitors. VEGF-A targeted therapy has been shown to be efficacious as a single agent in AMD, DME, renal cell carcinoma and hepatocellular carcinoma, whereas it is only of benefit when combined with chemotherapy for patients with metastatic colorectal, non-small-cell lung and metastatic breast cancer (Narayanan, R. et al., Nat Rev. Drug Discov. *5*, 815-816, 2005; Ellis and Hicklin, loc. cit).

Beside antibodies other binding domains can be used to neutralize a ligand or a receptor (Skerra, A., J. Mol. Recog. *13*, 167-187, 2000; Binz, H. K., Amstutz, P. and Plückthun, A., Nat. Biotechnol. *23*, 1257-1268, 2005). One such novel class of binding domains are based on designed repeat domains (WO 02/20565; Binz, H. K., Amstutz, P., Kohl, A., Stumpp, M. T., Briand, C., Forrer, P., Grütter, M. G., and Plückthun, A., Nat. Biotechnol. *22*, 575-582, 2004). WO 02/20565 describes how large libraries of repeat proteins can be

Regeneron Exhibit 1002.0042

constructed and their general application. Nevertheless, WO 02/20565 does neither disclose the selection of repeat domains with binding specificity for VEGF-Axxx nor concrete repeat sequence motifs of repeat domains that specifically bind to VEGF-Axxx.

Targeting VEGF-A with currently available therapeutics is not effective in all patients, or for all diseases (e.g., EGFR-expressing cancers). It has even become increasingly apparent that the therapeutic benefit associated with VEGF-A targeted therapy is complex and probably involves multiple mechanisms (Ellis and Hicklin, loc. cit.). For example, marketed anti-VEGF drugs, such as bevacizumab (Avastin®) or ranibizumab (Lucentis®) (see WO 96/030046, WO 98/045331 and WO 98/045332) or drugs in clinical development, such as VEGF-Trap® (WO 00/075319) do not distinguish between the pro- and anti-angiogenic forms of VEGF-A, so they do inhibit both. As a result, they inhibit angiogenesis, but also deprive healthy tissues of an essential survival factor, namely VEGF-Axxxb, resulting in cytotoxicity and dose-limiting side effects, which in turn limit efficacy. Side effects common to current anti-VEGF-A therapies are gastrointestinal perforations, bleeding, hypertension, thromboembolic events and proteinuria (Kamba, T. and McDonald, D.M., Br. J. Cancer *96*, 1788-95, 2007). Thus, a need exists for improved anti-angiogenic agents for treating cancer and other pathological conditions.

The technical problem underlying the present invention is to identify novel anti-angiogenic agents, such as repeat domains with binding specificity to VEGF-Axxx, for an improved treatment of cancer and other pathological conditions, e.g. eye diseases such as AMD or DME. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Summary of the invention

The present invention relates to a binding protein comprising a binding domain, wherein said binding domain inhibits VEGF-Axxx binding to VEGFR-2 and wherein said binding domain has a midpoint denaturation temperature (Tm) above 40°C upon thermal unfolding and forms less than 5% (w/w) insoluble aggregates at concentrations up to 10 g/L when incubated at 37°C for 1 day in PBS. More specifically the invention relates to a recombinant binding protein comprising at least one repeat domain, wherein said repeat domain binds VEGF-Axxx with a Kd below 10^{-7}M and inhibits VEGF-Axxx binding to VEGFR-2. In particular such a binding protein inhibits sprouting of HUVEC spheroids with an IC_{50} value below 10 nM, and such a binding protein has a dissociation constant K_d for

the interaction with VEGF-Axxxb that is at least 10-fold higher compared to its K_d for the interaction with VEGF-Axxx.

In particular, the invention relates to a recombinant binding protein comprising a binding domain with specificity for VEGF-A, which is a repeat domain, for example an ankyrin repeat domain, in particular an ankyrin repeat domain comprising a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GHLEIVEVLLK7GADVNA (SEQ ID NO:1)
wherein 1, 2, 3, 4, 5, 6, and 7, represent, independently of each other, an amino acid residue selected from the group consisting of A, D, E, F, H, I, K, L, M, N, Q, R, S, T, V, W and Y.

The invention also relates to a recombinant binding protein comprising a repeat domain with binding specificity for VEGF-A, which has at least 70% amino acid sequence identity with an ankyrin repeat domain of the present invention, or which comprises a repeat module with at least 70% amino acid sequence identity with an ankyrin repeat module of the present invention, or wherein one or more of the amino acid residues of the ankyrin repeat modules are exchanged by an amino acid residue found at the corresponding position on alignment of an ankyrin repeat unit.

The invention further relates to binding proteins comprising a recombinant binding protein of the present invention bound to one or more additional moieties, for example, a moiety that also binds to VEGFR-2 or to a different target, a labeling moiety, a moiety that facilitates protein purification, or a moiety that provides improved pharmacokinetics, for example a polyethylene glycol moiety. In certain embodiments, the additional moiety is a proteinaceous moiety. In certain other embodiments, the additional moiety is a non-proteinaceous polymer moiety.

The invention further relates to nucleic acid molecules encoding the recombinant binding proteins of the present invention, and to a pharmaceutical composition comprising one or more of the above mentioned binding proteins or nucleic acid molecules.

The invention further relates to a method of treatment of cancer and other pathological conditions, e.g. eye diseases such as AMD or DME, using the binding proteins of the invention.

Brief Description of the Figures

Figure 1. *Specific dog VEGF-A164 binding of selected designed ankyrin repeat proteins.*
The interaction of selected clones with dog VEGF-A164 (VEGF) and a negative control protein (MBP, *E. coli* maltose binding protein) is shown by crude extract ELISA. The biotinylated dog VEGF-A164 and MBP were immobilized over NeutrAvidin. The numbers refer to single DARPin clones selected in ribosome display against dog VEGF-A164 or the corresponding human VEGF-A165. A = Absorbance. White bars indicate binding to dog VEGF-A164, black bars show non-specific background binding to MBP.

Figure 2. *Spheroid outgrowth inhibition by a selected DARPin.*
The length of sprouts in a spheroid outgrowth inhibition assay are shown in presence of various concentrations of (a) DARPin #30 (SEQ ID NO:29), a DARPin with specificity to VEGF-Axxx, or (b) DARPin NC, a negative control DARPin with no specificity for VEGF-Axxx.

Figure 3. *Specific recognition of VEGF-A isoforms.*
Surface Plasmon Resonance (SPR) analysis of binding proteins on VEGF-A isoforms.
(a) and (b): SPR analysis of Avastin®. 250 nM of Avastin® was applied to a flow cell with immobilized dog VEGF-A164 (a) or dog VEGF-A164b (b) for 100 seconds, followed by washing with buffer flow.
(c) and (d): SPR analysis of DARPin #27 (SEQ ID NO:16). 250 nM of DARPin #27 was applied to a flow cell with immobilized dog VEGF-A164 (c) or dog VEGF-A164b (d) for 100 seconds, followed by washing with buffer flow. RU = Resonance Units.

Figure 4. *Efficient inhibition of human VEGF-A165 in the rabbit eye.*
Vascular leakage rabbit model to show the efficacy of a DARPin in inhibiting human VEGF-A165 in the eye in comparison to Lucentis®. At day 1 either PBS, DARPin #30 or Lucentis® is applied by an intravitreal injection into one eye of each rabbit (treated eye). At day 4 or day 30 both eyes of each rabbit were challenged by intravitreal injection of 500 ng of human VEGF-A165. All eyes were evaluated 48 hours after the VEGF-A165 injection by measuring the fluorescein content in the vitreous and retina of all eyes one hour after intravenous injection of sodium fluorescein.
R = ratio of fluorescein measurements treated eye / untreated eye. Standard deviations are shown by an error bar. 4-PBS = ratio 4 days after injection of PBS (control); 4-D = ratio 4 days after injection of DARPin #30; 30-D = ratio 30 days after injection of DARPin

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#30; 4-L = ratio 4 days after injection of Lucentis®; 30-L = ratio 30 days after injection of Lucentis®.

Detailed description of the invention

Mammalian VEGF-A exists as two families of alternative spliced isoforms: (i) the pro-angiogenic "VEGF-Axxx" isoforms generated by proximal splicing of exon 8 and (ii) the anti-angiogenic "VEGF-Axxxb" isoforms generated by distal splicing of exon 8. Preferably, the binding domain according to the invention is specific for the pro-angiogenic VEGF-Axxx of dog, rabbit, monkey or human origin. More preferably, the binding domain according to the invention is specific for the pro-angiogenic VEGF-Axxx of human origin. Most preferred, the binding domain according to the invention is specific for human VEGF-A165.

The term "protein" refers to a polypeptide, wherein at least part of the polypeptide has, or is able to, acquire a defined three-dimensional arrangement by forming secondary, tertiary, or quaternary structures within and/or between its polypeptide chain(s). If a protein comprises two or more polypeptides, the individual polypeptide chains may be linked non-covalently or covalently, e.g. by a disulfide bond between two polypeptides. A part of a protein, which individually has, or is able to acquire a defined three-dimensional arrangement by forming secondary or tertiary structures, is termed "protein domain". Such protein domains are well known to the practitioner skilled in the art.

The term "recombinant" as used in recombinant protein, recombinant protein domain and the like, means that said polypeptides are produced by the use of recombinant DNA technologies well known by the practitioner skilled in the relevant art. For example, a recombinant DNA molecule (e.g. produced by gene synthesis) encoding a polypeptide can be cloned into a bacterial expression plasmid (e.g. pQE30, Qiagen). When such a constructed recombinant expression plasmid is inserted into a bacteria (e.g. *E. coli*), this bacteria can produce the polypeptide encoded by this recombinant DNA. The correspondingly produced polypeptide is called a recombinant polypeptide.

The term "polypeptide tag" refers to an amino acid sequence attached to a polypeptide/protein, wherein said amino acid sequence is useful for the purification, detection, or targeting of said polypeptide/protein, or wherein said amino acid sequence improves the physicochemical behavior of the polypeptide/protein, or wherein said amino

acid sequence possesses an effector function. The individual polypeptide tags, moieties and/or domains of a binding protein may be connected to each other directly or via polypeptide linkers. These polypeptide tags are all well known in the art and are fully available to the person skilled in the art. Examples of polypeptide tags are small polypeptide sequences, for example, His, myc, FLAG, or Strep-tags or moieties such as enzymes (for example enzymes like alkaline phosphatase), which allow the detection of said polypeptide/protein, or moieties which can be used for targeting (such as immunoglobulins or fragments thereof) and/or as effector molecules.

The term "polypeptide linker" refers to an amino acid sequence, which is able to link, for example, two protein domains, a polypeptide tag and a protein domain, a protein domain and a non-polypeptide moiety such as polyethylene glycol or two sequence tags. Such additional domains, tags, non-polypeptide moieties and linkers are known to the person skilled in the relevant art. A list of example is provided in the description of the patent application WO 02/20565. Particular examples of such linkers are glycine-serine-linkers of variable lengths; preferably, said linkers have a length between 2 and 16 amino acids.

In the context of the present invention, the term "polypeptide" relates to a molecule consisting of one or more chains of multiple, i.e. two or more, amino acids linked via peptide bonds. Preferably, a polypeptide consists of more than eight amino acids linked via peptide bonds.

The term "binding protein" refers to a protein comprising one or more binding domains as further explained below. Preferably, said binding protein comprises up to four binding domains. More preferably, said binding protein comprises up to two binding domains. Most preferably, said binding protein comprises only one binding domain. Furthermore, any such binding protein may comprise additional protein domains that are not binding domains, multimerization moieties, polypeptide tags, polypeptide linkers and/or non-proteinaceous polymer molecules. Examples of multimerization moieties are immunoglobulin heavy chain constant regions which pair to provide functional immunoglobulin Fc domains, and leucine zippers or polypeptides comprising a free thiol which forms an intermolecular disulfide bond between two such polypeptides. Examples of non-proteinaceous polymer molecules are hydroxyethyl starch (HES), polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylene.

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The term “PEGylated” means that a PEG moiety is covalently attached to, for example, a polypeptide of the invention.

The term “binding domain” means a protein domain exhibiting the same "fold" (three-dimensional arrangement) as a protein scaffold and having a predetermined property, as defined below. Such a binding domain may be obtained by rational, or most commonly, combinatorial protein engineering techniques, skills which are known in the art (Skerra, 2000, loc. cit.; Binz et al., 2005, loc. cit.). For example, a binding domain having a predetermined property can be obtained by a method comprising the steps of (a) providing a diverse collection of protein domains exhibiting the same fold as a protein scaffold as defined further below; and (b) screening said diverse collection and/or selecting from said diverse collection to obtain at least one protein domain having said predetermined property. The diverse collection of protein domains may be provided by several methods in accordance with the screening and/or selection system being used, and may comprise the use of methods well known to the person skilled in the art, such as phage display or ribosome display.

The term "protein scaffold" means a protein with exposed surface areas in which amino acid insertions, substitutions or deletions are highly tolerable. Examples of protein scaffolds that can be used to generate binding domains of the present invention are antibodies or fragments thereof such as single-chain Fv or Fab fragments, protein A from *Staphylococcus aureus*, the bilin binding protein from *Pieris brassicae* or other lipocalins, ankyrin repeat proteins or other repeat proteins, and human fibronectin. Protein scaffolds are known to the person skilled in the art (Binz et al., 2005, loc. cit.; Binz et al., 2004, loc. cit.).

The term "predetermined property" refers to a property such as binding to a target, blocking of a target, activation of a target-mediated reaction, enzymatic activity, and related further properties. Depending on the type of desired property, one of ordinary skill will be able to identify format and necessary steps for performing screening and/or selection of a binding domain with the desired property. Preferably, said predetermined property is binding to a target.

Preferably, the binding protein of the invention is not an antibody or a fragment thereof, such as Fab or scFv fragments. Antibodies and fragments thereof are well known to the person skilled in the art.

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Also preferably, the binding domain of the invention does not comprise an immunoglobulin fold as present in antibodies and/or the fibronectin type III domain. An immunoglobulin fold is a common all-β protein fold that consists of a 2-layer sandwich of about 7 anti-parallel β-strands arranged in two β-sheets. Immunoglobulin folds are well known to the person skilled in the art. For example, such binding domains comprising an immunoglobulin fold are described in WO 07/080392 or WO 08/097497.

Further preferably, the binding domain of the invention does not comprise an immunoglobulin-like domain as found in VEGFR-1 or VEGFR-2. Such binding domains are described in WO 00/075319.

A preferred binding domain is a binding domain having anti-angiogenic effects. The anti-angiogenic effect of a binding domain can be determined by assays well know to the person skilled in the art, such as the sprouting assay of HUVEC spheroids described in Example 2.

Further preferred is a binding domain comprising between 70 and 300 amino acids, in particular between 100 and 200 amino acids.

Further preferred is a binding domain devoid of a free Cys residue. A free Cys residue is not involved in the formation of a disulfide bond. Even more preferred is a binding domain free of any Cys residue.

A preferred binding domain of the invention is a repeat domain or a designed repeat domain, preferably as described in WO 02/20565.

A particularly preferred binding domain is a designed ankyrin repeat domain (Binz, H. K. et al., 2004, loc. cit.), preferably as described in WO 02/20565. Examples of designed ankyrin repeat domains are shown in the Examples.

The definitions hereinafter for repeat proteins are based on those in patent application WO 02/20565. Patent application WO 02/20565 further contains a general description of repeat protein features, techniques and applications.

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The term "repeat proteins" refers to a protein comprising one or more repeat domains. Preferably, each of said repeat proteins comprises up to four repeat domains. More preferably, each of said repeat proteins comprises up to two repeat domains. Most preferably, each of the repeat proteins comprises only one repeat domain. Furthermore, said repeat protein may comprise additional non-repeat protein domains, polypeptide tags and/or polypeptide linkers.

The term "repeat domain" refers to a protein domain comprising two or more consecutive repeat units (modules) as structural units, wherein said structural units have the same fold, and stack tightly to create, for example, a superhelical structure having a joint hydrophobic core.

The term "designed repeat protein" and "designed repeat domain" refer to a repeat protein or repeat domain, respectively, obtained as the result of the inventive procedure explained in patent application WO 02/20565. Designed repeat proteins and designed repeat domains are synthetic and not from nature. They are man-made proteins or domains, respectively, obtained by expression of correspondingly designed nucleic acids. Preferably, the expression is done in eukaryotic or prokaryotic cells, such as bacterial cells, or by using a cell-free *in vitro* expression system.

The term "structural unit" refers to a locally ordered part of a polypeptide, formed by three-dimensional interactions between two or more segments of secondary structure that are near one another along the polypeptide chain. Such a structural unit exhibits a structural motif. The term "structural motif" refers to a three-dimensional arrangement of secondary structure elements present in at least one structural unit. Structural motifs are well known to the person skilled in the art. Structural units alone are not able to acquire a defined three-dimensional arrangement; however, their consecutive arrangement, for example as repeat modules in a repeat domain, leads to a mutual stabilization of neighboring units resulting in a superhelical structure.

The term "repeat unit" refers to amino acid sequences comprising repeat sequence motifs of one or more naturally occurring repeat proteins, wherein said "repeat units" are found in multiple copies, and which exhibit a defined folding topology common to all said motifs determining the fold of the protein. Such repeat units comprise framework residues and interaction residues. Examples of such repeat units are armadillo repeat units, leucine-rich repeat units, ankyrin repeat units, tetratricopeptide repeat units, HEAT repeat units, and

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leucine-rich variant repeat units. Naturally occurring proteins containing two or more such repeat units are referred to as "naturally occurring repeat proteins". The amino acid sequences of the individual repeat units of a repeat protein may have a significant number of mutations, substitutions, additions and/or deletions when compared to each other, while still substantially retaining the general pattern, or motif, of the repeat units.

The term "framework residues" relates to amino acid residues of the repeat units, or the corresponding amino acid residues of the repeat modules, which contribute to the folding topology, i.e. which contribute to the fold of said repeat unit (or module) or which contribute to the interaction with a neighboring unit (or module). Such contribution might be the interaction with other residues in the repeat unit (module), or the influence on the polypeptide backbone conformation as found in α-helices or β-sheets, or amino acid stretches forming linear polypeptides or loops.

The term "target interaction residues" refers to amino acid residues of the repeat units, or the corresponding amino acid residues of the repeat modules, which contribute to the interaction with target substances. Such contribution might be the direct interaction with the target substances, or the influence on other directly interacting residues, e.g. by stabilizing the conformation of the polypeptide of a repeat unit (module) to allow or enhance the interaction of directly interacting residues with said target. Such framework and target interaction residues may be identified by analysis of the structural data obtained by physicochemical methods, such as X-ray crystallography, NMR and/or CD spectroscopy, or by comparison with known and related structural information well known to practitioners in structural biology and/or bioinformatics.

Preferably, the repeat units used for the deduction of a repeat sequence motif are homologous repeat units, wherein the repeat units comprise the same structural motif and wherein more than 70% of the framework residues of said repeat units are homologous to each other. Preferably, more than 80% of the framework residues of said repeat units are homologous. Most preferably, more than 90% of the framework residues of said repeat units are homologous. Computer programs to determine the percentage of homology between polypeptides, such as Fasta, Blast or Gap, are known to the person skilled in the art. Further preferably, the repeat units used for the deduction of a repeat sequence motif are homologous repeat units obtained from repeat domains selected on a target, for example as described in Example 1 and having the same target-specificity.

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The term "repeat sequence motif" refers to an amino acid sequence, which is deduced from one or more repeat units. Preferably, said repeat units are from repeat domains having binding specificity for the same target. Such repeat sequence motifs comprise framework residue positions and target interaction residue positions. Said framework residue positions correspond to the positions of framework residues of the repeat units. Likewise, said target interaction residue positions correspond to the positions of target interaction residues of the repeat units. Repeat sequence motifs comprise fixed positions and randomized positions. The term "fixed position" refers to an amino acid position in a repeat sequence motif, wherein said position is set to a particular amino acid. Most often, such fixed positions correspond to the positions of framework residues and/or the positions of target interaction residues that are specific for a certain target. The term "randomized position" refers to an amino acid position in a repeat sequence motif, wherein two or more amino acids are allowed at said amino acid position, for example, wherein any of the usual twenty naturally occurring amino acids are allowed, or wherein most of the twenty naturally occurring amino acids are allowed, such as amino acids other than cysteine, or amino acids other than glycine, cysteine and proline. Most often, such randomized positions correspond to the positions of target interaction residues. However, some positions of framework residues may also be randomized.

The term "folding topology" refers to the tertiary structure of said repeat units. The folding topology will be determined by stretches of amino acids forming at least parts of α-helices or β-sheets, or amino acid stretches forming linear polypeptides or loops, or any combination of α-helices, β-sheets and/or linear polypeptides/loops.

The term "consecutive" refers to an arrangement, wherein the repeat units or repeat modules are arranged in tandem. In designed repeat proteins, there are at least 2, usually about 2 to 6, in particular at least about 6, frequently 20 or more repeat units. In most cases, repeat units will exhibit a high degree of sequence identity (same amino acid residues at corresponding positions) or sequence similarity (amino acid residues being different, but having similar physicochemical properties), and some of the amino acid residues might be key residues being strongly conserved in the different repeat units found in naturally occurring proteins. However, a high degree of sequence variability by amino acid insertions and/or deletions, and/or substitutions between the different repeat units found in naturally occurring proteins will be possible as long as the common folding topology is maintained.

Methods for directly determining the folding topology of repeat proteins by physico-chemical means such as X-ray crystallography, NMR or CD spectroscopy, are well known to the practitioner skilled in the art. Methods for identifying and determining repeat units or repeat sequence motifs or for identifying families of related proteins comprising such repeat units or motifs, such as homology searches (BLAST etc.), are well established in the field of bioinformatics, and are well known to the practitioner in the art. The step of refining an initial repeat sequence motif may comprise an iterative process.

The term "repeat modules" refers to the repeated amino acid sequences of the designed repeat domains, which are originally derived from the repeat units of naturally occurring repeat proteins. Each repeat module comprised in a repeat domain is derived from one or more repeat units of the family or subfamily of naturally occurring repeat proteins, e.g. the family of armadillo repeat proteins or ankyrin repeat proteins.

"Repeat modules" may comprise positions with amino acid residues present in all copies of corresponding repeat modules ("fixed positions") and positions with differing or "randomized" amino acid residues ("randomized positions").

The term “capping module” refers to a polypeptide fused to the N- or C-terminal repeat module of a repeat domain, wherein said capping module forms tight tertiary interactions with said repeat module thereby providing a cap that shields the hydrophobic core of said repeat module at the side not in contact with the consecutive repeat module from the solvent. Said N- and/or C-terminal capping module may be, or may be derived from, a capping unit or other domain found in a naturally occurring repeat protein adjacent to a repeat unit. The term “capping unit” refers to a naturally occurring folded polypeptide, wherein said polypeptide defines a particular structural unit which is N- or C-terminally fused to a repeat unit, wherein said polypeptide forms tight tertiary interactions with said repeat unit thereby providing a cap that shields the hydrophobic core of said repeat unit at one side from the solvent. Such capping units may have sequence similarities to said repeat sequence motif. Capping modules and capping repeats are described in WO 02/020565. For example, the N-terminal capping module of SEQ ID NO:21 is encoded by the amino acids from position 1 to 32. Also preferred is such an N-terminal capping module having a glycine or aspartate residue at position 5.

The term "target" refers to an individual molecule such as a nucleic acid molecule, a polypeptide or protein, a carbohydrate, or any other naturally occurring molecule,

including any part of such individual molecule, or complexes of two or more of such molecules. The target may be a whole cell or a tissue sample, or it may be any non-natural molecule or moiety. Preferably, the target is a naturally occurring or non-natural polypeptide or a polypeptide containing chemical modifications, for example modified by natural or non-natural phosphorylation, acetylation, or methylation. In the particular application of the present invention, the target is VEGF-Axxx or VEGFR-2.

The term "consensus sequence" refers to an amino acid sequence, wherein said consensus sequence is obtained by structural and/or sequence aligning of multiple repeat units. Using two or more structural and/or sequence aligned repeat units, and allowing for gaps in the alignment, it is possible to determine the most frequent amino acid residue at each position. The consensus sequence is that sequence which comprises the amino acids which are most frequently represented at each position. In the event that two or more amino acids are represented above-average at a single position, the consensus sequence may include a subset of those amino acids. Said two or more repeat units may be taken from the repeat units comprised in a single repeat protein, or from two or more different repeat proteins.

Consensus sequences and methods to determine them are well known to the person skilled in the art.

A “consensus amino acid residue” is the amino acid found at a certain position in a consensus sequence. If two or more, e.g. three, four or five, amino acid residues are found with a similar probability in said two or more repeat units, the consensus amino acid may be one of the most frequently found amino acids or a combination of said two or more amino acid residues.

Further preferred are non-naturally occurring binding proteins or binding domains.

The term “non-naturally occurring” means synthetic or not from nature, more specifically, the term means made from the hand of man. The term “non-naturally occurring binding protein" or "non-naturally occurring binding domain” means that said binding protein or said binding domain is synthetic (i.e. produced by chemical synthesis from amino acids) or recombinant and not from nature. “Non-naturally occurring binding protein" or "non-naturally occurring binding domain” is a man-made protein or domain, respectively, obtained by expression of correspondingly designed nucleic acids. Preferably, the

expression is done in eukaryotic or bacterial cells, or by using a cell-free *in vitro* expression system. Further, the term means that the sequence of said binding protein or said binding domain is not present as a non-artificial sequence entry in a sequence database, for example in GenBank, EMBL-Bank or Swiss-Prot. These databases and other similar sequence databases are well known to the person skilled in the art.

The invention relates to a binding protein comprising a binding domain, wherein said binding domain inhibits VEGF-Axxx binding to VEGFR-2 and wherein said binding protein and/or binding domain has a midpoint denaturation temperature (Tm) above 40°C upon thermal unfolding and forms less than 5% (w/w) insoluble aggregates at concentrations up to 10 g/L when incubated at 37°C for 1 day in phosphate buffered saline (PBS).

A binding domain can inhibit VEGF-Axxx binding to VEGFR-2 either by binding to VEGF-Axxx or by binding to VEGFR-2 in a way that the apparent dissociation constant (K_d) between VEGF-Axxx and VEGFR-2 is increased more than 10^2-fold, preferably more than 10^3-fold, more preferably more than 10^4-fold, more preferably more than 10^5-fold, and most preferably more than 10^6-fold. Preferably, the K_d for the interaction of the binding domain to either VEGF-Axxx or VEGFR-2 is below 10^{-7}M, preferably below 10^{-8}M, more preferably below 10^{-9}M, more preferably below 10^{-10}M, and most preferably below 10^{-11}M. Methods, to determine dissociation constants of protein-protein interactions, such as surface plasmon resonance (SPR) based technologies, are well known to the person skilled in the art.

A preferred binding domain binds VEGF-Axxx. Even more preferred is a binding domain that binds human VEGF-A165.

The term "PBS" means a phosphate buffered water solution containing 137 mM NaCl, 10 mM phosphate and 2.7 mM KCl and having a pH of 7.4.

Preferably, the binding protein and/or binding domain has a midpoint denaturation temperature (Tm) above 45°C, more preferably above 50°C, more preferably above 55°C, and most preferably above 60°C upon thermal unfolding. A binding protein or a binding domain of the invention possesses a defined secondary and tertiary structure under physiological conditions. Thermal unfolding of such a polypeptide results in a loss of its tertiary and secondary structure, which can be followed, for example, by circular dichroism (CD) measurements. The midpoint denaturation temperature of a binding protein or

binding domain upon thermal unfolding corresponds to the temperature at the midpoint of the cooperative transition in physiological buffer upon heat denaturation of said protein or domain by slowly increasing the temperature from 10°C to about 100°C. The determination of a midpoint denaturation temperature upon thermal unfolding is well known to the person skilled in the art. This midpoint denaturation temperature of a binding protein or binding domain upon thermal unfolding is indicative of the thermal stability of said polypeptide.

Also preferred is a binding protein and/or binding domain forming less than 5% (w/w) insoluble aggregates at concentrations up to 20 g/l, preferably up 40 g/L, more preferably up to 60 g/L, even more preferably up to 80 g/L, and most preferably up to 100 g/L when incubated for over 5 days, preferably over 10 days, more preferably over 20 days, more preferably over 40 days, and most preferably over 100 days at 37°C in PBS. The formation of insoluble aggregates can be detected by the appearance of visual precipitations, gel filtration or dynamic light scattering, which strongly increases upon formation of insoluble aggregates. Insoluble aggregates can be removed from a protein sample by centrifugation at 10'000xg for 10 minutes. Preferably, a binding protein and/or binding domain forms less than 2%, 1%, 0.5%, 0.2%, 0.1%, or 0.05% (w/w) insoluble aggregates under the mentioned incubation conditions at 37°C in PBS. Percentages of insoluble aggregates can be determined by separation of the insoluble aggregates from soluble protein, followed by determination of the protein amounts in the soluble and insoluble fraction by standard quantification methods.

Also preferred is a binding protein and/or binding domain that does not lose its native three-dimensional structure upon incubation in PBS containing 100 mM dithiothreitol (DTT) for 1 or 10 hours at 37°C.

In one particular embodiment the invention relates to a binding protein comprising a binding domain inhibiting VEGF-Axxx binding to VEGFR-2 and having the indicated or preferred midpoint denaturation temperature and non-aggregating properties as defined above, wherein said binding protein inhibits sprouting of HUVEC spheroids with an IC_{50} value below 100 nM.

The term "HUVEC" means human umbilical vein endothelial cells, which can be isolated from normal human umbilical vein and which are responsive to VEGF-A stimulation.

Assays to measure the sprouting of HUVEC spheroids, such as that described in Example 2, are well known to the person skilled in the art.

An IC_{50} value is the concentration of a substance, such as a binding protein or binding domain, which is required for 50% inhibition *in vitro* of an experimental determined parameter, such as the sprouting of HUVEC spheroids. IC_{50} values can be readily determined by the person skilled in the art (Korff T. and Augustin H.G., J. Cell Biol. *143(5)*, 1341-52, 1998).

Preferred is a binding protein and/or binding domain that inhibits the sprouting of HUVEC spheroid with an IC_{50} value below 10 nM, preferably below 1 nM, more preferably below 0.1 nM, and most preferably below 0.05 nM.

Further preferred is a monomeric binding protein and/or binding domain that inhibits the sprouting of HUVEC spheroids with an IC_{50} value lower than the corresponding IC_{50} value of ranibizumab (Lucentis®, a registered trademark of Genentech), bevacizumab (Avastin®, a registered trademark of Genentech), aflibercept (VEGF Trap®, a registered trademark of Regeneron), or pegaptanib (Macugen®, a registered trademark of Pfizer).

In particular the invention relates to a binding protein comprising a binding domain inhibiting VEGF-Axxx binding to VEGFR-2 and having the indicated or preferred midpoint denaturation temperature and non-aggregating properties as defined above, wherein the K_d for the interaction of said binding domain to VEGF-Axxxb is at least 10-fold higher compared to the K_d for the interaction of said binding domain to the corresponding VEGF-Axxx.

Preferably, the K_d for the interaction of the binding domain to VEGF-Axxxb is at least 10^2-fold higher, preferably 10^3-fold higher, more preferably 10^4-fold higher, more preferably 10^5-fold higher, and most preferably 10^6-fold higher compared to the K_d for the interaction of the binding domain to the corresponding VEGF-Axxx.

Also preferably, the K_d for the interaction of a binding domain to VEGF-Axxxb is above 10^3 nM and the K_d for the interaction of the binding domain to VEGF-Axxx is below 10 or 1 nM.

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The K_d for the interaction of a preferred binding domain to VEGF-B, VEGF-C, VEGF-D, PlGF or PDGF is above 1 nM, preferably above 10 nM, more preferably above 10^2 nM, even more preferably above 10^3 nM, and most preferably above10^4 nM.

Preferably, VEGF-Axxx is either dog VEGF-A164 or simian VEGF-A165 or human VEGF-A165, and VEGF-Axxxb is either dog VEGF-A164b or simian VEGF-A165b or human VEGF-A165b.

Another preferred embodiment is a recombinant binding protein comprising a binding domain, wherein said binding domain inhibits VEGF-Axxx binding to VEGFR-2 and wherein said binding domain is a repeat domain or a designed repeat domain. Such a repeat domain may comprise one, two, three or more internal repeat modules that will participate in binding to VEGF-Axxx. Preferably, such a repeat domain comprises an N-terminal capping module, two to four internal repeat modules, and a C-terminal capping module. Preferably, said binding domain is an ankyrin repeat domain or designed ankyrin repeat domain.

Preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GHLEIVEVLLK7GADVNA (SEQ ID NO:1)
wherein 1, 2, 3, 4, 5, 6, and 7, represent, independently of each other, an amino acid residue selected from the group A, D, E, F, H, I, K, L, M, N, Q, R, S, T, V, W and Y.

Particularly preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23GWTPLHLAA45GHLEIVEVLLK6GADVNA (SEQ ID NO:2)
wherein
1 represents an amino acid residue selected from the group consisting of F, T, N, R, V, A, I, K, Q, S and Y; preferably F, T, N, R and V; more preferably F and T;
2 represents an amino acid residue selected from the group consisting of W, Y, H and F; preferably W, Y and H;
3 represents an amino acid residue selected from the group consisting of M, I, F and V; preferably M and I;

4 represents an amino acid residue selected from the group consisting of H, A, K, G, L, M, N, T, V, W and Y; preferably H, A and K;
5 represents an amino acid residue selected from the group consisting of E, Y, F, V, H, I, L, N and R; preferably E, Y, F, V and H; more preferably E, Y, F and V; and
6 represents an amino acid residue selected from the group consisting of A, N, Y, H and R.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GHLEIVEVLLK7GADVN8 (SEQ ID NO:3)
wherein
1 represents an amino acid residue selected from the group consisting of T, E, A, D, F, K, N, Q, R, S, W and Y; preferably T and E;
2 represents an amino acid residue selected from the group consisting of V, F, Y, A, H, I, K, R, T and W; preferably V, F and Y;
3 represents an amino acid residue selected from the group consisting of S, A, N, F and M; preferably S, A and N; more preferably S and A;
4 represents an amino acid residue selected from the group consisting of Y, F, S and W;
5 represents an amino acid residue selected from the group consisting of A, S, L and Y; preferably A and S;
6 represents an amino acid residue selected from the group consisting of D, N, M, A, I, K and Y; preferably D, N and M; more preferably D and N;
7 represents an amino acid residue selected from the group consisting of A, Y, H, N and D; and
8 represents the amino acid residue T or A.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23GWTPLHL4ADLG5LEIVEVLLK6GADVN7 (SEQ ID NO:4)
wherein
1 represents an amino acid residue selected from the group consisting of K, T and Y;
2 represents the amino acid residue N or M;
3 represents the amino acid residue T or F;
4 represents the amino acid residue S or A;

5 represents the amino acid residue H or R;
6 represents an amino acid residue selected from the group consisting of A, Y, H and N; and
7 represents the amino acid residue A or T.

Even more preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:3, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:2 and/or followed by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:4.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GH7EIVEVLLK8GADVNA (SEQ ID NO:5)
wherein
1 represents an amino acid residue selected from the group consisting of A, N, R, V, Y, E, H, I, K, L, Q, S and T; preferably A, N, R, V and Y; more preferably A and R;
2 represents an amino acid residue selected from the group consisting of S, A, N, R, D, F, L, P, T and Y; preferably S, A, N and R;
3 represents an amino acid residue selected from the group consisting of T, V, S, A, L and F; preferably T, V, S, A and L; more preferably T, V and S;
4 represents an amino acid residue selected from the group consisting of W, F and H;
5 represents an amino acid residue selected from the group consisting of P, I, A, L, S, T, V and Y; preferably P and I;
6 represents an amino acid residue selected from the group consisting of W, F, I, L, T and V;
7 represents the amino acid residue L or P; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GHLEIVEVLLK7GADVNA (SEQ ID NO:6)
wherein

1 represents an amino acid residue selected from the group consisting of H, Q, A, K, R, D, I, L, M, N, V and Y; preferably H, Q, A, K and R; more preferably A and R;
2 represents an amino acid residue selected from the group consisting of Y, F and H;
3 represents an amino acid residue selected from the group consisting of Q, F and T;
4 represents an amino acid residue selected from the group consisting of W, M, G, H, N and T; preferably W and M;
5 represents an amino acid residue selected from the group consisting of T, A, M, L and V; preferably T, A and M;
6 represents an amino acid residue selected from the group consisting of I, L, V, D and T; preferably I, L and V; and
7 represents an amino acid residue selected from the group consisting of A, H, N and Y.

Even more preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:6, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:5.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23GWTPLHLAA45GHLEIVEVLLK6GADVNA (SEQ ID NO:7)
wherein
1 represents an amino acid residue selected from the group consisting of K, M, N, R and V;
2 represents an amino acid residue selected from the group consisting of Y, H, M and V;
3 represents an amino acid residue selected from the group consisting of F, L, M and V;
4 represents an amino acid residue selected from the group consisting of R, H, V, A, K and N; preferably R, H, V and A;
5 represents an amino acid residue selected from the group consisting of F, D, H, T, Y, M and K; preferably F, D, H, T and Y; and
6 represents an amino acid residue selected from the group consisting of A, H, N and Y.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GHLEIVEVLLK7GADVN8 (SEQ ID NO:8)

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wherein
1 represents an amino acid residue selected from the group consisting of T, A, H, I, N and S;
2 represents an amino acid residue selected from the group consisting of F, I, Q, R, V and N;
3 represents an amino acid residue selected from the group consisting of A, G, N, Q and V;
4 represents the amino acid residue W or Y;
5 represents an amino acid residue selected from the group consisting of A, S, T and M;
6 represents an amino acid residue selected from the group consisting of N, V, S, F, M and W;
7 represents an amino acid residue selected from the group consisting of A, H, N and Y; and
8 represents the amino acid residue T or A.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHL5A67GHLEIVEVLLK8GADVNA (SEQ ID NO:9)
wherein
1 represents an amino acid residue selected from the group consisting of K, A, V and N;
2 represents an amino acid residue selected from the group consisting of N, I and Y;
3 represents an amino acid residue selected from the group consisting of T, F, Y and W;
4 represents an amino acid residue selected from the group consisting of W, D and Y;
5 represents the amino acid residue S or A;
6 represents an amino acid residue selected from the group consisting of D, I and M;
7 represents an amino acid residue selected from the group consisting of L, T and Y; and
8 represents an amino acid residue selected from the group consisting of A, H, Y and N;

Even more preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:8, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:7 and/or followed by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:9.

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Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif

1DFK2DTPLHLAA34GH5EIVEVLLK6GADVNA (SEQ ID NO:10)

wherein

1 represents an amino acid residue selected from the group consisting of L, S and T;

2 represents an amino acid residue selected from the group consisting of G, S and C; preferably G and S;

3 represents the amino acid residue S or A;

4 represents an amino acid residue selected from the group consisting of Q, S, M and N; preferably Q and S;

5 represents an amino acid residue selected from the group consisting of L, M and Q; and

6 represents an amino acid residue selected from the group consisting of A, H, N, Y and D.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif

1D2L34TPLHLA567GHLEIVEVLLK8GADVNA (SEQ ID NO:11)

wherein

1 represents an amino acid residue selected from the group consisting of Y, H, F, I, L and W; preferably Y and H;

2 represents an amino acid residue selected from the group consisting of M, D, I, L, V; preferably M and D;

3 represents an amino acid residue selected from the group consisting of G, S and V;

4 represents the amino acid residue W or F;

5 represents an amino acid residue selected from the group consisting of A, G and T;

6 represents the amino acid residue D or W;

7 represents the amino acid residue L or F; and

8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

Even more preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:11, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:10.

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Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif

1D23G4TPL5LAA67GHLEIVEVLLK8GADVNA (SEQ ID NO:12)

wherein

1 represents an amino acid residue selected from the group consisting of K, S, I, N, T and V; preferably K and S;

2 represents an amino acid residue selected from the group consisting of K, N, W, A, H, M, Q and S; preferably K and N;

3 represents an amino acid residue selected from the group consisting of F, Q, L, H and V; preferably F, Q and L;

4 represents the amino acid residue F or T;

5 represents the amino acid residue Q or H;

6 represents the amino acid residue Y or S;

7 represents an amino acid residue selected from the group consisting of N, H, Y and M; preferably N and H; and

8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

Further preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif

1D23GWT4LHLAADLG5LEIVEVLLK6GADVNA (SEQ ID NO:13)

wherein

1 represents an amino acid residue selected from the group consisting of F, Y, H and W; preferably F, Y and H;

2 represents an amino acid residue selected from the group consisting of I, M, D and V; preferably I, M and D;

3 represents the amino acid residue F or L;

4 represents the amino acid residue L or P;

5 represents an amino acid residue selected from the group consisting of H, L and Y; and

6 represents an amino acid residue selected from the group consisting of A, H, N, C and Y.

Even more preferred is a recombinant binding protein, wherein said ankyrin repeat domain or said designed ankyrin repeat domain comprises a repeat module with the

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ankyrin repeat sequence motif of SEQ ID NO:13, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:12.

Another preferred embodiment is a recombinant binding protein comprising at least one repeat domain with binding specificity for VEGF-Axxx, wherein said repeat domain competes for binding to VEGF-Axxx with a repeat domain selected from the group consisting of SEQ ID NOs:16, 22, 23, 29, 30 and 33, or a repeat domain selected from the group consisting of SEQ ID NOs:16, 22, 23, 29, 30, 33, 34, 36, 39 and 40.

The term "compete for binding" means the inability of two different binding domains of the invention to bind simultaneously to the same target, while both are able to bind the same target individually. Thus, such two binding domains compete for binding to said target. Methods, such as competition ELISA or competition SPR measurements (e.g. by using the Proteon instrument from BioRad), to determine if two binding domains compete for binding to a target are well known to the practitioner in the art.

A recombinant binding protein that competes for binding to VEGF-Axxx with a selected repeat protein can be identified by methods well know to the person skilled in the art, such as a competition Enzyme-Linked ImmunoSorbent Assay (ELISA).

Another preferred embodiment is a recombinant binding protein comprising a repeat domain with binding specificity for VEGF-Axxx selected from the group consisting of SEQ ID NOs:14 to 33, or selected from the group consisting of SEQ ID NOs:14 to 40.

Further preferred is a recombinant binding protein, wherein said repeat domain with binding specificity for VEGF-Axxx comprises an amino acid sequence that has at least 70% amino acid sequence identity with a repeat domain of said group of repeat domains. Preferably, said amino acid sequence identity is at least 75%, more preferably 80%, more preferably 85%, more preferably 90%, and most preferably 95%.

Further preferred is a recombinant binding protein, wherein said repeat domain with binding specificity for VEGF-Axxx comprises a repeat module that has at least 70% amino acid sequence identity with a repeat module of a repeat domain of said group of repeat domains. Preferably, said amino acid sequence identity is at least 75%, more preferably 80%, more preferably 85%, more preferably 90%, and most preferably 95%.

In a further preferred embodiment of a recombinant binding protein comprising a repeat domain according to the present invention, one or more of the amino acid residues of the repeat modules of said repeat domain are exchanged by an amino acid residue found at the corresponding position on alignment of a repeat unit. Preferably, up to 30% of the amino acid residues are exchanged, more preferably, up to 20%, and even more preferably, up to 10% of the amino acid residues are exchanged. Most preferably, such a repeat unit is a naturally occurring repeat unit. Even more preferably, said repeat domain has binding specificity for VEGF-Axxx or VEGFR-2.

In still another particular embodiment, up to 30% of the amino acid residues, more preferably, up to 20%, and even more preferably, up to 10% of the amino acid residues are exchanged with amino acids which are not found in the corresponding positions of repeat units.

In further embodiments, any of the VEGF-Axxx binding proteins or domains described herein may be covalently bound to one or more additional moieties, including, for example, a moiety that also binds to VEGFR-2 (e.g. a VEGFR-2 binding polypeptide), a moiety that binds to a different target, such as PlGF, human serum albumin, a cellular receptor (e.g. Her2), an immunoglobulin (e.g. IgG1), a cytokine (e.g. TNF-alpha or an interleukin) or a growth factor to create a dual-specificity binding agent, a labeling moiety (e.g. a fluorescent label such as fluorescein, or a radioactive tracer), a moiety that facilitates protein purification (e.g. a small peptide tag, such as a His- or strep-tag), a moiety that provides effector functions for improved therapeutic efficacy (e.g. the Fc part of an antibody to provide antibody-dependent cell-mediated cytotoxicity, a toxic protein moiety such as *Pseudomonas aeruginosa* exotoxin A (ETA) or a small molecular toxic agent such as maytansinoids or DNA alkylating agents) or a moiety that provides improved pharmacokinetics. Improved pharmacokinetics may be assessed according to the perceived therapeutic need. Often it is desirable to increase bioavailability and/or increase the time between doses, possibly by increasing the time that a protein remains available in the serum after dosing. In some instances, it is desirable to improve the continuity of the serum concentration of the protein over time (e.g., decrease the difference in serum concentration of the protein shortly after administration and shortly before the next administration). Moieties that tend to slow clearance of a protein from the blood include hydroxyethyl starch (HES), polyethylene glycol (PEG), sugars (e.g. sialic acid), well-tolerated protein moieties (e.g. Fc fragment or serum albumin), and binding domains or peptides with specificity and affinity for abundant serum proteins, such as

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antibody Fc fragments or serum albumin. The recombinant binding protein of the invention may be attached to a moiety that reduces the clearance rate of polypeptides in a mammal (e.g. in mouse, rat, or human) by greater than three-fold relative to the unmodified polypeptides.

One ore more polyethylene glycol moieties may be attached at different positions in the binding protein, and such attachment may be achieved by reaction with amines, thiols or other suitable reactive groups. Attachment of polyethylene glycol moieties (PEGylation) may be site-directed, wherein a suitable reactive group is introduced into the protein to create a site where PEGylation preferentially occurs, or is originally present in the binding protein. The thiol group may be present in a cysteine residue; and the amine group may be, for example, a primary amine found at the N-terminus of the polypeptide or an amine group present in the side chain of an amino acid, such as lysine or arginine. In a preferred embodiment, the binding protein is modified so as to have a cysteine residue at a desired position, permitting site directed PEGylation on the cysteine, for example by reaction with a polyethylene glycol derivative carrying a maleimide function. The polyethylene glycol moiety may vary widely in molecular weight (i.e. from about 1 kDa to about 100 kDa) and may be branched or linear. Preferably, the polyethylene glycol has a molecular weight of about 1 to about 50 kDa, preferably about 10 to about 40 kDa, even more preferably about 15 to about 30 kDa, and most preferably about 20 kDa.

In a further embodiment, the invention relates to nucleic acid molecules encoding the particular recombinant binding proteins. Further, a vector comprising said nucleic acid molecule is considered.

Further, a pharmaceutical composition comprising one or more of the above mentioned binding proteins, in particular recombinant binding proteins comprising repeat domains, or nucleic acid molecules encoding the particular recombinant binding proteins, and optionally a pharmaceutical acceptable carrier and/or diluent is considered. Pharmaceutical acceptable carriers and/or diluents are known to the person skilled in the art and are explained in more detail below. Even further, a diagnostic composition comprising one or more of the above mentioned recombinant binding proteins, in particular binding proteins comprising repeat domains, is considered.

The binding protein of the invention suppresses or prevents VEGF induced pathological angiogenesis, vascular leakage (edema), pulmonary hypertension, tumor formation and/or

inflammatory disorders. With "suppression" it is understood that the recombinant protein prevents the mentioned pathologies to some extent, e.g. to 10% or 20%, more preferably 50%, in particular 70%, 80% or 90%, or even 95%.

The term “edema” means a condition that is caused by vascular leakage. Vasodilation and increased permeability during inflammation can be predominant pathogenetic mechanisms. For instance, edema contributes to infarct expansion after stroke and may cause life-threatening intracranial hypertension in cancer patients. Further, extravasation of plasma proteins favors metastatic spread of occult tumors, and airway congestion may cause fatal asthmatic attacks. The increased vascular leakage which occurs during inflammation can lead to respiratory distress, ascites, peritoneal sclerosis (in dialysis patients), adhesion formation (abdominal surgery) and metastatic spreading.

The term “angiogenesis” means a fundamental process by which new blood vessels are formed. The primary angiogenic period in humans takes place during the first three months of embryonic development but angiogenesis also occurs as a normal physiological process during periods of tissue growth, such as an increase in muscle or fat and during the menstrual cycle and pregnancy.

The term “pathological angiogenesis” refers to the formation and growth of blood vessels during the maintenance and the progression of several disease states. Particular examples of pathological angiogenesis are found in blood vessels (atherosclerosis, hemangioma, hemangioendothelioma), bone and joints (rheumatoid arthritis, synovitis, bone and cartilage destruction, osteomyelitis, pannus growth, osteophyte formation, neoplasms and metastasis), skin (warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic edema, neoplasms), liver, kidney, lung, ear and other epithelia (inflammatory and infectious processes including hepatitis, glomerulonephritis, pneumonia; and asthma, nasal polyps, otitis, transplantation disorders, liver regeneration disorders, neoplasms and metastasis), uterus, ovary and placenta (dysfunctional uterine bleeding due to intra-uterine contraceptive devices, follicular cyst formation, ovarian hyperstimulation syndrome, endometriosis, neoplasms), brain, nerves and eye (retinopathy of prematurity, diabetic retinopathy, choroidal and other intraocular disorders, leukomalacia, neoplasms and metastasis), heart and skeletal muscle due to work overload, adipose tissue (obesity), endocrine organs (thyroiditis, thyroid enlargement, pancreas transplantation disorders), hematopoiesis (Kaposi syndrome in AIDS),

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hematologic malignancies (leukemias), and lymph vessels (tumor metastasis, lymphoproliferative disorders).

The term "retinal ischemic diseases" means that the retina's supply of blood and oxygen is decreased, the peripheral portions of the retina lose their source of nutrition and stop functioning properly. A particular example of a retinal ischemic disease is retinopathy. Common diseases which lead to retinopathy are diabetic retinopathy, central retinal vein occlusion, stenosis of the carotid artery, and sickle cell retinopathy. Diabetic retinopathy is a major cause of visual loss in diabetic patients. In the ischemic retina the growth of new blood vessels occurs (neovascularisation). These vessels often grow on the surface of the retina, at the optic nerve, or in the front of the eye on the iris. The new vessels cannot replace the flow of necessary nutrients and, instead, can cause many problems such as vitreous hemorrhage, retinal detachment, and uncontrolled glaucoma. These problems occur because new vessels are fragile and are prone to bleed. If caught in its early stages, proliferative diabetic retinopathy can sometimes be arrested with panretinal photocoagulation. However, in some cases, vitrectomy surgery is the only option.

Beside these retinopathies, vascular diseases of the eye also include ocular neovascularization diseases, such as macular degeneration and diabetic macular edema (DME). Macular degeneration results from the neovascular growth of the choroid vessel underneath the macula. There are two types of macular degeneration: dry and wet. While wet macular degeneration only comprises 15% of all macular degeneration, nearly all wet macular degeneration leads to blindness. In addition, wet macular degeneration nearly always results from dry macular degeneration. Once one eye is affected by wet macular degeneration, the condition almost always affects the other eye. Wet macular degeneration is often called age-related wet macular degeneration of wet-AMD as it is mostly found in elderly persons.

Diabetic retinopathy (DR) and DME are leading causes of blindness in the working-age population of most developed countries. The increasing number of individuals with diabetes worldwide suggests that DR and DME will continue to be major contributors to vision loss and associated functional impairment for years to come. Several biochemical mechanisms, including protein kinase C–β activation, increased vascular endothelial growth factor production, oxidative stress, and accumulation of intracellular sorbitol and advanced glycosylation end products, may contribute to the vascular disruptions that

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characterize DR/DME. The inhibition of these pathways holds the promise of intervention for DR and DME.

The term "pulmonary hypertension" means a disorder in which the blood pressure in the pulmonary arteries is abnormally high. In the absence of other diseases of the heart or lungs it is called primary pulmonary hypertension. Diffuse narrowing of the pulmonary arterioles occurs as a result of pathological arteriogenesis followed by pulmonary hypertension as a response to the increased resistance to blood flow. The incidence is 8 out of 100'000 people. However, pulmonary hypertension can also occur as a complication of Chronic Obstructive Pulmonary Diseases (COPD) such as emphysema, chronic bronchitis or diffuse interstitial fibrosis and in patients with asthmatiform COPD. The incidence of COPD is approximately 5 out of 10'000 people.

Furthermore the binding proteins of the invention can be used to treat inflammation and more specifically inflammatory disorders.

The term "inflammation" as used herein means, the local reaction to injury of living tissues, especially the local reaction of the small blood vessels, their contents, and their associated structures. The passage of blood constituents through the vessel walls into the tissues is the hallmark of inflammation, and the tissue collection so formed is termed the exudates or edema. Any noxious process that damages living tissue, e.g. infection with bacteria, excessive heat, cold, mechanical injury such as crushing, acids, alkalis, irradiation, or infection with viruses can cause inflammation irrespective of the organ or tissue involved. It should be clear that diseases classified as "inflammatory diseases" and tissue reactions ranging from burns to pneumonia, leprosy, tuberculosis, and rheumatoid arthritis are all "inflammations".

The binding proteins according to the invention can be used to treat tumor formation. The term "tumor" means a mass of abnormal tissue that arises without obvious cause from pre-existing body cells, has no purposeful function, and is characterized by a tendency to autonomous and unrestrained growth. Tumors are quite different from inflammatory or other swellings because the cells in tumors are abnormal in their appearance and other characteristics. Abnormal cells, i.e. the kind of cells that generally make up tumors, differ from normal cells in having undergone one or more of the following alterations: (1) hypertrophy, or an increase in the size of individual cells; (2) hyperplasia or an increase in the number of cells within a given zone; (3) anaplasia, or a regression of the

physical characteristics of a cell toward a more primitive or undifferentiated type. Tumors may be benign, for example lipomas, angiomas, osteomas, chondromas, and adenomas. Examples of malignant tumors are carcinomas (such as the breast tumors, carcinomas in the respiratory and gastrointestinal tracts, the endocrine glands, and the genitourinary system), sarcomas (in connective tissues, including fibrous tissues, adipose (fat) tissues, muscle, blood vessels, bone, and cartilage), carcinosarcoma (in both epithelial and connective tissue) leukemias and lymphomas, tumors of nerve tissues (including the brain), and melanoma (a cancer of the pigmented skin cells). The use of the binding proteins of the present invention against tumors can also be in combination with any other tumor therapy known in the art such as irradiation, photo-dynamic therapy, chemotherapy or surgery.

A pharmaceutical composition comprises binding proteins as described above and a pharmaceutically acceptable carrier, excipient or stabilizer (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]). Suitable carriers, excipients or stabilizers known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids and amino acid copolymers. A pharmaceutical composition may also be a combination formulation, comprising an additional active agent, such as an anti-cancer agent or an anti-angiogenic agent (for example human VEGF-Axxxb; preferably, human VEGF-A165b).

A preferred pharmaceutical composition for the treatment of eye diseases comprises binding proteins as described above and a detergent such as polysorbate 20 (e.g. about 0.04%), a buffer such as histidine, phosphate or lactic acid and a sugar such as sucrose or trehalose. Preferably, such a composition comprises binding proteins as described above and PBS. Said pharmaceutical compositions may be administered locally, either topically to a portion of the eye or be injected into the eye for instance into the subconjunctivital, peri- or retrobulbar space or directly into the eye. Alternatively, said compositions may be administered systemically by parental administration. Preferably, said pharmaceutical composition is applied to the eye by an intravitreous injection. Also preferably, said pharmaceutical composition is applied to the eye topically and as an eye drop. The eye drop may be applied to the cornea (clear part in the centre of the eye)

thereby allowing the molecules to permeate into the eye. For the treatment of a disease affecting the posterior of the eye, it may be most desirable that the binding protein penetrates the sclera when injected under the conjunctiva or around the globe. The administering of the binding protein may be performed after a preliminary step of modulating the surface of the eye to improve penetration of the molecules. Preferably, the epithelial layer such as the corneal epithelium is modulated by a penetration enhancer to allow for a sufficient and rapid penetration of the molecules as for example described above. The use of the binding proteins of the present invention against eye diseases can also be in combination with any other therapy known in the art such as photo-dynamic therapy.

The formulations to be used for in vivo administration must be aseptic or sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. In one embodiment of the invention, an intraocular implant can be used for providing the binding protein of the invention. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing a polypeptide of the invention, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides, copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT® (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The pharmaceutical composition may be administered by any suitable method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parental administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. The dosage and mode of administration will depend on the individual to be treated and the particular disease. Generally, the pharmaceutical composition is administered so that the binding protein of the present invention is given at a dose between 1 μg/kg and 20 mg/kg, more preferably between 10 μg/kg and 5 mg/kg, most preferably between 0.1 and 2 mg/kg. Preferably, it is given as a bolus dose. Continuous infusion may also be used and includes continuous subcutaneous delivery via an osmotic minipump. If so, the pharmaceutical composition

may be infused at a dose between 5 and 20 μg/kg/minute, more preferably between 7 and 15 μg/kg/minute. In particular, the pharmaceutical composition is administered by injections into the eye so that the binding protein of the invention is given at a dose between 0.1 mg and 10 mg per injection, more preferably between 0.3 and 6 mg per injection, most preferably between 1 mg and 4 mg per injection. Further, the pharmaceutical composition is administered by eye drops to the eye so that a single drop of a solution containing a concentration of the binding protein of the invention between 10 and 120 mg/ml, more preferably between 20 and 100 mg/ml, most preferably between 40 and 80 mg/ml is applied to the eye.

In another embodiment of the invention a binding protein inhibiting the activity of VEGF-Axxx, as described above, can be used in combination with a binding protein or small molecule inhibiting the activity of PlGF, with the same inhibition levels of PlGF as described above for VEGF-Axxx. This embodiment is based on the fact that PlGF is found to be angiogenic at sites where VEGF-Axxx levels are increased. Further, a binding protein inhibiting the activity of VEGF-Axxx, as described above, can be used in combination with a binding protein or small molecule inhibiting the activity of platelet-derived growth factor (PDGF), VEGF-C or other members of the VEGF family of proteins, tumor necrosis factor alpha (TNFalpha), delta-ligand like 4 (Dll4), interleukin 6 (IL-6), neuropilin or angiopoietin 2 (Ang2).

The invention further provides novel methods of treatment. In one aspect, a method of treating a retinopathy is provided, the method comprising administering, to a patient in need thereof, a therapeutically effective amount of a binding protein of the invention, in particular a binding protein that inhibits the interaction between human VEGF-Axxx and human VEGFR-2, but not the interaction between human VEGF-Axxxb and human VEGFR-2, and the binding protein inhibits VEGFR-2 mediated angiogenesis.

The invention further relates to methods for using a binding protein as described to inhibit a VEGF-A biological activity in a cell or to inhibit a biological activity mediated by VEGFR-2. The cell may be situated *in vivo* or *ex vivo*, and may be, for example, a cell of a living organism, a cultured cell or a cell in a tissue sample. The method may comprise contacting said cell with any of the VEGF-A/VEGFR-2 interaction inhibiting binding proteins disclosed herein, in an amount and for a time sufficient to inhibit such biological activity.

The invention provides a method for treating a subject having a condition which responds to the inhibition of VEGF-Axxx or VEGFR-2. Such a method comprises administering to said subject an effective amount of a binding protein described herein. A condition may be one that is characterized by inappropriate angiogenesis. A condition may be a hyper-proliferative condition. Examples of conditions (or disorders) suitable for treatment include autoimmune disorders, inflammatory disorders, retinopathies (particularly proliferative retinopathies), and cancers, in particular one of the diseases described above. Any of the binding proteins described herein may be used for the preparation of a medicament for the treatment of such a disorder, particularly a disorder selected from the group consisting of: an autoimmune disorder, an inflammatory disorder, a retinopathy, and a cancer. Preferred conditions (or disorders) suitable for treatment are first-line metastatic renal cell carcinoma, relapsed glioblastoma multiforme, adjuvant colon cancer, adjuvant HER2-negative breast cancer, adjuvant HER2-positive breast cancer, adjuvant non-small cell lung cancer, diffuse large B-cell lymphoma, first-line advanced gastric cancer, first-line HER2-negative metastatic breast cancer, first-line HER2-positive metastatic breast cancer, first-line metastatic ovarian cancer, gastrointestinal stromal tumors, high risk carcinoid, hormone refractory prostate cancer, newly diagnosed glioblastoma multiforme, metastatic head and neck cancer, relapsed platinum-sensitive ovarian cancer, second-line metastatic breast cancer, extensive small cell lung cancer, non-squamous, non-small cell lung cancer with previously treated CNS metastases and relapsed multiple myeloma, prostate cancer, non-small cell lung cancer (NSCLC), colorectal cancer and pancreatic cancer, advanced ovarian cancer (AOC), AOC patients with symptomatic malignant ascites and non-Hodgkin's lymphoma.

The recombinant binding protein according to the invention may be obtained and/or further evolved by several methods such as display on the surface of bacteriophages (WO 90/02809, WO 07/006665) or bacterial cells (WO 93/10214), ribosomal display (WO 98/48008), display on plasmids (WO 93/08278) or by using covalent RNA-repeat protein hybrid constructs (WO 00/32823), or intracellular expression and selection / screening such as by protein complementation assay (WO 98/341120). Such methods are known to the person skilled in the art.

A library of ankyrin repeat proteins used for the selection/screening of a recombinant binding protein according to the invention may be obtained according to protocols known to the person skilled in the art (WO 02/020565, Binz, H.K. et al., JMB, *332*, 489-503, 2003, and Binz et al., 2004, loc. cit). The use of such a library for the selection VEGF-Axxx

specific DARPins is given in Example 1. In analogy, the ankyrin repeat sequence motifs as presented above can used to build libraries of ankyrin repeat proteins that may be used for the selection or screening of VEGF-Axxx specific DARPins. Furthermore, repeat domains of the present invention may be modularly assembled from repeat modules according the current inventions and appropriate capping modules (Forrer, P., et al., FEBS letters *539*, 2-6, 2003) using standard recombinant DNA technologies (e.g. WO 02/020565, Binz et al., 2003, loc. cit. and Binz et al., 2004, loc. cit).

The invention is not restricted to the particular embodiments described in the Examples. Other sources may be used and processed following the general outline described below.

Examples

All of the starting materials and reagents disclosed below are known to those skilled in the art, and are available commercially or can be prepared using well-known techniques.

Materials

Chemicals were purchased from Fluka (Switzerland). Oligonucleotides were from Microsynth (Switzerland). Unless stated otherwise, DNA polymerases, restriction enzymes and buffers were from New England Biolabs (USA) or Fermentas (Lithuania). The cloning and protein production strain was *E. coli* XL1-blue (Stratagene, USA). VEGF variants were from R&D Systems (Minneapolis, USA) or were produced in Chinese Hamster Ovary Cells or in *Pichia pastoris* and purified according to standard protocols (Rennel, E. S. et al., European J. Cancer *44*, 1883-94, 2008; Pichia expression system from Invitrogen). Biotinylated VEGF variants were obtained chemically via coupling of the biotin moiety to primary amines of the purified VEGF variants using standard biotinylation reagents and methods (Pierce, USA).

Molecular Biology

Unless stated otherwise, methods are performed according to described protocols (Sambrook J., Fritsch E. F. and Maniatis T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory 1989, New York).

Designed ankyrin repeat protein libraries

The N2C and N3C designed ankyrin repeat protein libraries are described (WO 02/20565; Binz et al. 2003, loc. cit.; Binz et al. 2004, loc. cit.). The digit in N2C and N3C describes

the number of randomized repeat modules present between the N-terminal and C-terminal capping modules. The nomenclature used to define the positions inside the repeat units and modules is based on Binz et al. 2004, loc. cit. with the modification that borders of the repeat modules and repeat units are shifted by one amino acid position. For example, position 1 of a repeat module of Binz et al. 2004 (loc. cit.) corresponds to position 2 of a repeat module of the current disclosure and consequently position 33 of a repeat module of Binz et al. 2004, loc. cit. corresponds to position 1 of a following repeat module of the current disclosure.

All the DNA sequences were confirmed by sequencing, and the calculated molecular weight of all described proteins was confirmed by mass spectrometry.

Example 1: *Selection of binding proteins comprising a repeat domain with binding specificity for VEGF-Axxx*

Using ribosome display (Hanes, J. and Plückthun, A., PNAS *94*, 4937-42, 1997) many designed ankyrin repeat proteins (DARPins) with binding specificity for VEGF-Axxx were selected from the N2C or N3C DARPin libraries described by Binz et al. 2004 (loc. cit.). The binding of the selected clones toward specific (VEGF-Axxx) and unspecific (MBP, *E. coli* maltose binding protein) targets was assessed by crude extract ELISA indicating that VEGF-Axxx binding proteins were successfully selected (Fig. 1). SEQ ID NO:14 to 40 constitute amino acid sequences of selected binding proteins comprising a repeat domain with binding specificity for VEGF-Axxx. Sequence analysis of selected binders revealed specific ankyrin repeat sequence motifs inherent to certain selected families of binders. Such ankyrin repeat sequence motifs present in repeat domains with binding specificity for VEGF-Axxx are provided in SEQ ID NO:1 to 13.

Selection of VEGF-Axxx specific ankyrin repeat proteins by ribosome display

The selection of VEGF-Axxx specific ankyrin repeat proteins was performed by ribosome display (Hanes and Plückthun, loc. cit.) using dog VEGF-A164 or human VEGF-A165 as target proteins, the library of designed ankyrin repeat proteins as described (WO 02/020565, Binz et al., 2003, loc. cit. and Binz et al., 2004, loc. cit) and established protocols (Zahnd, C., Amstutz, P. and Plückthun, A., Nat. Methods *4*, 69-79, 2007). Ribosome-display selection rounds were performed on dog or human VEGF variants (including biotinylated variants immobilized over neutravidin or streptavidin) with both the N2C and N3C DARPin libraries using established protocols (Binz et al. 2004, loc. cit.).

The number of reverse transcription (RT)-PCR cycles after each selection round was constantly reduced from 40 to 30, adjusting to the yield due to enrichment of binders. Four initial selection rounds on dog VEGF yielded pools of nanomolar-affinity DARPins, as revealed by ELISA and SPR measurements of single clones. To find DARPins with further improved affinities, additional off-rate selections were performed on biotinylated human or dog VEGF immobilized over neutravidin or streptavidin, taking pools after the second and third initial ribosome-display selection rounds, followed by an on-rate selection round on human VEGF.

Selected clones bind specifically to VEGF-Axxx as shown by crude extract ELISA
Individual selected DARPins specifically binding VEGF-Axxx were identified by an enzyme-linked immunosorbent assay (ELISA) using crude *Escherichia coli* extracts of DARPin expression cells using standard protocols. Selected clones were cloned into the pQE30 (Qiagen) expression vector, transformed into *E. coli* XL1-Blue (Stratagene) and then grown overnight at 37°C in a 96-deep-well plate (each clone in a single well) containing 1 ml growth medium (2YT containing 1% glucose and 100 µg/ml ampicillin). 1 ml of fresh 2YT containing 50 µg/ml ampicillin was inoculated with 100 µl of the overnight culture in a fresh 96-deep-well plate. After incubation for 2 h at 37°C, expression was induced with IPTG (1 mM final concentration) and continued for 3 h. Cells were harvested, resuspended in 100 µl B-PERII (Pierce) and incubated for 15 min at room temperature with shaking. Then, 900 µl PBS-TB (PBS supplemented with 0.2% BSA, 0.1% Tween 20, pH 7.4) were added and cell debris were removed by centrifugation. 100 µl of each lysed clone were applied to a well of a NeutrAvidin coated MaxiSorp plate containing either a VEGF-Axxx variant or the unrelated MBP immobilized via their biotin moiety and incubated for 1 h at RT. After extensive washing with PBS-T (PBS supplemented with 0.1% Tween 20, pH 7.4) the plate was developed using standard ELISA procedures using the monoclonal anti-RGS(His)$_4$ antibody (34650, Qiagen) as primary antibody and a polyclonal goat anti-mouse antibody conjugated with alkaline phosphatase (A3562, Sigma) as secondary reagent. Binding was then detected by using disodium 4-nitrophenyl phosphate (4NPP, Fluka) as a substrate for alkaline phosphatase. The color development was measured at 405 nm. The results from an example crude extract ELISA used to identify DARPins binding to VEGF-Axxx is shown in Fig. 1. Screening of several hundred clones by such a crude cell extract ELISA revealed more than hundred different DARPins with specificity for VEGF-Axxx. These binding proteins were chosen for further analysis. Examples of amino acid sequences of selected ankyrin repeat domains that specifically bind to VEGF-Axxx are provided in SEQ ID NO:14 to 40.

Regeneron Exhibit 1002.0077

Deducing repeat sequence motives from selected repeat domains with binding specificity for VEGF-Axxx

The amino acid sequences of selected repeat domains with binding specificity for VEGF-Axxx were further analyzed by sequence analyzing tools known to the practitioner in the art (WO 02/020565; Forrer et al., 2003, loc. cit.; Forrer, P., Binz, H.K., Stumpp, M.T. and Plückthun, A., ChemBioChem, *5(2)*, 183-189, 2004). Nevertheless, in contrast to WO 02/020565 where naturally occurring repeat motifs were used to deduce repeat sequence motifs, here the repeat sequence motifs were deduced from the repeat units of selected repeat domains with binding specificity for VEGF-Axxx. Thereby families of selected repeat domains comprising a common repeat sequence motif were determined. Such repeat sequence motifs present in repeat domains with binding specificity for VEGF-Axxx are provided in SEQ ID NO:1 to 13.

High level and soluble expression of DARPins

For further analysis, the selected clones showing specific VEGF-Axxx binding in the crude cell extract ELISA as described above were expressed in *E. coli* XL1-blue cells and purified using their His-tag using standard protocols. 25 ml of stationary overnight cultures (LB, 1% glucose, 100 mg/l of ampicillin; 37°C) were used to inoculate 1 l cultures (same medium). At A(600) = 0.7, the cultures were induced with 0.5 mM IPTG and incubated at 37°C for 4 h. The cultures were centrifuged and the resulting pellets were resuspended in 40 ml of TBS500 (50 mM Tris–HCl, 500 mM NaCl, pH 8) and sonicated. The lysate was recentrifuged, and glycerol (10% (v/v) final concentration) and imidazole (20 mM final concentration) were added to the resulting supernatant. Proteins were purified over a Ni-nitrilotriacetic acid column (2.5 ml column volume) according to the manufacturer's instructions (QIAgen, Germany). Up to 200 mg of highly soluble DARPins with binding specificity to VEGF-Axxx could be purified from one litre of *E. coli* culture with a purity > 95% as estimated from SDS-15% PAGE. Such purified DARPins are used for further characterizations.

Example 2: *Determination of IC_{50} values of selected DARPins with binding specificity to VEGF-Axxx in a spheroid outgrowth assay*

Addition of VEGF-Axxx to HUVEC spheroids embedded in collagen matrices leads to spheroid sprouting. Addition of an inhibitor of VEGF-Axxx will block sprout formation, which can be quantified statistically by the numbers and lengths of sprouts. By adding

different concentration of inhibitor and a constant amount of VEGF, the IC_{50} can be determined.

Inhibition of spheroid sprouting by VEGF-Axxx specific DARPins

Spheroid outgrowth assays were done according to standard protocols (Korff et al., loc. cit.). DARPins with specificity for VEGF-Axxx were selected and purified to > 96% purity as described in Example 1. Human umbilical vein cells were grown to confluency in monolayer culture. After trypsinization, the cell suspension was placed in a hanging drop to form spheroids, i.e. approximately 500 organized aggregated HUVECs. Spheroids were embedded in a collagen matrix and stimulated with VEGF-A165 to initiate sprout outgrowth. Sprouting inhibitors were added additionally to observe their effects on sprouting inhibition. Sprout numbers per spheroid and sprout lengths were quantified using a graphical software.

The results from two example spheroid sprouting assays are shown in Fig. 2a (DARPin #30 with binding specificity for VEGF-Axxx) and Fig. 2b (DARPin NC, a negative control DARPin with no binding specificity for VEGF-Axxx; e.g. DARPin E3_5 (Binz et al., 2005, loc. cit.). The best performing DARPins in this assay showed IC_{50} values in the range of 10 to 50 pM, while Avastin®, Lucentis® and Macugen® showed IC_{50} values in parallel experiments in the range of 150 and 500 pM.

Example 3: *Determination of the target specificity of DARPin #27 in comparison to Avastin® by Surface Plasmon Resonance analysis*

Dog VEGF-A164 or Dog VEGF-A164b were immobilized in a flow cell and the interaction of DARPin #27 (SEQ ID NO:16) and Avastin® with the immobilized targets were analyzed.

Surface Plasmon Resonance (SPR) analysis

SPR was measured using a ProteOn instrument (BioRad). The running buffer was 20 mM HEPES, pH 7.4, 150 mM NaCl and 0.005% Tween 20. About 1200 RU of dog VEGF-A164 or dog VEGF-A164b were immobilized on a GLC chip (BioRad). The interactions were measured at a flow of 60 µl/min with 5 min buffer flow, 100 seconds injection of Avastin® or DARPin #27 at a concentration of 250 nM and an off-rate measurement of a few minutes with buffer flow. The signal of an uncoated reference cell was subtracted from the measurements.

The results are shown in Fig. 3a (Avastin interaction with dog VEGF-A164), Fig. 3b (Avastin interaction with dog VEGF-A164b), Fig. 3c (DARPin #27 interaction with dog VEGF-A164) and Fig. 3d (DARPin #27 interaction with dog VEGF-A164b). Whereas Avastin clearly interacts with both immobilized VEGF isoforms, the DARPin #27 shows only interaction with VEGF-A164 and not VEGF-A164b.

Example 4: *In vivo efficacy of DARPin #30 in inhibiting VEGF-A165 in a vascular leakage rabbit model.*

Pegylated DARPin #30 (SEQ ID NO:29) or Lucentis® is applied by intravitreal injection into an eye of a rabbit to test their efficacy to inhibit vascular leakage induced by a subsequent intravitreous injection of human VEGF-A165.

Vascular leakage inhibition measurements in rabbits

At day 1 either PBS, PEGylated DARPin #30 (125 µg) or the equimolar amount of Lucentis® (162 µg) is applied by an intravitreal injection into one eye of each rabbit (treated eye). At day 4 or day 30 the treated eye of each rabbit was challenged by intravitreal injection of 500 ng of human VEGF-A165. Both eyes of all animals were evaluated 48 hours after the VEGF-A165 injection by measuring the fluorescein content in all eyes 1 h after intravenous injection of sodium fluorescein (50 mg/kg animal body weight, 10%(w/v) in 0.9% (w/v)saline solution). The ratios of the amounts of fluorescence in the treated and untreated eyes were calculated for every animal. A ratio of one corresponds to absence of additional fluorescence leakage in the treated eye, a ratio greater than one indicates more fluorescence leakage in the treated eye than in the untreated control eye.

Preparation of PEGylated DARPin

The PEGylation of protein by making use of a single Cys residue and maleimide chemistry is well known to the person skilled in the art and can be performed according to established protocols (e.g. from Pierce). DARPin #30 comprising an additional C-terminal linker (GGGSGGGSC, SEQ ID NO:41) was purified to near homogeneity using standard chromatographic methods. The protein is completely reduced using DTT and purified by gel-filtration to remove the DTT and to exchange the buffer by PBS. PEG-maleimide (methoxy-poly(ethylene glycol)-oxopropylamino-propyl maleimide; NOF, no. Sunbright ME-200MA) dissolved in PBS is mixed with the DARPin in PBS at about 15% molar

excess of PEG-maleimide for 2-4 hours at room temperature. The PEGylated DARPin is then separated from non-reactive DARPin and non-reactive PEG moieties by using standard anion exchange chromatography.

The results are shown in Fig. 4. Both PEGylated DARPin #30 and Lucentis® were able to protect the rabbit eye from VEGF-A165 induced vascular leakage 4 days after they were applied by intravitreal injections. Nevertheless, only the PEGylated DARPin #30, and not Lucentis®, was able to protect the rabbit eye from VEGF-A165 induced vascular leakage up to 30 days after the intravitreal injection.

Regeneron Exhibit 1002.0081

Claims

1. A recombinant binding protein comprising at least one repeat domain, wherein said repeat domain binds VEGF-Axxx with a Kd below 10^{-7}M and inhibits VEGF-Axxx binding to VEGFR-2.

2. The binding protein of claim 1, wherein said repeat domain inhibits sprouting of HUVEC spheroids with an IC_{50} value below 10 nM.

3. The binding protein of claim 1, wherein the K_d for the interaction of said repeat domain with VEGF-Axxxb is at least 10-fold higher compared to the K_d for the interaction of said repeat domain with the corresponding VEGF-Axxx.

4. The binding protein of claim 1, wherein said repeat domain is an ankyrin repeat domain.

5. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23GWTPLHL4ADLG5LEIVEVLLK6GADVN7 (SEQ ID NO:4)
wherein
1 represents an amino acid residue selected from the group consisting of K, T and Y;
2 represents the amino acid residue N or M;
3 represents the amino acid residue T or F;
4 represents the amino acid residue S or A;
5 represents the amino acid residue H or R;
6 represents an amino acid residue selected from the group consisting of A, Y, H, and N; and
7 represents the amino acid residue A or T.

6. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPLHLAA56GH7EIVEVLLK8GADVNA (SEQ ID NO:5)
wherein
1 represents an amino acid residue selected from the group consisting of A, N, R, V, Y, E, H, I, K, L, Q, S and T;
2 represents an amino acid residue selected from the group consisting of S, A, N, R, D, F, L, P, T and Y;

3 represents an amino acid residue selected from the group consisting of T, V, S, A, L and F;
4 represents an amino acid residue selected from the group consisting of W, F and H;
5 represents an amino acid residue selected from the group consisting of P, I, A, L, S, T, V and Y;
6 represents an amino acid residue selected from the group consisting of W, F, I, L, T and V;
7 represents the amino acid residue L or P; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

7. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23GWTPLHLAA45GHLEIVEVLLK6GADVNA (SEQ ID NO:7)
wherein
1 represents an amino acid residue selected from the group consisting of K, M, N, R and V;
2 represents an amino acid residue selected from the group consisting of Y, H, M and V;
3 represents an amino acid residue selected from the group consisting of F, L, M and V;
4 represents an amino acid residue selected from the group consisting of R, H, V, A, K and N;
5 represents an amino acid residue selected from the group consisting of F, D, H, T, Y, M and K; and
6 represents an amino acid residue selected from the group consisting of A, H, N and Y.

8. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1DFK2DTPLHLAA34GH5EIVEVLLK6GADVNA (SEQ ID NO:10)
wherein
1 represents an amino acid residue selected from the group consisting of L, S and T;
2 represents an amino acid residue selected from the group consisting of G, S and C;
3 represents the amino acid residue S or A;
4 represents an amino acid residue selected from the group consisting of Q, S, M and N;
5 represents an amino acid residue selected from the group consisting of L, M and Q; and
6 represents an amino acid residue selected from the group consisting of A, H, N, Y and D.

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9. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif
1D23G4TPL5LAA67GHLEIVEVLLK8GADVNA (SEQ ID NO:12)
wherein
1 represents an amino acid residue selected from the group consisting of K, S, I, N, T and V;
2 represents an amino acid residue selected from the group consisting of K, N, W, A, H, M, Q and S;
3 represents an amino acid residue selected from the group consisting of F, Q, L, H and V;
4 represents the amino acid residue F or T;
5 represents the amino acid residue Q or H;
6 represents the amino acid residue Y or S;
7 represents an amino acid residue selected from the group consisting of N, H, Y and M; and
8 represents an amino acid residue selected from the group consisting of A, H, N and Y.

10. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:6, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:5.

11. The binding protein of claim 4, wherein said ankyrin repeat domain comprises a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:3, wherein said repeat module is preceded by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:2 and/or followed by a repeat module with the ankyrin repeat sequence motif of SEQ ID NO:4.

12. The binding protein of claim 4, wherein said repeat domain competes for binding to VEGF-Axxx with an ankyrin repeat domain selected from the group consisting of SEQ ID NOs:16, 22, 23, 29, 30, 33, 34, 36, 39 and 40.

13. The binding protein of claim 4, wherein said ankyrin repeat domain is selected from the group consisting of SEQ ID NOs:14 to 40.

Regeneron Exhibit 1002.0084

14. The binding protein of claim 4, wherein said ankyrin repeat domain comprises an amino acid sequence that has at least 75% amino acid sequence identity with one ankyrin repeat domain selected from the group consisting of SEQ ID NOs:14 to 40.

15. The binding protein of claim 4, wherein one or more of the amino acid residues of the repeat modules of said repeat domain are exchanged by an amino acid residue found at the corresponding position on alignment of a repeat unit.

16. A binding protein of any one of claims 1 to 15 additionally comprising a non-proteinaceous polymer moiety.

17. A nucleic acid encoding a binding protein according to any one of claims 1 to 15.

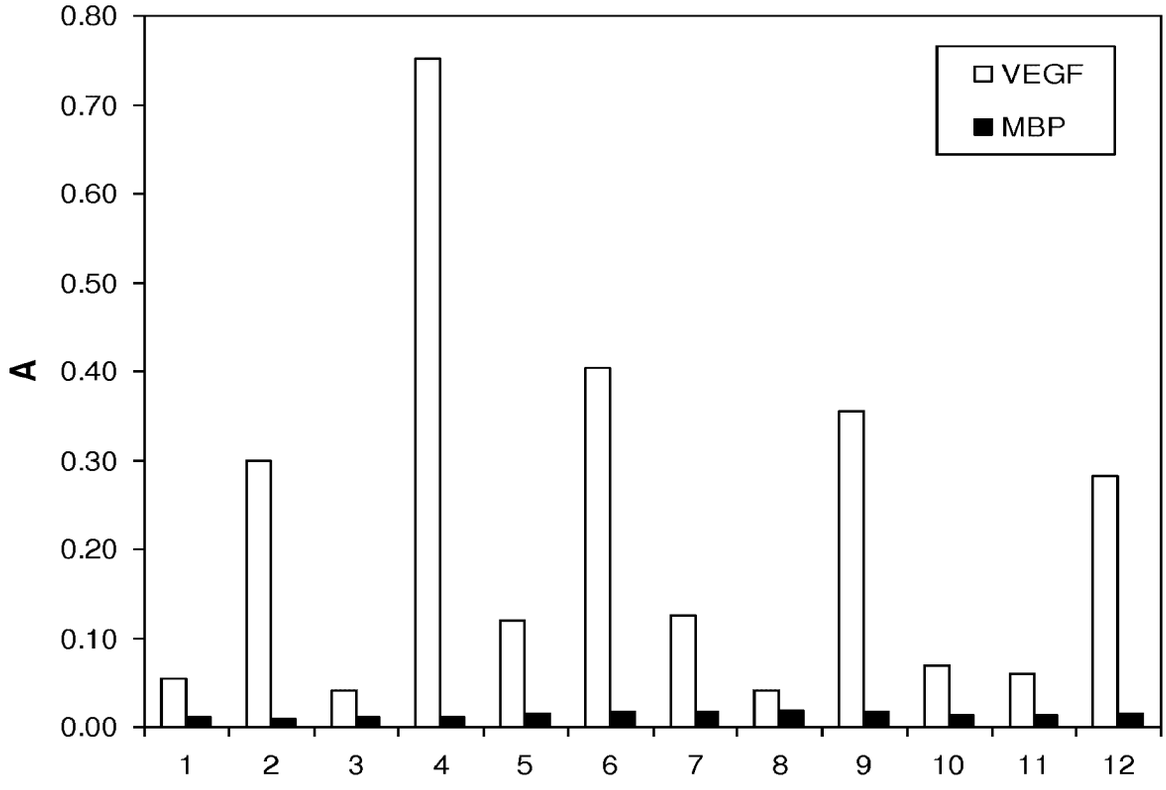
18. A pharmaceutical composition comprising the binding protein of any one of claims 1 to 16 or the nucleic acid of claim 17, and optionally a pharmaceutical acceptable carrier and/or diluent.

19. A pharmaceutical composition according to claim 18 for the treatment of an eye disorder.

20. A method of treating pathological angiogenesis in a mammal including man, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1 to 17.

Regeneron Exhibit 1002.0085

Fig. 1



Regeneron Exhibit 1002.0086

Fig. 2a

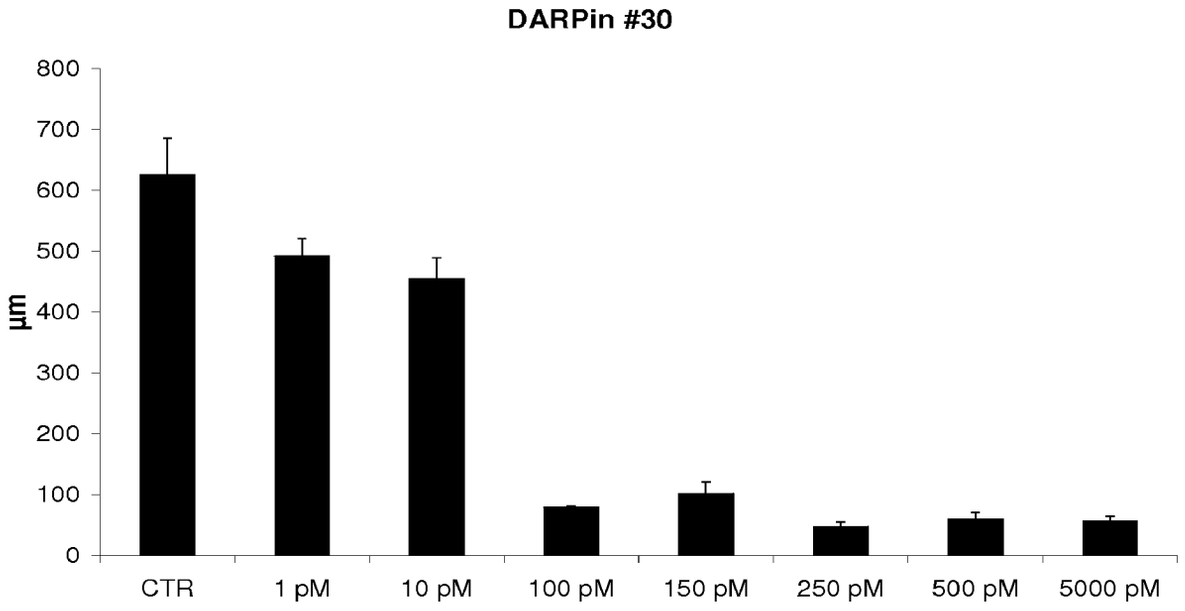
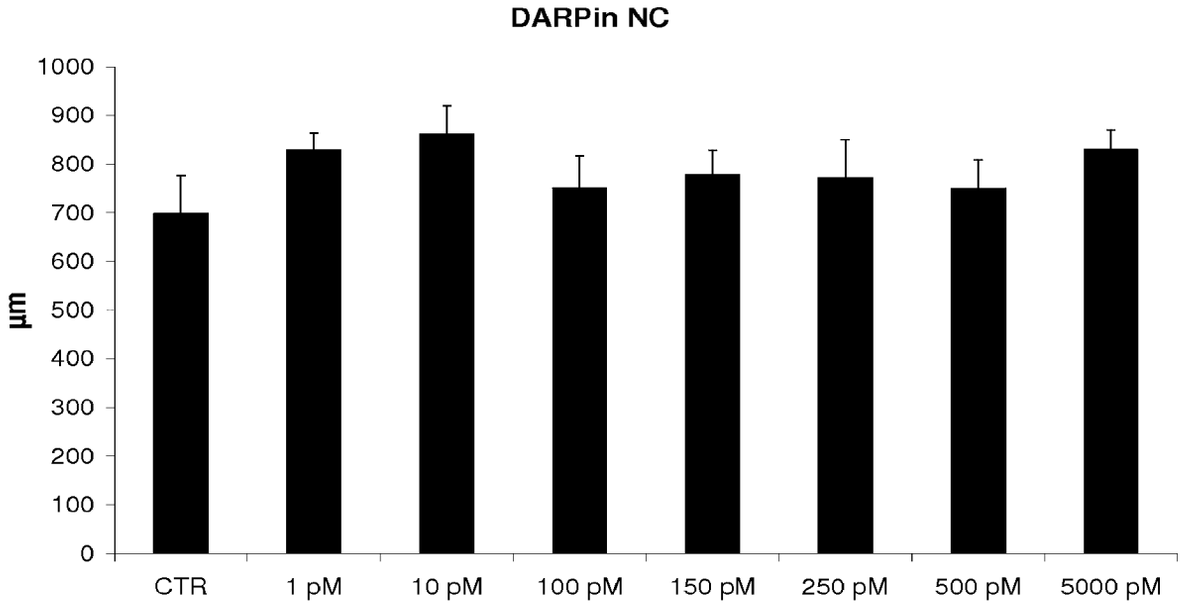


Fig. 2b



Regeneron Exhibit 1002.0087

Fig. 3a

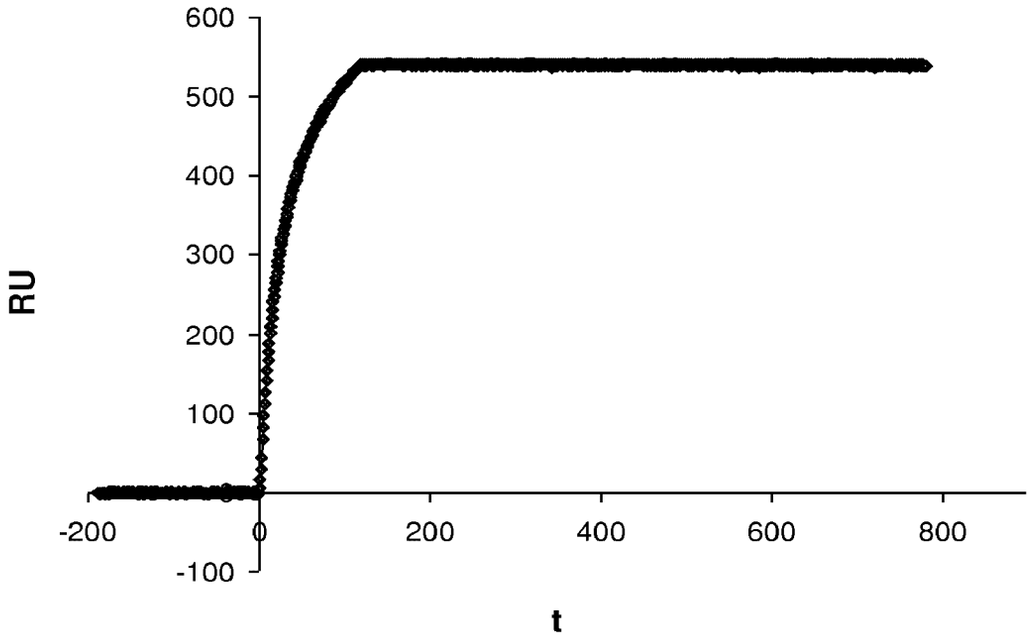

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Fig. 3b

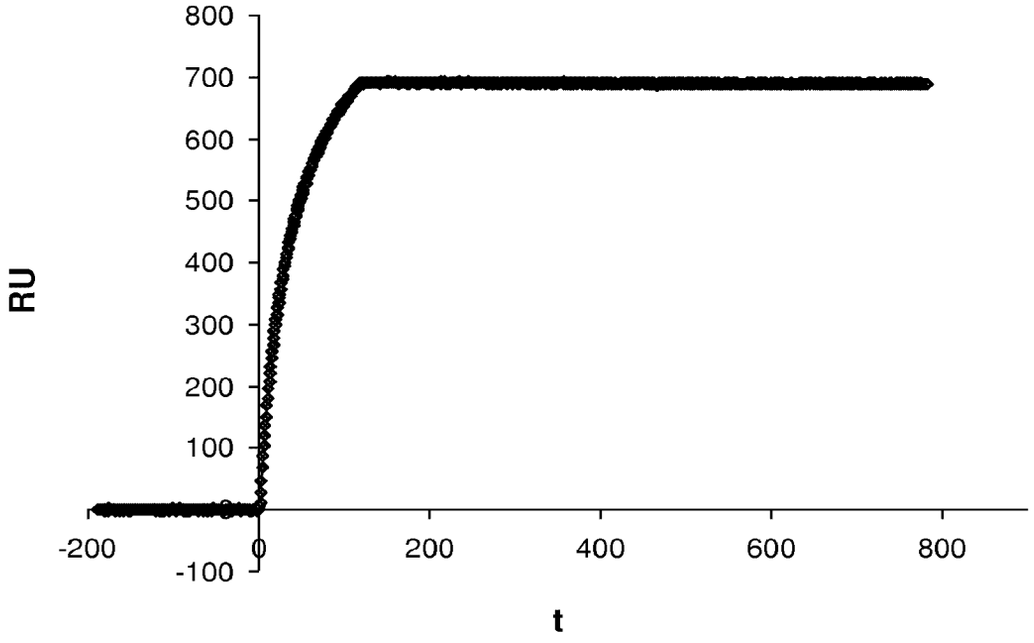
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Fig. 3c

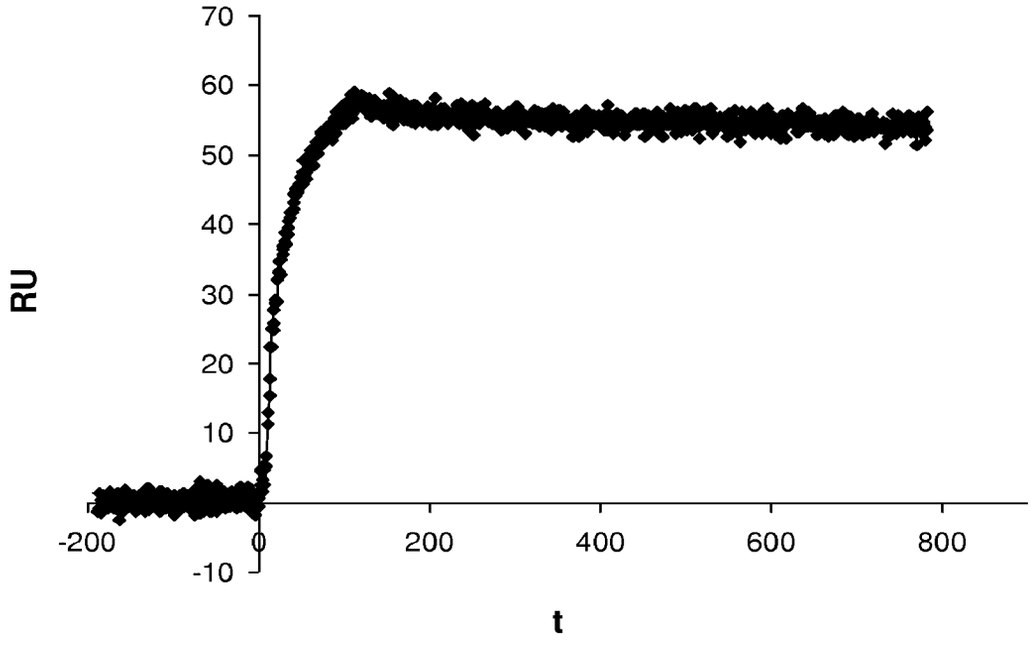
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Fig. 3d

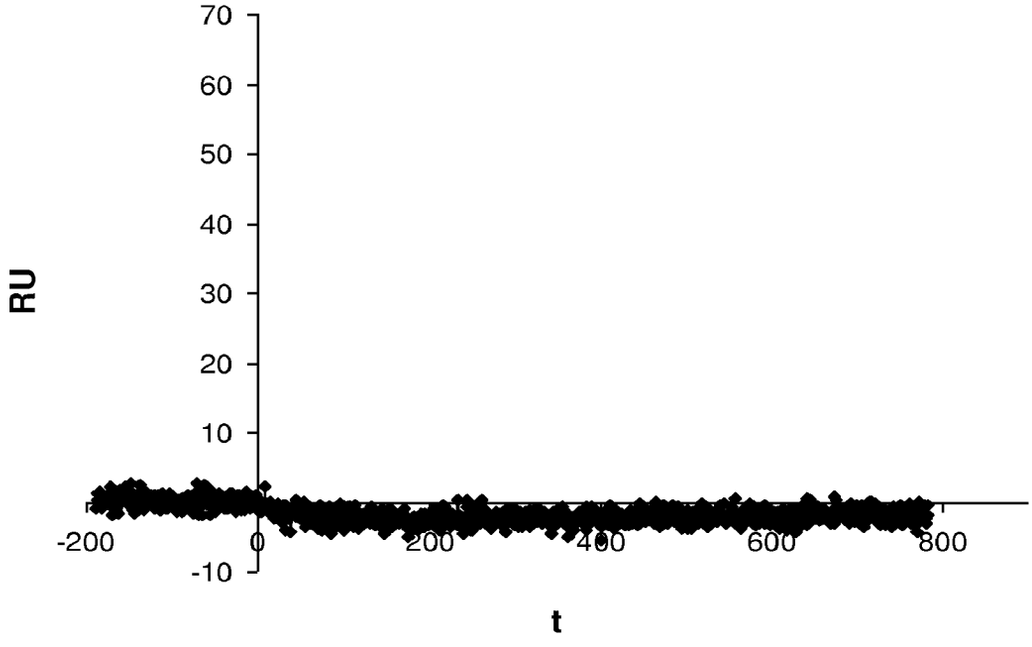
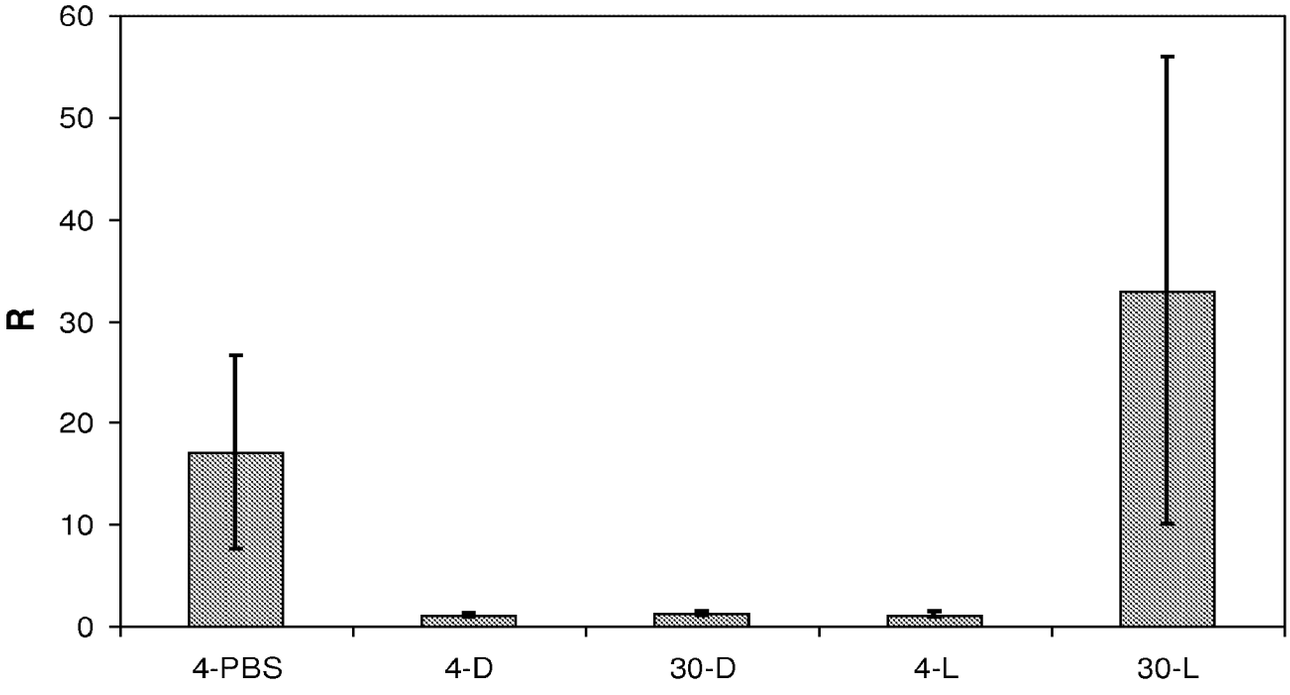

70
60
50
40
30
20
10
-10
RU
-200
0
200
400
600
800
t

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/064483

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INOKI ISAO ET AL: "Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis." THE FASEB JOURNAL : OFFICIAL PUBLICATION OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY FEB 2002 LNKD-PUBMED:11744618, vol. 16, no. 2, February 2002 (2002-02), pages 219-221, XP002577430 ISSN: 1530-6860	1
Y	the whole document	2-20
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2010	06/05/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Luo, Xinmei

Form PCT/ISA/210 (second sheet) (April 2005)

Regeneron Exhibit 1002.0091

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/064483

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STUMPP M T ET AL: "DARPins: A new generation of protein therapeutics" DRUG DISCOVERY TODAY, ELSEVIER, RAHWAY, NJ, US LNKD- DOI:10.1016/J.DRUDIS.2008.04.013, vol. 13, no. 15-16, 1 August 2008 (2008-08-01), pages 695-701, XP023440383 ISSN: 1359-6446 [retrieved on 2008-07-11] the whole document	2-20
A	FORRER P ET AL: "Consensus design of repeat proteins" CHEMBIOCHEM - A EUROPEAN JOURNAL OF CHEMICAL BIOLOGY, WILEY VCH, WEINHEIM, DE LNKD- DOI:10.1002/CBIC.200300762, vol. 5, no. 2, 6 February 2004 (2004-02-06), pages 183-189, XP002382372 ISSN: 1439-4227	1-20
A	WO 02/20565 A (UNIV ZUERICH [CH]; STUMPP MICHAEL TOBIAS [CH]; FORRER PATRICK [CH]; BI) 14 March 2002 (2002-03-14) the whole document	1-20
A	BINZ H K ET AL: "Designing Repeat Proteins: Well-expressed, Soluble and Stable Proteins from Combinatorial Libraries of Consensus Ankyrin Repeat Proteins" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 332, no. 2, 12 September 2003 (2003-09-12), pages 489-503, XP004450112 ISSN: 0022-2836	1-20
A	BINZ H KASPAR ET AL: "High-affinity binders selected from designed ankyrin repeat protein libraries" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 22, no. 5, 1 May 2004 (2004-05-01), pages 575-582, XP002343919 ISSN: 1087-0156	1-20
A	WO 2005/056764 A (COMPOUND THERAPEUTICS INC [US]; CHEN YAN; GETMANOVA ELENA; WRIGHT MART) 23 June 2005 (2005-06-23)	1-20
A	WO 2008/066752 A2 (ADNEXUS A BRISTOL MEYERS SQUIB [US]; CAMPHAUSEN RAY [US]; FABRIZIO DAV) 5 June 2008 (2008-06-05)	1-20

Regeneron Exhibit 1002.0092

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2009/064483

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Regeneron Exhibit 1002.0093

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/EP2009/064483

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0220565 A	14-03-2002	AT 448301 T	15-11-2009
		AU 1816602 A	22-03-2002
		CA 2421447 A1	14-03-2002
		EP 1332209 A2	06-08-2003
		EP 2149604 A1	03-02-2010
		ES 2335861 T3	06-04-2010
		JP 2004508033 T	18-03-2004
		US 2009082274 A1	26-03-2009
		US 2004132028 A1	08-07-2004
WO 2005056764 A	23-06-2005	AU 2004296376 A1	23-06-2005
		BR PI0417302 A	06-03-2007
		CA 2552435 A1	23-06-2005
		CN 1946417 A	11-04-2007
		EP 1711196 A2	18-10-2006
		JP 2007516707 T	28-06-2007
		KR 20060129246 A	15-12-2006
		SG 149004 A1	29-01-2009
WO 2008066752 A2	05-06-2008	AU 2007325838 A1	05-06-2008
		CA 2670471 A1	05-06-2008
		EP 2121743 A2	25-11-2009

Regeneron Exhibit 1002.0094

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 November 2011 (03.11.2011)

PCT

(10) International Publication Number
WO 2011/135067 A1

(51) **International Patent Classification**:
A61K 47/48 (2006.01) ***A61P 35/00*** (2006.01)
A61P 27/02 (2006.01)

(21) **International Application Number**:
PCT/EP2011/056824

(22) **International Filing Date**:
29 April 2011 (29.04.2011)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
10161685.2 30 April 2010 (30.04.2010) EP

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(74) **Agent**: **BECKER, Konrad**; Aeschenvorstadt 24, Postfach 318, CH-4010 Basel (CH).

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

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2011/135067 A1

(54) **Title**: MODIFIED BINDING PROTEINS INHIBITING THE VEGF-A RECEPTOR INTERACTION

(57) **Abstract**: The present invention relates to binding proteins specific for VEGF-A, in particular to recombinant binding proteins comprising a polyethylene glycol moiety and a binding domain, which inhibits VEGF-Axxx binding to VEGFR-2. Examples of such recombinant binding proteins are proteins which comprise an ankyrin repeat domain with the desired binding specificity, and a polyethylene glycol moiety. The binding proteins are useful in the treatment of cancer and other pathological conditions, e.g. eye diseases such as age-related macular degeneration.

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Modified binding proteins inhibiting the VEGF-A receptor interaction

Field of the invention

The present invention relates to modified recombinant binding proteins specific for VEGF-A, as well as pharmaceutical compositions comprising such proteins, and the use of such proteins in the treatment of tumors and eye diseases.

Background of the invention

Angiogenesis, the growth of new blood vessels from pre-existing vasculature, is a key process in several pathological conditions, including tumor growth and eye diseases, in particular ocular neovascularization diseases such as age-related macular degeneration (AMD) or diabetic macular edema (DME) (Carmeliet, P., Nature *438*, 932–936, 2005). Vascular endothelial growth factors (VEGFs) stimulate angiogenesis and lymphangiogenesis by activating VEGF receptor (VEGFR) tyrosine kinases in endothelial cells (Ferrara, N., Gerber, H. P. and LeCouter, J., Nature Med. *9*, 669–676, 2003).

The mammalian VEGF family consists of five glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D (also known as FIGF) and placenta growth factor (PlGF, also known as PGF). VEGF-A has been shown to be an effective target for anti-angiogenic therapy (Ellis, L. M. and Hicklin, D. J., Nature Rev. Cancer *8*, 579-591, 2008). The VEGF-A ligands bind to and activate three structurally similar type III receptor tyrosine kinases, designated VEGFR-1 (also known as FLT1), VEGFR-2 (also known as KDR) and VEGFR-3 (also known as FLT4). The VEGF ligands have distinctive binding specificities for each of these tyrosine kinase receptors, which contribute to their diversity of function. In response to ligand binding, the VEGFR tyrosine kinases activate a network of distinct downstream signaling pathways. VEGFR-1 and VEGFR-2 are primarily found on the vascular endothelium whereas VEGFR-3 is mostly found on the lymphatic endothelium. These receptors all have an extracellular domain, a single transmembrane region and a consensus tyrosine kinase sequence interrupted by a kinase-insert domain. More recently neuropilin (NRP-1), originally identified as a receptor for the semaphorin / collapsin family of neuronal guidance mediators, was shown to act as an isoform specific receptor for VEGF-A.

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Various isoforms of VEGF-A are known that are generated by alternative splicing from eight exons within the *VEGF-A* gene. All isoforms contain exons 1–5 and the terminal exon, exon 8. Exons 6 and 7, which encode heparin-binding domains, can be included or excluded. This gives rise to a family of proteins termed according to their amino acid number: VEGF-A165, VEGF-A121, VEGF-A189, and so on. Exon 8, however, contains two 3' splice sites in the nucleotide sequences, which can be used by the cell to generate two families of isoforms with identical length, but differing C-terminal amino acid sequences (Varey, A.H.R. et al., British J. Cancer *98*, 1366-1379, 2008). VEGF-Axxx ("xxx" denotes the amino acid number of the mature protein), the pro-angiogenic family of isoforms, is generated by use of the most proximal sequence in exon 8 (resulting in the inclusion of exon 8a). The more recently described anti-angiogenic VEGF-Axxxb isoforms are generated by the use of a distal splice site, 66 bp further along the gene from the proximal splice site. This results in splicing out of exon 8a and the production of mRNA sequences that encode the VEGF-Axxxb family. VEGF-A165 is the predominant pro-angiogenic isoform and is commonly overexpressed in a variety of human solid tumors. VEGF-A165b was the first of the exon 8b-encoded isoforms identified and was shown to have anti-angiogenic effects (Varey et al., loc. cit.; Konopatskaya, O. et al., Molecular Vision *12*, 626-632, 2006). It is an endogenous inhibitory form of VEGF-A, which decreases VEGF-A induced proliferation and migration of endothelial cells. Although it can bind to VEGFR-2, VEGF-A165b binding does not result in receptor phosphorylation or activation of the downstream signaling pathways.

There are several approaches to inhibiting VEGF-A signaling, including neutralization of the ligand or receptor by antibodies, and blocking VEGF-A receptor activation and signaling with tyrosine kinase inhibitors. VEGF-A targeted therapy has been shown to be efficacious as a single agent in AMD, DME, renal cell carcinoma and hepatocellular carcinoma, whereas it is only of benefit when combined with chemotherapy for patients with metastatic colorectal, non-small-cell lung and metastatic breast cancer (Narayanan, R. et al., Nat Rev. Drug Discov. *5*, 815-816, 2005; Ellis and Hicklin, loc. cit).

Beside antibodies other binding domains can be used to neutralize a ligand or a receptor (Skerra, A., J. Mol. Recog. *13*, 167-187, 2000; Binz, H. K., Amstutz, P. and Plückthun, A., Nat. Biotechnol. *23*, 1257-1268, 2005). One such novel class of binding domains are based on designed repeat domains (WO 02/20565; Binz, H. K., Amstutz, P., Kohl, A., Stumpp, M. T., Briand, C., Forrer, P., Grütter, M. G., and Plückthun, A., Nat. Biotechnol. *22*, 575-582, 2004). WO 02/20565 describes how large libraries of repeat proteins can be

constructed and their general application. Nevertheless, WO 02/20565 does neither disclose the selection of repeat domains with binding specificity for VEGF-Axxx nor concrete repeat sequence motifs of repeat domains that specifically bind to VEGF-Axxx.

Targeting VEGF-A with currently available therapeutics is not effective in all patients, or for all diseases (e.g., EGFR-expressing cancers). It has even become increasingly apparent that the therapeutic benefit associated with VEGF-A targeted therapy is complex and probably involves multiple mechanisms (Ellis and Hicklin, loc. cit.). For example, marketed anti-VEGF drugs, such as bevacizumab (Avastin®) or ranibizumab (Lucentis®) (see WO 96/030046, WO 98/045331 and WO 98/045332) or drugs in clinical development, such as VEGF-Trap® (WO 00/075319) do not distinguish between the pro- and anti-angiogenic forms of VEGF-A, so they do inhibit both. As a result, they inhibit angiogenesis, but also deprive healthy tissues of an essential survival factor, namely VEGF-Axxxb, resulting in cytotoxicity and dose-limiting side effects, which in turn limit efficacy. Side effects common to current anti-VEGF-A therapies are gastrointestinal perforations, bleeding, hypertension, thromboembolic events and proteinuria (Kamba, T. and McDonald, D.M., Br. J. Cancer *96*, 1788-95, 2007). Another marketed anti-VEGF drug for the treatment of AMD is pegaptanib (WO 98/018480; Macugen®, a registered trademark of Pfizer). Pegaptanib is a PEGylated anti-VEGF aptamer, a single strand of nucleic acid that binds with specificity to the target protein. For the treatment of neovascular AMD there is ample evidence that vision outcomes with Lucentis® are superior to those with Macugen®, and there is no definitive evidence to suggest a difference in safety between the drugs. As a result, Macugen® is not a commonly used therapy for this disease.

Overall, a need exists for improved anti-angiogenic agents for treating cancer and other pathological conditions.

The technical problem underlying the present invention is to identify novel anti-angiogenic agents, such as repeat domains with binding specificity to VEGF-Axxx, for an improved treatment of cancer and other pathological conditions, e.g. eye diseases such as AMD or DME. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Summary of the invention

The present invention relates to a recombinant binding protein comprising an ankyrin repeat domain and a polyethylene glycol moiety of at least 5 kDa molecular weight, wherein said ankyrin domain binds VEGF-Axxx with a Kd below 10^{-9}M and inhibits VEGF-Axxx binding to VEGFR-2.

In a preferred embodiment, the polyethylene glycol moiety is coupled to a single Cys residue of the binding domain.

The invention further relates to a pharmaceutical composition comprising one or more of the above mentioned binding proteins or nucleic acid molecules.

The invention further relates to a method of treatment of cancer and other pathological conditions, e.g. eye diseases such as AMD or DME, using the binding proteins of the invention.

Brief Description of the Figures

Figure 1. *Specific dog VEGF-A164 binding of selected designed ankyrin repeat proteins.*
The interaction of selected clones with dog VEGF-A164 (VEGF) and a negative control protein (MBP, *Escherichia coli* maltose binding protein) is shown by crude extract ELISA. The biotinylated dog VEGF-A164 and MBP were immobilized over NeutrAvidin. The numbers refer to single DARPin clones selected in ribosome display against dog VEGF-A164 or the corresponding human VEGF-A165.
A = Absorbance. White bars indicate binding to dog VEGF-A164, black bars show non-specific background binding to MBP.

Figure 2. *Spheroid outgrowth inhibition by a selected DARPin.*
The length of sprouts in a spheroid outgrowth inhibition assay are shown in presence of various concentrations of (a) DARPin #30 (amino acids 1 to 126 of SEQ ID NO:4), a DARPin with specificity to VEGF-Axxx, or (b) DARPin NC, a negative control DARPin with no specificity for VEGF-Axxx.

Figure 3. *Specific recognition of VEGF-A isoforms.*
Surface Plasmon Resonance (SPR) analysis of binding proteins on VEGF-A isoforms.

(a) and (b): SPR analysis of Avastin®. 250 nM of Avastin® was applied to a flow cell with immobilized dog VEGF-A164 (a) or dog VEGF-A164b (b) for 100 seconds, followed by washing with buffer flow.
(c) and (d): SPR analysis of DARPin #27 (amino acids 1 to 159 of SEQ ID NO:1). 250 nM of DARPin #27 was applied to a flow cell with immobilized dog VEGF-A164 (c) or dog VEGF-A164b (d) for 100 seconds, followed by washing with buffer flow.
RU = Resonance Units.

Figure 4. *Efficient inhibition of human VEGF-A165 in the rabbit eye.*
Vascular leakage rabbit model to show the efficacy of a DARPin in inhibiting human VEGF-A165 in the eye in comparison to Lucentis®. At day 1 either PBS, DARPin #30 or Lucentis® is applied by an intravitreal injection into one eye of each rabbit (treated eye). At day 4 or day 30 both eyes of each rabbit were challenged by intravitreal injection of 500 ng of human VEGF-A165. All eyes were evaluated 48 hours after the VEGF-A165 injection by measuring the fluorescein content in the vitreous and retina of all eyes one hour after intravenous injection of sodium fluorescein.
R = ratio of fluorescein measurements treated eye / untreated eye. Standard deviations are shown by an error bar. 4-PBS = ratio 4 days after injection of PBS (control); 4-D = ratio 4 days after injection of DARPin #30; 30-D = ratio 30 days after injection of DARPin #30; 4-L = ratio 4 days after injection of Lucentis®; 30-L = ratio 30 days after injection of Lucentis®.

Detailed description of the invention

Mammalian VEGF-A exists as two families of alternative spliced isoforms: (i) the pro-angiogenic "VEGF-Axxx" isoforms generated by proximal splicing of exon 8 and (ii) the anti-angiogenic "VEGF-Axxxb" isoforms generated by distal splicing of exon 8. Preferably, the binding domain according to the invention is specific for the pro-angiogenic VEGF-Axxx of dog, rabbit, monkey or human origin. More preferably, the binding domain according to the invention is specific for the pro-angiogenic VEGF-Axxx of human origin. Most preferred, the binding domain according to the invention is specific for human VEGF-A165.

The term "protein" refers to a polypeptide, wherein at least part of the polypeptide has, or is able to acquire a defined three-dimensional arrangement by forming secondary, tertiary, or quaternary structures within and/or between its polypeptide chain(s). If a protein

comprises two or more polypeptides, the individual polypeptide chains may be linked non-covalently or covalently, e.g. by a disulfide bond between two polypeptides. A part of a protein, which individually has, or is able to acquire a defined three-dimensional arrangement by forming secondary or tertiary structures, is termed "protein domain". Such protein domains are well known to the practitioner skilled in the art.

The term “recombinant” as used in recombinant protein, recombinant protein domain and the like, means that said polypeptides are produced by the use of recombinant DNA technologies well known by the practitioner skilled in the relevant art. For example, a recombinant DNA molecule (e.g. produced by gene synthesis) encoding a polypeptide can be cloned into a bacterial expression plasmid (e.g. pQE30, Qiagen). When such a constructed recombinant expression plasmid is inserted into a bacteria (e.g. *E. coli*), this bacteria can produce the polypeptide encoded by this recombinant DNA. The correspondingly produced polypeptide is called a recombinant polypeptide.

The term "polypeptide tag" refers to an amino acid sequence attached to a polypeptide/protein, wherein said amino acid sequence is useful for the purification, detection, or targeting of said polypeptide/protein, or wherein said amino acid sequence improves the physicochemical behavior of the polypeptide/protein, or wherein said amino acid sequence possesses an effector function. The individual polypeptide tags, moieties and/or domains of a binding protein may be connected to each other directly or via polypeptide linkers. These polypeptide tags are all well known in the art and are fully available to the person skilled in the art. Examples of polypeptide tags are small polypeptide sequences, for example, His, myc, FLAG, or Strep-tags or moieties such as enzymes (for example enzymes like alkaline phosphatase), which allow the detection of said polypeptide/protein, or moieties which can be used for targeting (such as immunoglobulins or fragments thereof) and/or as effector molecules.

The term "polypeptide linker" refers to an amino acid sequence, which is able to link, for example, two protein domains, a polypeptide tag and a protein domain, a protein domain and a non-polypeptide moiety such as polyethylene glycol or two sequence tags. Such additional domains, tags, non-polypeptide moieties and linkers are known to the person skilled in the relevant art. A list of example is provided in the description of the patent application WO 02/20565. Particular examples of such linkers are glycine-serine-linkers and proline-threonine-linkers of variable lengths; preferably, said linkers have a length

between 2 and 24 amino acids; more preferably, said linkers have a length between 2 and 16 amino acids.

In the context of the present invention, the term "polypeptide" relates to a molecule consisting of one or more chains of multiple, i.e. two or more, amino acids linked via peptide bonds. Preferably, a polypeptide consists of more than eight amino acids linked via peptide bonds.

The term “polymer moiety” refers to either a proteinaceous polymer moiety or a non-proteinaceous polymer moiety. A “proteinaceous polymer moiety” preferably is a polypeptide that does not form a stable tertiary structure while not forming more than 10% (preferably, not more than 5%; also preferred, not more than 2%; even more preferably, not more than 1%; and most preferably, no detectable amounts, as determined by size exclusion chromatography (SEC)) of oligomers or aggregates when stored at a concentration of about 0.1 mM in PBS at RT for one month. Such proteinaceous polymer moieties run at an apparent molecular weight in SEC that is higher than their effective molecular weight when using globular proteins as molecular weight standards for the SEC. Preferably, the apparent molecular weight of said proteinaceous polymer moieties determined by SEC is 1.5x, 2x or 2.5x higher than their effective molecular weight calculated from their amino acid sequence. Also preferably, the apparent molecular weights of said non-proteinaceous polymer moieties determined by SEC is 2x, 4x or 8x higher than their effective molecular weight calculated from their molecular composition. Preferably, more than 50%, 70% or even 90% of the amino acids of said proteinaceous polymer moiety do not form stable secondary structures at a concentration of about 0.1 mM in PBS at RT as determined by Circular Dichroism (CD) measurements. Most preferably, said proteinaceous polymer shows a typical near UV CD-spectra of a random coil conformation. Such CD analyses are well known to the person skilled in the art. Also preferable are proteinaceous polymer moieties that consist of more than 50, 100, 200, 300, 400, 500, 600, 700 or 800 amino acids. Examples of proteinaceous polymer moieties are XTEN® (a registered trademark of Amunix; WO 07/103515) polypeptides, or polypeptides comprising proline, alanine and serine residues as described in WO 08/155134. Such proteinaceous polymer moieties can be covalently attached to, for example, a binding domain of the invention by the generation of genetic fusion polypeptides using standard DNA cloning technologies, followed by their standard expression and purification. Examples of binding proteins comprising a repeat domain binding VEGF-Axxx and such a proteinaceous polymer moiety are shown in SEQ ID NO:1

and SEQ ID NO:4. The amino acid positions from 1 to 159 of SEQ ID NO:1 correspond to the repeat domain and the amino acid position 161 to 1'025 of SEQ ID NO:1 correspond to the proteinaceous polymer moiety. The amino acid positions from 1 to 126 of SEQ ID NO:4 correspond to the repeat domain and the amino acid positions 131 to 640 of SEQ ID NO:4 correspond to the proteinaceous polymer moiety.

A polymer moiety of the invention may vary widely in molecular weight (i.e. from about 1 kDa to about 150 kDa). Preferably, the polymer moiety has a molecular weight of at least 2, 5, 10, 20, 30, 50, 70 or 100 kDa.

Preferably, said polymer moiety is connected by a polypeptide linker to a binding domain. Examples of such polypeptide linkers are the amino acids 1 to 8 of SEQ ID NO:8 and SEQ ID NO:9.

Examples of non-proteinaceous polymer moieties are hydroxyethyl starch (HES), polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylene. The term "PEGylated" means that a PEG moiety is covalently attached to, for example, a polypeptide of the invention. Examples of repeat proteins containing a polypeptide linker between the repeat domain and a C-terminal Cys residue useful for binding a non-proteinaceous polymer moiety are SEQ ID NO:2, 3, 5, 6 and 7.

In a specific embodiment, a PEG moiety or any other non-proteinaceous polymer can, e.g., be coupled to a cysteine thiol via a maleimide linker with the cysteine being coupled via a peptide linker to the N- or C-terminus of a binding domain as described herein (e.g. SEQ ID NO:3).

The term "binding protein" refers to a protein comprising one or more binding domains and one or more polymer moieties as further explained below. Preferably, said binding protein comprises up to four binding domains. More preferably, said binding protein comprises up to two binding domains. Most preferably, said binding protein comprises only one binding domain. Furthermore, any such binding protein may comprise additional protein domains that are not binding domains, multimerization moieties, polypeptide tags, polypeptide linkers and/or a single Cys residue. Examples of multimerization moieties are immunoglobulin heavy chain constant regions which pair to provide functional immunoglobulin Fc domains, and leucine zippers or polypeptides comprising a free thiol which forms an intermolecular disulfide bond between two such polypeptides. The single

Cys residue may be used for conjugating other moieties to the polypeptide, for example, by using the maleimide chemistry well known to the person skilled in the art.

Preferably, said binding protein comprises up to four polymer moieties. More preferably, said binding protein comprises up to two polymer moieties. Most preferably, said binding protein comprises only one polymer moiety.

Also preferably, said binding protein has an apparent molecular weight of at least 70, 100, 200, 300, 500 or 800 kDa when analyzed at a concentration of 0.1 mM in PBS at RT by SEC using globular proteins as molecular weight standards.

The term “binding domain” means a protein domain exhibiting the same "fold" (three-dimensional arrangement) as a protein scaffold and having a predetermined property, as defined below. Such a binding domain may be obtained by rational, or most commonly, combinatorial protein engineering techniques, skills which are known in the art (Skerra, 2000, loc. cit.; Binz et al., 2005, loc. cit.). For example, a binding domain having a predetermined property can be obtained by a method comprising the steps of (a) providing a diverse collection of protein domains exhibiting the same fold as a protein scaffold as defined further below; and (b) screening said diverse collection and/or selecting from said diverse collection to obtain at least one protein domain having said predetermined property. The diverse collection of protein domains may be provided by several methods in accordance with the screening and/or selection system being used, and may comprise the use of methods well known to the person skilled in the art, such as phage display or ribosome display.

The term "protein scaffold" means a protein with exposed surface areas in which amino acid insertions, substitutions or deletions are highly tolerable. Examples of protein scaffolds that can be used to generate binding domains of the present invention are antibodies or fragments thereof such as single-chain Fv or Fab fragments, protein A from *Staphylococcus aureus*, the bilin binding protein from *Pieris brassicae* or other lipocalins, ankyrin repeat proteins or other repeat proteins, and human fibronectin. Protein scaffolds are known to the person skilled in the art (Binz et al., 2005, loc. cit.; Binz et al., 2004, loc. cit.).

The term "predetermined property" refers to a property such as binding to a target, blocking of a target, activation of a target-mediated reaction, enzymatic activity, and

related further properties. Depending on the type of desired property, one of ordinary skill will be able to identify format and necessary steps for performing screening and/or selection of a binding domain with the desired property. Preferably, said predetermined property is binding to a target.

Preferably, the binding protein of the invention is not an antibody or a fragment thereof, such as Fab or scFv fragments. Antibodies and fragments thereof are well known to the person skilled in the art.

Also preferably, the binding domain of the invention does not comprise an immunoglobulin fold as present in antibodies and/or the fibronectin type III domain. An immunoglobulin fold is a common all-β protein fold that consists of a 2-layer sandwich of about 7 anti-parallel β-strands arranged in two β-sheets. Immunoglobulin folds are well known to the person skilled in the art. For example, such binding domains comprising an immunoglobulin fold are described in WO 07/080392 or WO 08/097497.

Further preferably, the binding domain of the invention does not comprise an immunoglobulin-like domain as found in VEGFR-1 or VEGFR-2. Such binding domains are described in WO 00/075319.

A preferred binding domain is a binding domain having anti-angiogenic effects. The anti-angiogenic effect of a binding domain can be determined by assays well know to the person skilled in the art, such as the sprouting assay of HUVEC spheroids described in Example 2.

Further preferred is a binding domain comprising between 70 and 300 amino acids, in particular between 100 and 200 amino acids.

Further preferred is a binding domain devoid of a free Cys residue. A free Cys residue is not involved in the formation of a disulfide bond. Even more preferred is a binding domain free of any Cys residue.

A preferred binding domain of the invention is a repeat domain or a designed repeat domain, preferably as described in WO 02/20565.

A particularly preferred binding domain is a designed ankyrin repeat domain (Binz, H. K. et al., 2004, loc. cit.), preferably as described in WO 02/20565. Examples of designed ankyrin repeat domains are shown in the Examples.

The definitions hereinafter for repeat proteins are based on those in patent application WO 02/20565. Patent application WO 02/20565 further contains a general description of repeat protein features, techniques and applications.

The term "repeat proteins" refers to a protein comprising one or more repeat domains. Preferably, each of said repeat proteins comprises up to four repeat domains. More preferably, each of said repeat proteins comprises up to two repeat domains. Most preferably, each of the repeat proteins comprises only one repeat domain. Furthermore, said repeat protein may comprise additional non-repeat protein domains, polypeptide tags and/or polypeptide linkers.

The term "repeat domain" refers to a protein domain comprising two or more consecutive repeat units (modules) as structural units, wherein said structural units have the same fold, and stack tightly to create, for example, a superhelical structure having a joint hydrophobic core.

The term "designed repeat protein" and "designed repeat domain" refer to a repeat protein or repeat domain, respectively, obtained as the result of the inventive procedure explained in patent application WO 02/20565. Designed repeat proteins and designed repeat domains are synthetic and not from nature. They are man-made proteins or domains, respectively, obtained by expression of correspondingly designed nucleic acids. Preferably, the expression is done in eukaryotic or prokaryotic cells, such as bacterial cells, or by using a cell-free *in vitro* expression system.

The term "structural unit" refers to a locally ordered part of a polypeptide, formed by three-dimensional interactions between two or more segments of secondary structure that are near one another along the polypeptide chain. Such a structural unit exhibits a structural motif. The term "structural motif" refers to a three-dimensional arrangement of secondary structure elements present in at least one structural unit. Structural motifs are well known to the person skilled in the art. Structural units alone are not able to acquire a defined three-dimensional arrangement; however, their consecutive arrangement, for example as

repeat modules in a repeat domain, leads to a mutual stabilization of neighboring units resulting in a superhelical structure.

The term "repeat unit" refers to amino acid sequences comprising repeat sequence motifs of one or more naturally occurring repeat proteins, wherein said "repeat units" are found in multiple copies, and which exhibit a defined folding topology common to all said motifs determining the fold of the protein. Examples of such repeat units are armadillo repeat units, leucine-rich repeat units, ankyrin repeat units, tetratricopeptide repeat units, HEAT repeat units, and leucine-rich variant repeat units. Naturally occurring proteins containing two or more such repeat units are referred to as "naturally occurring repeat proteins". The amino acid sequences of the individual repeat units of a repeat protein may have a significant number of mutations, substitutions, additions and/or deletions when compared to each other, while still substantially retaining the general pattern, or motif, of the repeat units.

Preferably, the repeat units used for the deduction of a repeat sequence motif are homologous repeat units obtained from repeat domains selected on a target, for example as described in Example 1 and having the same target-specificity.

The term "repeat sequence motif" refers to an amino acid sequence, which is deduced from one or more repeat units. Preferably, said repeat units are from repeat domains having binding specificity for the same target.
The term "folding topology" refers to the tertiary structure of said repeat units. The folding topology will be determined by stretches of amino acids forming at least parts of α-helices or β-sheets, or amino acid stretches forming linear polypeptides or loops, or any combination of α-helices, β-sheets and/or linear polypeptides/loops.

The term "consecutive" refers to an arrangement, wherein the repeat units or repeat modules are arranged in tandem. In designed repeat proteins, there are at least 2, usually about 2 to 6, in particular at least about 6, frequently 20 or more repeat units. In most cases, repeat units will exhibit a high degree of sequence identity (same amino acid residues at corresponding positions) or sequence similarity (amino acid residues being different, but having similar physicochemical properties), and some of the amino acid residues might be key residues being strongly conserved in the different repeat units found in naturally occurring proteins. However, a high degree of sequence variability by amino acid insertions and/or deletions, and/or substitutions between the different repeat

units found in naturally occurring proteins will be possible as long as the common folding topology is maintained.

Methods for directly determining the folding topology of repeat proteins by physico-chemical means such as X-ray crystallography, NMR or CD spectroscopy, are well known to the practitioner skilled in the art. Methods for identifying and determining repeat units or repeat sequence motifs or for identifying families of related proteins comprising such repeat units or motifs, such as homology searches (BLAST etc.), are well established in the field of bioinformatics, and are well known to the practitioner in the art. The step of refining an initial repeat sequence motif may comprise an iterative process.

The term "repeat modules" refers to the repeated amino acid sequences of the designed repeat domains, which are originally derived from the repeat units of naturally occurring repeat proteins. Each repeat module comprised in a repeat domain is derived from one or more repeat units of the family or subfamily of naturally occurring repeat proteins, e.g. the family of armadillo repeat proteins or ankyrin repeat proteins.

"Repeat modules" may comprise positions with amino acid residues present in all copies of corresponding repeat modules ("fixed positions") and positions with differing or "randomized" amino acid residues ("randomized positions").

The term “capping module” refers to a polypeptide fused to the N- or C-terminal repeat module of a repeat domain, wherein said capping module forms tight tertiary interactions with said repeat module thereby providing a cap that shields the hydrophobic core of said repeat module at the side not in contact with the consecutive repeat module from the solvent. Said N- and/or C-terminal capping module may be, or may be derived from, a capping unit or other domain found in a naturally occurring repeat protein adjacent to a repeat unit. The term “capping unit” refers to a naturally occurring folded polypeptide, wherein said polypeptide defines a particular structural unit which is N- or C-terminally fused to a repeat unit, wherein said polypeptide forms tight tertiary interactions with said repeat unit thereby providing a cap that shields the hydrophobic core of said repeat unit at one side from the solvent. Such capping units may have sequence similarities to said repeat sequence motif. Capping modules and capping repeats are described in WO 02/020565. For example, the N-terminal capping module of SEQ ID NO:2 is encoded by the amino acids from position 1 to 32. Also preferred is such an N-terminal capping module having a glycine or aspartate residue at position 5.

The term "target" refers to an individual molecule such as a nucleic acid molecule, a polypeptide or protein, a carbohydrate, or any other naturally occurring molecule, including any part of such individual molecule, or complexes of two or more of such molecules. The target may be a whole cell or a tissue sample, or it may be any non-natural molecule or moiety. Preferably, the target is a naturally occurring or non-natural polypeptide or a polypeptide containing chemical modifications, for example modified by natural or non-natural phosphorylation, acetylation, or methylation. In the particular application of the present invention, the target is VEGF-Axxx or VEGFR-2.

The term "consensus sequence" refers to an amino acid sequence, wherein said consensus sequence is obtained by structural and/or sequence aligning of multiple repeat units. Using two or more structural and/or sequence aligned repeat units, and allowing for gaps in the alignment, it is possible to determine the most frequent amino acid residue at each position. The consensus sequence is that sequence which comprises the amino acids which are most frequently represented at each position. In the event that two or more amino acids are represented above-average at a single position, the consensus sequence may include a subset of those amino acids. Said two or more repeat units may be taken from the repeat units comprised in a single repeat protein, or from two or more different repeat proteins.

Consensus sequences and methods to determine them are well known to the person skilled in the art.

A “consensus amino acid residue” is the amino acid found at a certain position in a consensus sequence. If two or more, e.g. three, four or five, amino acid residues are found with a similar probability in said two or more repeat units, the consensus amino acid may be one of the most frequently found amino acids or a combination of said two or more amino acid residues.

Further preferred are non-naturally occurring binding proteins or binding domains.

The term “non-naturally occurring” means synthetic or not from nature, more specifically, the term means made from the hand of man. The term “non-naturally occurring binding protein" or "non-naturally occurring binding domain” means that said binding protein or said binding domain is synthetic (i.e. produced by chemical synthesis from amino acids) or

recombinant and not from nature. “Non-naturally occurring binding protein" or "non-naturally occurring binding domain” is a man-made protein or domain, respectively, obtained by expression of correspondingly designed nucleic acids. Preferably, the expression is done in eukaryotic or bacterial cells, or by using a cell-free *in vitro* expression system. Further, the term means that the sequence of said binding protein or said binding domain is not present as a non-artificial sequence entry in a sequence database, for example in GenBank, EMBL-Bank or Swiss-Prot. These databases and other similar sequence databases are well known to the person skilled in the art.

A binding domain can inhibit VEGF-Axxx binding to VEGFR-2 either by binding to VEGF-Axxx or by binding to VEGFR-2 in a way that the apparent dissociation constant (K_d) between VEGF-Axxx and VEGFR-2 is increased more than 10^2-fold, preferably more than 10^3-fold, more preferably more than 10^4-fold, more preferably more than 10^5-fold, and most preferably more than 10^6-fold. Preferably, the K_d for the interaction of the binding domain to either VEGF-Axxx or VEGFR-2 is below 10^{-7}M, preferably below 10^{-8}M, more preferably below 10^{-9}M, more preferably below 10^{-10}M, and most preferably below 10^{-11}M. Methods, to determine dissociation constants of protein-protein interactions, such as surface plasmon resonance (SPR) based technologies, are well known to the person skilled in the art.

A preferred binding domain binds VEGF-Axxx. Even more preferred is a binding domain that binds human VEGF-A165.

The term “PBS” means a phosphate buffered water solution containing 137 mM NaCl, 10 mM phosphate and 2.7 mM KCl and having a pH of 7.4.

Preferred is a binding protein and/or binding domain that does not lose its native three-dimensional structure upon incubation in PBS containing 100 mM dithiothreitol (DTT) for 1 or 10 hours at 37°C.

In one particular embodiment the invention relates to a binding protein comprising a binding domain inhibiting VEGF-Axxx binding to VEGFR-2 and having the indicated or preferred midpoint denaturation temperature and non-aggregating properties as defined above, wherein said binding protein inhibits sprouting of HUVEC spheroids with an IC_{50} value below 100 nM.

The term "HUVEC" means human umbilical vein endothelial cells, which can be isolated from normal human umbilical vein and which are responsive to VEGF-A stimulation. Assays to measure the sprouting of HUVEC spheroids, such as that described in Example 2, are well known to the person skilled in the art.

An IC_{50} value is the concentration of a substance, such as a binding protein or binding domain, which is required for 50% inhibition *in vitro* of an experimental determined parameter, such as the sprouting of HUVEC spheroids. IC_{50} values can be readily determined by the person skilled in the art (Korff T. and Augustin H.G., J. Cell Biol. *143(5)*, 1341-52, 1998).

Preferred is a binding protein and/or binding domain that inhibits the sprouting of HUVEC spheroid with an IC_{50} value below 10 nM, preferably below 1 nM, more preferably below 0.1 nM, and most preferably below 0.05 nM.

Further preferred is a monomeric binding protein and/or binding domain that inhibits the sprouting of HUVEC spheroids with an IC_{50} value lower than the corresponding IC_{50} value of ranibizumab (Lucentis®, a registered trademark of Genentech), bevacizumab (Avastin®, a registered trademark of Genentech), aflibercept (VEGF Trap®, a registered trademark of Regeneron), or pegaptanib (Macugen®, a registered trademark of Pfizer).

The K_d for the interaction of a preferred binding domain to VEGF-B, VEGF-C, VEGF-D, PlGF or PDGF is above 1 nM, preferably above 10 nM, more preferably above 10^2 nM, even more preferably above 10^3 nM, and most preferably above10^4 nM.

Preferably, VEGF-Axxx is either dog VEGF-A164 or simian VEGF-A165 or human VEGF-A165, and VEGF-Axxxb is either dog VEGF-A164b or simian VEGF-A165b or human VEGF-A165b.

Another preferred embodiment is a recombinant binding protein comprising a binding domain, wherein said binding domain inhibits VEGF-Axxx binding to VEGFR-2 and wherein said binding domain is a repeat domain or a designed repeat domain. Such a repeat domain may comprise one, two, three or more internal repeat modules that will participate in binding to VEGF-Axxx. Preferably, such a repeat domain comprises an N-terminal capping module, two to four internal repeat modules, and a C-terminal capping

module. Preferably, said binding domain is an ankyrin repeat domain or designed ankyrin repeat domain.

A preferred recombinant binding protein comprises a binding domain as described herein, conjugated to a polyethylene glycol (PEG) moiety, preferably wherein said PEG moiety is coupled to a single Cys residue of said binding domain. Preferably, said Cys residue is genetically introduced at the C-terminal end of said binding domain. The PEG moiety can then be coupled by chemical means, for example, by using maleimide chemistry well known to the person skilled in the art. Examples of such binding proteins comprising a PEG moiety conjugated to a single Cys residue are given in the Examples.

A preferred embodiment of the invention comprises a recombinant binding protein comprising a binding domain as described herein, wherein said binding domain is conjugated at its C-terminus via a peptide bond to SEQ ID NO:8, which is in turn conjugated at the C-terminal cysteine thiol to a maleimide-coupled PEG, such as α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene (NOF, Sunbright ME-200MA (20kD) or Sunbright ME-400MA (40kD)). In one embodiment the α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene has a molecular weight of at least about 2, 5, 10, 20, 30, 40, 50, 70, or 100 kD. In certain embodiments the α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene has a molecular weight of at least about 20 or at least about 40 kD.

Another preferred embodiment is a recombinant binding protein as defined above comprising at least one repeat domain with binding specificity for VEGF-Axxx, wherein said repeat domain competes for binding to VEGF-Axxx with a repeat domain selected from the group of the repeat domains of SEQ ID NO:1 to 7. Preferably, said repeat domain competes for binding to VEGF-Axxx with the repeat domain of SEQ ID NO:1 or 3. More preferably, said repeat domain competes for binding to VEGF-Axxx with the repeat domain of SEQ ID NO:3.

The term "compete for binding" means the inability of two different binding domains of the invention to bind simultaneously to the same target, while both are able to bind the same target individually. Thus, such two binding domains compete for binding to said target. Methods, such as competition ELISA or competition SPR measurements (e.g. by using the Proteon instrument from BioRad), to determine if two binding domains compete for binding to a target are well known to the practitioner in the art.

A recombinant binding protein that competes for binding to VEGF-Axxx with a selected repeat protein can be identified by methods well know to the person skilled in the art, such as a competition Enzyme-Linked ImmunoSorbent Assay (ELISA).

Another preferred embodiment is a recombinant binding protein comprising a repeat domain with binding specificity for VEGF-Axxx selected from the group consisting of the repeat domains of SEQ ID NO:1 to 7. Preferably, said repeat domain is selected from the repeat domains of SEQ ID NO:2 or 3. More preferably, said repeat domain is the repeat domain of SEQ ID NO:3.

One ore more polyethylene glycol moieties may be attached at different positions in the binding protein, and such attachment may be achieved by reaction with amines, thiols or other suitable reactive groups. Attachment of polyethylene glycol moieties (PEGylation) may be site-directed, wherein a suitable reactive group is introduced into the protein to create a site where PEGylation preferentially occurs, or is originally present in the binding protein. The thiol group may be present in a cysteine residue; and the amine group may be, for example, a primary amine found at the N-terminus of the polypeptide or an amine group present in the side chain of an amino acid, such as lysine or arginine. In a preferred embodiment, the binding protein is modified so as to have a cysteine residue at a desired position, permitting site directed PEGylation on the cysteine, for example by reaction with a polyethylene glycol derivative carrying a maleimide function. The polyethylene glycol moiety may vary widely in molecular weight (i.e. from about 1 kDa to about 100 kDa) and may be branched or linear. Preferably, the polyethylene glycol has a molecular weight of about 1 to about 50 kDa, preferably about 10 to about 40 kDa, even more preferably about 15 to about 30 kDa, and most preferably about 20 kDa.

In a further embodiment, the invention relates to nucleic acid molecules encoding the particular recombinant binding proteins. Further, a vector comprising said nucleic acid molecule is considered.

Further, a pharmaceutical composition comprising one or more of the above mentioned binding proteins, in particular recombinant binding proteins comprising repeat domains, or nucleic acid molecules encoding the particular recombinant binding proteins, and optionally a pharmaceutical acceptable carrier and/or diluent is considered. Pharmaceutical acceptable carriers and/or diluents are known to the person skilled in the

art and are explained in more detail below. Even further, a diagnostic composition comprising one or more of the above mentioned recombinant binding proteins, in particular binding proteins comprising repeat domains, is considered.

The binding protein of the invention suppresses or prevents VEGF induced pathological angiogenesis, vascular leakage (edema), pulmonary hypertension, tumor formation and/or inflammatory disorders. With "suppression" it is understood that the recombinant protein prevents the mentioned pathologies to some extent, e.g. to 10% or 20%, more preferably 50%, in particular 70%, 80% or 90%, or even 95%.

The term “edema” means a condition that is caused by vascular leakage. Vasodilation and increased permeability during inflammation can be predominant pathogenetic mechanisms. For instance, edema contributes to infarct expansion after stroke and may cause life-threatening intracranial hypertension in cancer patients. Further, extravasation of plasma proteins favors metastatic spread of occult tumors, and airway congestion may cause fatal asthmatic attacks. The increased vascular leakage which occurs during inflammation can lead to respiratory distress, ascites, peritoneal sclerosis (in dialysis patients), adhesion formation (abdominal surgery) and metastatic spreading.

The term “angiogenesis” means a fundamental process by which new blood vessels are formed. The primary angiogenic period in humans takes place during the first three months of embryonic development but angiogenesis also occurs as a normal physiological process during periods of tissue growth, such as an increase in muscle or fat and during the menstrual cycle and pregnancy.

The term “pathological angiogenesis” refers to the formation and growth of blood vessels during the maintenance and the progression of several disease states. Particular examples of pathological angiogenesis are found in blood vessels (atherosclerosis, hemangioma, hemangioendothelioma), bone and joints (rheumatoid arthritis, synovitis, bone and cartilage destruction, osteomyelitis, pannus growth, osteophyte formation, neoplasms and metastasis), skin (warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic edema, neoplasms), liver, kidney, lung, ear and other epithelia (inflammatory and infectious processes including hepatitis, glomerulonephritis, pneumonia; and asthma, nasal polyps, otitis, transplantation disorders, liver regeneration disorders, neoplasms and metastasis), uterus, ovary and placenta (dysfunctional uterine bleeding due to intra-uterine contraceptive devices, follicular cyst formation, ovarian

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hyperstimulation syndrome, endometriosis, neoplasms), brain, nerves and eye (retinopathy of prematurity, diabetic retinopathy, choroidal and other intraocular disorders, leukomalacia, neoplasms and metastasis), heart and skeletal muscle due to work overload, adipose tissue (obesity), endocrine organs (thyroiditis, thyroid enlargement, pancreas transplantation disorders), hematopoiesis (Kaposi syndrome in AIDS), hematologic malignancies (leukemias), and lymph vessels (tumor metastasis, lymphoproliferative disorders).

The term "retinal ischemic diseases" means that the retina's supply of blood and oxygen is decreased, the peripheral portions of the retina lose their source of nutrition and stop functioning properly. A particular example of a retinal ischemic disease is retinopathy. Common diseases which lead to retinopathy are diabetic retinopathy, central retinal vein occlusion, stenosis of the carotid artery, and sickle cell retinopathy. Diabetic retinopathy is a major cause of visual loss in diabetic patients. In the ischemic retina the growth of new blood vessels occurs (neovascularisation). These vessels often grow on the surface of the retina, at the optic nerve, or in the front of the eye on the iris. The new vessels cannot replace the flow of necessary nutrients and, instead, can cause many problems such as vitreous hemorrhage, retinal detachment, and uncontrolled glaucoma. These problems occur because new vessels are fragile and are prone to bleed. If caught in its early stages, proliferative diabetic retinopathy can sometimes be arrested with panretinal photocoagulation. However, in some cases, vitrectomy surgery is the only option.

Beside these retinopathies, vascular diseases of the eye also include ocular neovascularization diseases, such as macular degeneration and diabetic macular edema (DME). Macular degeneration results from the neovascular growth of the choroid vessel underneath the macula. There are two types of macular degeneration: dry and wet. While wet macular degeneration only comprises 15% of all macular degeneration, nearly all wet macular degeneration leads to blindness. In addition, wet macular degeneration nearly always results from dry macular degeneration. Once one eye is affected by wet macular degeneration, the condition almost always affects the other eye. Wet macular degeneration is often called age-related wet macular degeneration of wet-AMD as it is mostly found in elderly persons.

Diabetic retinopathy (DR) and DME are leading causes of blindness in the working-age population of most developed countries. The increasing number of individuals with diabetes worldwide suggests that DR and DME will continue to be major contributors to

vision loss and associated functional impairment for years to come. Several biochemical mechanisms, including protein kinase C–β activation, increased vascular endothelial growth factor production, oxidative stress, and accumulation of intracellular sorbitol and advanced glycosylation end products, may contribute to the vascular disruptions that characterize DR/DME. The inhibition of these pathways holds the promise of intervention for DR and DME.

The term "pulmonary hypertension" means a disorder in which the blood pressure in the pulmonary arteries is abnormally high. In the absence of other diseases of the heart or lungs it is called primary pulmonary hypertension. Diffuse narrowing of the pulmonary arterioles occurs as a result of pathological arteriogenesis followed by pulmonary hypertension as a response to the increased resistance to blood flow. The incidence is 8 out of 100'000 people. However, pulmonary hypertension can also occur as a complication of Chronic Obstructive Pulmonary Diseases (COPD) such as emphysema, chronic bronchitis or diffuse interstitial fibrosis and in patients with asthmatiform COPD. The incidence of COPD is approximately 5 out of 10'000 people.

Furthermore the binding proteins of the invention can be used to treat inflammation and more specifically inflammatory disorders.

The term "inflammation" as used herein means, the local reaction to injury of living tissues, especially the local reaction of the small blood vessels, their contents, and their associated structures. The passage of blood constituents through the vessel walls into the tissues is the hallmark of inflammation, and the tissue collection so formed is termed the exudates or edema. Any noxious process that damages living tissue, e.g. infection with bacteria, excessive heat, cold, mechanical injury such as crushing, acids, alkalis, irradiation, or infection with viruses can cause inflammation irrespective of the organ or tissue involved. It should be clear that diseases classified as "inflammatory diseases" and tissue reactions ranging from burns to pneumonia, leprosy, tuberculosis, and rheumatoid arthritis are all "inflammations".

The binding proteins according to the invention can be used to treat tumor formation. The term "tumor" means a mass of abnormal tissue that arises without obvious cause from pre-existing body cells, has no purposeful function, and is characterized by a tendency to autonomous and unrestrained growth. Tumors are quite different from inflammatory or other swellings because the cells in tumors are abnormal in their appearance and other

characteristics. Abnormal cells, i.e. the kind of cells that generally make up tumors, differ from normal cells in having undergone one or more of the following alterations: (1) hypertrophy, or an increase in the size of individual cells; (2) hyperplasia or an increase in the number of cells within a given zone; (3) anaplasia, or a regression of the physical characteristics of a cell toward a more primitive or undifferentiated type. Tumors may be benign, for example lipomas, angiomas, osteomas, chondromas, and adenomas. Examples of malignant tumors are carcinomas (such as the breast tumors, carcinomas in the respiratory and gastrointestinal tracts, the endocrine glands, and the genitourinary system), sarcomas (in connective tissues, including fibrous tissues, adipose (fat) tissues, muscle, blood vessels, bone, and cartilage), carcinosarcoma (in both epithelial and connective tissue) leukemias and lymphomas, tumors of nerve tissues (including the brain), and melanoma (a cancer of the pigmented skin cells). The use of the binding proteins of the present invention against tumors can also be in combination with any other tumor therapy known in the art such as irradiation, photo-dynamic therapy, chemotherapy or surgery.

A pharmaceutical composition comprises binding proteins as described above and a pharmaceutically acceptable carrier, excipient or stabilizer (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]). Suitable carriers, excipients or stabilizers known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids and amino acid copolymers. A pharmaceutical composition may also be a combination formulation, comprising an additional active agent, such as an anti-cancer agent or an anti-angiogenic agent (for example human VEGF-Axxxb; preferably, human VEGF-A165b).

A preferred pharmaceutical composition for the treatment of eye diseases comprises binding proteins as described above and a detergent such as nonionic detergent, including but not limited to polysorbate 20 (e.g. about 0.04%), a buffer such as histidine, phosphate or lactic acid and a sugar such as sucrose or trehalose. Preferably, such a composition comprises binding proteins as described above and PBS. Said or any other pharmaceutical compositions described herein may be administered locally, either topically to a portion of the eye or be injected into the eye for instance into the

subconjunctivital, peri- or retrobulbar space or directly into the eye. Alternatively, said or such other pharmaceutical compositions may be administered systemically by parental administration. Preferably, said or such other pharmaceutical composition is applied to the eye by an intravitreous injection. Also preferably, said pharmaceutical composition is applied to the eye topically and as an eye drop. The eye drop may be applied to the cornea (clear part in the centre of the eye) thereby allowing the molecules to permeate into the eye. For the treatment of a disease affecting the posterior of the eye, it may be most desirable that the binding protein penetrates the sclera when injected under the conjunctiva or around the globe. The administering of the binding protein may be performed after a preliminary step of modulating the surface of the eye to improve penetration of the molecules. Preferably, the epithelial layer such as the corneal epithelium is modulated by a penetration enhancer to allow for a sufficient and rapid penetration of the molecules as for example described above. The use of the binding proteins of the present invention against eye diseases can also be in combination with any other therapy known in the art such as photo-dynamic therapy.

The formulations to be used for in vivo administration must be aseptic or sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. In one embodiment of the invention, an intraocular implant can be used for providing the binding protein of the invention. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing a polypeptide of the invention, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides, copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT® (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The pharmaceutical composition may be administered by any suitable method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parental administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. The dosage and mode of administration will depend on the individual to be treated and the particular disease.

Generally, the pharmaceutical composition is administered so that the binding protein of the present invention is given at a dose between 1 μg/kg and 20 mg/kg, more preferably between 10 μg/kg and 5 mg/kg, most preferably between 0.1 and 2 mg/kg. Preferably, it is given as a bolus dose. Continuous infusion may also be used and includes continuous subcutaneous delivery via an osmotic minipump. If so, the pharmaceutical composition may be infused at a dose between 5 and 20 μg/kg/minute, more preferably between 7 and 15 μg/kg/minute. In particular, the pharmaceutical composition is administered by injections into the eye so that the binding protein of the invention is given at a dose between 0.1 mg and 10 mg per injection, more preferably between 0.3 and 6 mg per injection, most preferably between 1 mg and 4 mg per injection. Further, the pharmaceutical composition is administered by eye drops to the eye so that a single drop of a solution containing a concentration of the binding protein of the invention between 10 and 120 mg/ml, more preferably between 20 and 100 mg/ml, most preferably between 40 and 80 mg/ml is applied to the eye.

In another embodiment of the invention a binding protein inhibiting the activity of VEGF-Axxx, as described above, can be used in combination with a binding protein or small molecule inhibiting the activity of PlGF, with the same inhibition levels of PlGF as described above for VEGF-Axxx. This embodiment is based on the fact that PlGF is found to be angiogenic at sites where VEGF-Axxx levels are increased. Further, a binding protein inhibiting the activity of VEGF-Axxx, as described above, can be used in combination with a binding protein or small molecule inhibiting the activity of platelet-derived growth factor (PDGF), VEGF-C or other members of the VEGF family of proteins, tumor necrosis factor alpha (TNFalpha), delta-ligand like 4 (Dll4), interleukin 6 (IL-6), neuropilin or angiopoietin 2 (Ang2).

The invention further provides methods of treatment. In one aspect, a method of treating a retinopathy is provided, the method comprising administering, to a patient in need thereof, a therapeutically effective amount of a binding protein of the invention, in particular a binding protein that inhibits the interaction between human VEGF-Axxx and human VEGFR-2, but not the interaction between human VEGF-Axxxb and human VEGFR-2, and the binding protein inhibits VEGFR-2 mediated angiogenesis.

The invention further relates to methods for using a binding protein as described to inhibit a VEGF-A biological activity in a cell or to inhibit a biological activity mediated by VEGFR-2. The cell may be situated *in vivo* or *ex vivo*, and may be, for example, a cell of a living

organism, a cultured cell or a cell in a tissue sample. The method may comprise contacting said cell with any of the VEGF-A/VEGFR-2 interaction inhibiting binding proteins disclosed herein, in an amount and for a time sufficient to inhibit such biological activity.

The invention provides a method for treating a subject having a condition which responds to the inhibition of VEGF-Axxx or VEGFR-2. Such a method comprises administering to said subject an effective amount of a binding protein described herein. A condition may be one that is characterized by inappropriate angiogenesis. A condition may be a hyper-proliferative condition. Examples of conditions (or disorders) suitable for treatment include autoimmune disorders, inflammatory disorders, retinopathies (particularly proliferative retinopathies), and cancers, in particular one of the diseases described above. Any of the binding proteins described herein may be used for the preparation of a medicament for the treatment of such a disorder, particularly a disorder selected from the group consisting of: an autoimmune disorder, an inflammatory disorder, a retinopathy, and a cancer. Preferred conditions (or disorders) suitable for treatment are first-line metastatic renal cell carcinoma, relapsed glioblastoma multiforme, adjuvant colon cancer, adjuvant HER2-negative breast cancer, adjuvant HER2-positive breast cancer, adjuvant non-small cell lung cancer, diffuse large B-cell lymphoma, first-line advanced gastric cancer, first-line HER2-negative metastatic breast cancer, first-line HER2-positive metastatic breast cancer, first-line metastatic ovarian cancer, gastrointestinal stromal tumors, high risk carcinoid, hormone refractory prostate cancer, newly diagnosed glioblastoma multiforme, metastatic head and neck cancer, relapsed platinum-sensitive ovarian cancer, second-line metastatic breast cancer, extensive small cell lung cancer, non-squamous, non-small cell lung cancer with previously treated CNS metastases and relapsed multiple myeloma, prostate cancer, non-small cell lung cancer (NSCLC), colorectal cancer and pancreatic cancer, advanced ovarian cancer (AOC), AOC patients with symptomatic malignant ascites and non-Hodgkin's lymphoma.

The recombinant binding protein according to the invention may be obtained and/or further evolved by several methods such as display on the surface of bacteriophages (WO 90/02809, WO 07/006665) or bacterial cells (WO 93/10214), ribosomal display (WO 98/48008), display on plasmids (WO 93/08278) or by using covalent RNA-repeat protein hybrid constructs (WO 00/32823), or intracellular expression and selection / screening such as by protein complementation assay (WO 98/341120). Such methods are known to the person skilled in the art.

A library of ankyrin repeat proteins used for the selection/screening of a recombinant binding protein according to the invention may be obtained according to protocols known to the person skilled in the art (WO 02/020565, Binz, H.K. et al., JMB, *332*, 489-503, 2003, and Binz et al., 2004, loc. cit). The use of such a library for the selection VEGF-Axxx specific DARPins is given in Example 1. In analogy, the ankyrin repeat sequence motifs as presented above can used to build libraries of ankyrin repeat proteins that may be used for the selection or screening of VEGF-Axxx specific DARPins. Furthermore, repeat domains of the present invention may be modularly assembled from repeat modules according the current inventions and appropriate capping modules (Forrer, P., et al., FEBS letters *539*, 2-6, 2003) using standard recombinant DNA technologies (e.g. WO 02/020565, Binz et al., 2003, loc. cit. and Binz et al., 2004, loc. cit).

The invention is not restricted to the particular embodiments described in the Examples. Other sources may be used and processed following the general outline described below.

Examples

All of the starting materials and reagents disclosed below are known to those skilled in the art, and are available commercially or can be prepared using well-known techniques.

Materials

Chemicals were purchased from Fluka (Switzerland). Oligonucleotides were from Microsynth (Switzerland). Unless stated otherwise, DNA polymerases, restriction enzymes and buffers were from New England Biolabs (USA) or Fermentas (Lithuania). The cloning and protein production strain was *E. coli* XL1-blue (Stratagene, USA). VEGF variants were from R&D Systems (Minneapolis, USA) or were produced in Chinese Hamster Ovary Cells or in *Pichia pastoris* and purified according to standard protocols (Rennel, E. S. et al., European J. Cancer *44*, 1883-94, 2008; Pichia expression system from Invitrogen). Biotinylated VEGF variants were obtained chemically via coupling of the biotin moiety to primary amines of the purified VEGF variants using standard biotinylation reagents and methods (Pierce, USA).

Molecular Biology

Unless stated otherwise, methods are performed according to described protocols (Sambrook J., Fritsch E. F. and Maniatis T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory 1989, New York).

Designed ankyrin repeat protein libraries

The N2C and N3C designed ankyrin repeat protein libraries are described (WO 02/20565; Binz et al. 2003, loc. cit.; Binz et al. 2004, loc. cit.). The digit in N2C and N3C describes the number of randomized repeat modules present between the N-terminal and C-terminal capping modules. The nomenclature used to define the positions inside the repeat units and modules is based on Binz et al. 2004, loc. cit. with the modification that borders of the repeat modules and repeat units are shifted by one amino acid position. For example, position 1 of a repeat module of Binz et al. 2004 (loc. cit.) corresponds to position 2 of a repeat module of the current disclosure and consequently position 33 of a repeat module of Binz et al. 2004, loc. cit. corresponds to position 1 of a following repeat module of the current disclosure.

All the DNA sequences were confirmed by sequencing, and the calculated molecular weight of all described proteins was confirmed by mass spectrometry.

Example 1: *Selection of binding proteins comprising a repeat domain with binding specificity for VEGF-Axxx*

Using ribosome display (Hanes, J. and Plückthun, A., PNAS *94*, 4937-42, 1997) many designed ankyrin repeat proteins (DARPins) with binding specificity for VEGF-Axxx were selected from the N2C or N3C DARPin libraries described by Binz et al. 2004 (loc. cit.). The binding of the selected clones toward specific (VEGF-Axxx) and unspecific (MBP, *E. coli* maltose binding protein) targets was assessed by crude extract ELISA indicating that VEGF-Axxx binding proteins were successfully selected (Fig. 1). The repeat domains of SEQ ID NO:1 to 7 constitute amino acid sequences of selected binding proteins comprising a repeat domain with binding specificity for VEGF-Axxx. Sequence analysis of selected binders revealed specific ankyrin repeat sequence motifs inherent to certain selected families of binders.

Selection of VEGF-Axxx specific ankyrin repeat proteins by ribosome display

The selection of VEGF-Axxx specific ankyrin repeat proteins was performed by ribosome display (Hanes and Plückthun, loc. cit.) using dog VEGF-A164 or human VEGF-A165 as target proteins, the library of designed ankyrin repeat proteins as described (WO 02/020565, Binz et al., 2003, loc. cit. and Binz et al., 2004, loc. cit) and established protocols (Zahnd, C., Amstutz, P. and Plückthun, A., Nat. Methods *4*, 69-79, 2007). Ribosome-display selection rounds were performed on dog or human VEGF variants (including biotinylated variants immobilized over neutravidin or streptavidin) with both the N2C and N3C DARPin libraries using established protocols (Binz et al. 2004, loc. cit.). The number of reverse transcription (RT)-PCR cycles after each selection round was constantly reduced from 40 to 30, adjusting to the yield due to enrichment of binders. Four initial selection rounds on dog VEGF yielded pools of nanomolar-affinity DARPins, as revealed by ELISA and SPR measurements of single clones. To find DARPins with further improved affinities, additional off-rate selections were performed on biotinylated human or dog VEGF immobilized over neutravidin or streptavidin, taking pools after the second and third initial ribosome-display selection rounds, followed by an on-rate selection round on human VEGF.

Selected clones bind specifically to VEGF-Axxx as shown by crude extract ELISA

Individual selected DARPins specifically binding VEGF-Axxx were identified by an enzyme-linked immunosorbent assay (ELISA) using crude *Escherichia coli* extracts of DARPin expression cells using standard protocols. Selected clones were cloned into the pQE30 (Qiagen) expression vector, transformed into *E. coli* XL1-Blue (Stratagene) and then grown overnight at 37°C in a 96-deep-well plate (each clone in a single well) containing 1 ml growth medium (2YT containing 1% glucose and 100 µg/ml ampicillin). 1 ml of fresh 2YT containing 50 µg/ml ampicillin was inoculated with 100 µl of the overnight culture in a fresh 96-deep-well plate. After incubation for 2 h at 37°C, expression was induced with IPTG (1 mM final concentration) and continued for 3 h. Cells were harvested, resuspended in 100 µl B-PERII (Pierce) and incubated for 15 min at room temperature with shaking. Then, 900 µl PBS-TB (PBS supplemented with 0.2% BSA, 0.1% Tween 20, pH 7.4) were added and cell debris were removed by centrifugation. 100 µl of each lysed clone were applied to a well of a NeutrAvidin coated MaxiSorp plate containing either a VEGF-Axxx variant or the unrelated MBP immobilized via their biotin moiety and incubated for 1 h at RT. After extensive washing with PBS-T (PBS supplemented with 0.1% Tween 20, pH 7.4) the plate was developed using standard ELISA procedures using the monoclonal anti-RGS(His)$_4$ antibody (34650, Qiagen) as primary antibody and a polyclonal goat anti-mouse antibody conjugated with alkaline

phosphatase (A3562, Sigma) as secondary reagent. Binding was then detected by using disodium 4-nitrophenyl phosphate (4NPP, Fluka) as a substrate for alkaline phosphatase. The color development was measured at 405 nm. The results from an example crude extract ELISA used to identify DARPins binding to VEGF-Axxx is shown in Fig. 1. Screening of several hundred clones by such a crude cell extract ELISA revealed more than hundred different DARPins with specificity for VEGF-Axxx. These binding proteins were chosen for further analysis. Examples of amino acid sequences of selected ankyrin repeat domains that specifically bind to VEGF-Axxx are provided in SEQ ID NO:1 to 7.

Deducing repeat sequence motives from selected repeat domains with binding specificity for VEGF-Axxx

The amino acid sequences of selected repeat domains with binding specificity for VEGF-Axxx were further analyzed by sequence analyzing tools known to the practitioner in the art (WO 02/020565; Forrer et al., 2003, loc. cit.; Forrer, P., Binz, H.K., Stumpp, M.T. and Plückthun, A., ChemBioChem, *5(2)*, 183-189, 2004). Nevertheless, in contrast to WO 02/020565 where naturally occurring repeat motifs were used to deduce repeat sequence motifs, here the repeat sequence motifs were deduced from the repeat units of selected repeat domains with binding specificity for VEGF-Axxx. Thereby families of selected repeat domains comprising a common repeat sequence motif were determined.

High level and soluble expression of DARPins

For further analysis, the selected clones showing specific VEGF-Axxx binding in the crude cell extract ELISA as described above were expressed in *E. coli* XL1-blue cells and purified using their His-tag using standard protocols. 25 ml of stationary overnight cultures (LB, 1% glucose, 100 mg/l of ampicillin; 37°C) were used to inoculate 1 l cultures (same medium). At A(600) = 0.7, the cultures were induced with 0.5 mM IPTG and incubated at 37°C for 4 h. The cultures were centrifuged and the resulting pellets were resuspended in 40 ml of TBS500 (50 mM Tris–HCl, 500 mM NaCl, pH 8) and sonicated. The lysate was recentrifuged, and glycerol (10% (v/v) final concentration) and imidazole (20 mM final concentration) were added to the resulting supernatant. Proteins were purified over a Ni-nitrilotriacetic acid column (2.5 ml column volume) according to the manufacturer's instructions (QIAgen, Germany). Up to 200 mg of highly soluble DARPins with binding specificity to VEGF-Axxx could be purified from one litre of *E. coli* culture with a purity > 95% as estimated from SDS-15% PAGE. Such purified DARPins are used for further characterizations.

Example 2: *Determination of IC_{50} values of selected DARPins with binding specificity to VEGF-Axxx in a spheroid outgrowth assay*

Addition of VEGF-Axxx to HUVEC spheroids embedded in collagen matrices leads to spheroid sprouting. Addition of an inhibitor of VEGF-Axxx will block sprout formation, which can be quantified statistically by the numbers and lengths of sprouts. By adding different concentration of inhibitor and a constant amount of VEGF, the IC_{50} can be determined.

Inhibition of spheroid sprouting by VEGF-Axxx specific DARPins

Spheroid outgrowth assays were done according to standard protocols (Korff et al., loc. cit.). DARPins with specificity for VEGF-Axxx were selected and purified to > 96% purity as described in Example 1. Human umbilical vein cells were grown to confluency in monolayer culture. After trypsinization, the cell suspension was placed in a hanging drop to form spheroids, i.e. approximately 500 organized aggregated HUVECs. Spheroids were embedded in a collagen matrix and stimulated with VEGF-A165 to initiate sprout outgrowth. Sprouting inhibitors were added additionally to observe their effects on sprouting inhibition. Sprout numbers per spheroid and sprout lengths were quantified using a graphical software.

The results from two example spheroid sprouting assays are shown in Fig. 2a (DARPin #30 with binding specificity for VEGF-Axxx) and Fig. 2b (DARPin NC, a negative control DARPin with no binding specificity for VEGF-Axxx; e.g. DARPin E3_5 (Binz et al., 2005, loc. cit.). The best performing DARPins in this assay showed IC_{50} values in the range of 10 to 50 pM, while Avastin®, Lucentis® and Macugen® showed IC_{50} values in parallel experiments in the range of 150 and 500 pM.

Example 3: *Determination of the target specificity of DARPin #27 in comparison to Avastin® by Surface Plasmon Resonance analysis*

Dog VEGF-A164 or Dog VEGF-A164b were immobilized in a flow cell and the interaction of DARPin #27 (the repeat domain of SEQ ID NO:1, corresponding to amino acids 1 to 159) and Avastin® with the immobilized targets were analyzed.

Surface Plasmon Resonance (SPR) analysis

SPR was measured using a ProteOn instrument (BioRad). The running buffer was 20 mM HEPES, pH 7.4, 150 mM NaCl and 0.005% Tween 20. About 1200 RU of dog VEGF-A164 or dog VEGF-A164b were immobilized on a GLC chip (BioRad). The interactions were measured at a flow of 60 µl/min with 5 min buffer flow, 100 seconds injection of Avastin® or DARPin #27 at a concentration of 250 nM and an off-rate measurement of a few minutes with buffer flow. The signal of an uncoated reference cell was subtracted from the measurements.

The results are shown in Fig. 3a (Avastin interaction with dog VEGF-A164), Fig. 3b (Avastin interaction with dog VEGF-A164b), Fig. 3c (DARPin #27 interaction with dog VEGF-A164) and Fig. 3d (DARPin #27 interaction with dog VEGF-A164b). Whereas Avastin clearly interacts with both immobilized VEGF isoforms, the DARPin #27 shows only interaction with VEGF-A164 and not VEGF-A164b.

Example 4: *In vivo efficacy of DARPin #30 in inhibiting VEGF-A165 in a vascular leakage rabbit model.*

Pegylated DARPin #30 (the repeat domain of SEQ ID NO:4 corresponding to the amino acids 1 to 126) or Lucentis® is applied by intravitreal injection into an eye of a rabbit to test their efficacy to inhibit vascular leakage induced by a subsequent intravitreous injection of human VEGF-A165.

Vascular leakage inhibition measurements in rabbits

At day 1 either PBS, PEGylated DARPin #30 (125 µg) or the equimolar amount of Lucentis® (162 µg) is applied by an intravitreal injection into one eye of each rabbit (treated eye). At day 4 or day 30 the treated eye of each rabbit was challenged by intravitreal injection of 500 ng of human VEGF-A165. Both eyes of all animals were evaluated 48 hours after the VEGF-A165 injection by measuring the fluorescein content in all eyes 1 h after intravenous injection of sodium fluorescein (50 mg/kg animal body weight, 10%(w/v) in 0.9% (w/v)saline solution). The ratios of the amounts of fluorescence in the treated and untreated eyes were calculated for every animal. A ratio of one corresponds to absence of additional fluorescence leakage in the treated eye, a ratio greater than one indicates more fluorescence leakage in the treated eye than in the untreated control eye.

Preparation of PEGylated DARPin

The PEGylation of protein by making use of a single Cys residue and maleimide chemistry is well known to the person skilled in the art and can be performed according to established protocols (e.g. from Pierce). DARPin #30 comprising an additional C-terminal linker (GGGSGGGSC, SEQ ID NO:8) was purified to near homogeneity using standard chromatographic methods. The protein is completely reduced using DTT and purified by gel-filtration to remove the DTT and to exchange the buffer by PBS. PEG-maleimide (methoxy-poly(ethylene glycol)-oxopropylamino-propyl maleimide; NOF, no. Sunbright ME-200MA) dissolved in PBS is mixed with the DARPin in PBS at about 15% molar excess of PEG-maleimide for 2-4 hours at room temperature. The PEGylated DARPin is then separated from non-reactive DARPin and non-reactive PEG moieties by using standard anion exchange chromatography.

The results are shown in Fig. 4. Both PEGylated DARPin #30 and Lucentis® were able to protect the rabbit eye from VEGF-A165 induced vascular leakage 4 days after they were applied by intravitreal injections. Nevertheless, only the PEGylated DARPin #30, and not Lucentis®, was able to protect the rabbit eye from VEGF-A165 induced vascular leakage up to 30 days after the intravitreal injection.

In other experiments the intravitreal terminal half-lives of the different binding proteins of the invention were measured after intravitreal injections into rabbit eyes. DARPin #30 comprising an additional C-terminal linker (GGGSGGGSC, SEQ ID NO:8) was conjugated to a 20 kDa and a 40 kDa non-proteinaceous PEG moiety using the respective maleimide PEGs from NOF (see Example 5). The terminal half-lives were determined to be 3.5 days (+/- 0.3 days), 6.1 days (+/- 1.0 days) and 5.4 days (+/-0.8 days) for the DARPin #30, the DARPin #30 conjugated to the 20 kDa PEG moiety and the DARPin #30 conjugated to the 40 kDA PEG moiety. Surprisingly, increasing the molecular weight of the non-proteinaceous PEG moiety from 20 kDa to 40 kDa did not result in an increased terminal half-live. The same trend was observed in corresponding experiments were binding proteins comprising the repeat domain of SEQ ID NO:1 (amino acids 1 to 159) or SEQ ID NO:3 (amino acids 1 to 126) instead of the repeat domain of SEQ ID NO:4 were used.

Example 5: *Recombinant binding proteins*

Examples of recombinant binding proteins comprising a repeat domain binding VEGF-Axxx and a proteinaceous polymer moiety are SEQ ID NO:1 and 4. The repeat domain of SEQ ID NO:1 corresponds to amino acids 1 to 159 and the proteinaceous polymer moiety

of SEQ ID NO:1 corresponds to amino acids 160 to 1'024. The repeat domain of SEQ ID NO:4 corresponds to amino acids 1 to 126 and the proteinaceous polymer moiety of SEQ ID NO:4 corresponds to amino acids 127 to 536.

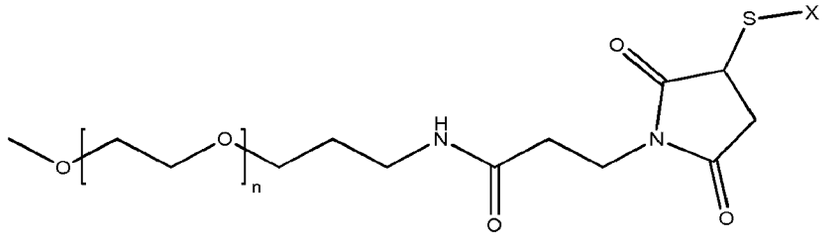
The binding proteins of SEQ ID NO:1 and 4 were expressed in the cytoplasm of *Escherichia coli* using standard techniques known to the person skilled in the art (see, for example, the pQE expression system from Qiagen (Germany)). The Met residue additionally encoded by the expression vector was efficiently cleaved off in the cytoplasm of *E. coli* from the expressed polypeptide since the start Met is followed by a small Gly residue (i.e. the amino acid at position 1 of SEQ ID NO:1 and 4). The cells were lysed (e.g. by using a French press) and the binding proteins were purified to near homogeneity from the crude cell extract by using standard chromatographic techniques known to the person skilled in the art.

Examples of recombinant binding proteins comprising one repeat domain binding VEGF-Axxx and one non-proteinaceous polymer moiety were produced using the repeat proteins of SEQ ID No:2, 3, 5, 6, and 7. These repeat proteins comprise an N-terminal repeat domain, followed by a polypeptide linker and a C-terminal Cys. The respective repeat domains correspond to amino acids 1 to 159 for SEQ ID NO:2 and 7, and to amino acids 1 to 126 for SEQ ID NO:3 to 6. The repeat proteins of SEQ ID NO:2, 3, 5, 6, and 7 were expressed in the cytoplasm of *Escherichia coli* using standard techniques known to the person skilled in the art (see, for example, The Expressionist from Qiagen (Germany)). The Met residue additionally encoded by the expression vector was efficiently cleaved off in the cytoplasm of *E. coli* from the expressed polypeptide since the start Met is followed by a small Gly residue (i.e. the amino acid at position 1 of SEQ ID NO:2, 3, 5, 6, and 7). The cells were lysed (.e.g. by using a French press) and the binding proteins were purified to near homogeneity from the crude cell extract by using standard chromatographic techniques known to the person skilled in the art.

The purified repeat proteins comprising a single Cys residue were then conjugated to a non-proteinaceous polymer moiety using standard maleimide chemistry as outlined in Example 4. Thereby, a binding protein of the invention comprising the repeat protein of SEQ ID NO:2 and a 40 kDa non-proteinaceous PEG moiety (e.g. a 40 kDa maleimide-PEG (α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene) from NOF, product no. Sunbright ME-400MA), the repeat protein of SEQ ID NO:3 and a 20 kDa non-proteinaceous PEG moiety (e.g. a 20 kDa maleimide-PEG (α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene) from NOF, product no. Sunbright

ME-200MA), the repeat protein of SEQ ID NO:5 and a 12 kDa non-proteinaceous PEG moiety (e.g. a 12 kDa maleimide-PEG (α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene) from NOF, product no. Sunbright ME-120MA), the repeat protein of SEQ ID NO:6 and a 5 kDa non-proteinaceous PEG moiety (e.g. a 5 kDa maleimide-PEG (α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene) from NOF, product no. Sunbright ME-050MA) and the repeat protein of SEQ ID NO:7 and a 2 kDa non-proteinaceous PEG moiety (e.g. a 2 kDa maleimide-PEG (α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene) from NOF, product no. Sunbright ME-020MA) were produced. The PEGylated repeat proteins were then further separated from non-PEGylated repeat proteins and excess PEG by standard chromatographic techniques known to the person skilled in the art.

Thus, SEQ ID NO:2, 3, 5, 6, and 7 were conjugated at the thiol of their C-terminal cysteine to a maleimide PEG (α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene). The following structure was thereby produced:



wherein X is SEQ ID NO: 2, 3, 5, 6, or 7; and n is a positive integer.

Claims

1. A recombinant binding protein comprising an ankyrin repeat domain and a polyethylene glycol moiety of at least 5 kDa molecular weight, wherein said ankyrin domain binds VEGF-Axxx with a Kd below 10^{-9}M and inhibits VEGF-Axxx binding to VEGFR-2.

2. The binding protein of claim 1, which has an apparent molecular weight of at least 100 kDa when analyzed at a concentration of 0.1 mM in PBS at room temperature by size exclusion chromatography using globular proteins as molecular weight standards.

3. The binding protein of claim 1, wherein the N-terminal capping module of said ankyrin repeat domain comprises an Asp residue at position 5.

4. The binding protein of claim 1, wherein said ankyrin repeat domain competes for binding to VEGF-Axxx with the ankyrin repeat domains of SEQ ID NO:1 or 3.

5. The binding protein of claim 1, wherein said ankyrin repeat domain is selected from the group consisting of the ankyrin repeat domains of SEQ ID NO:1 to 7.

6. The binding protein of any one of claims 1 to 5, wherein said ankyrin repeat domain is conjugated at its C-terminus via a peptide bond to a polypeptide linker and a C-terminal Cys residue, wherein the thiol of said C-terminal Cys is further conjugated to a maleimide-coupled polyethylene glycol.

7. The binding protein of claim 6, wherein said maleimide-coupled polyethylene glycol is α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene.

8. The binding protein of claim 1, wherein said binding protein is an ankyrin repeat protein selected from the group consisting of the ankyrin repeat proteins of SEQ ID NO:2, 3, 5, 6 or 7.

9. The binding protein of any one of claims 6 to 8, wherein said polyethylene glycol moiety has a molecular weight of around 20 kDa.

10. The binding protein of claim 1 comprising the ankyrin repeat protein of SEQ ID NO:3 wherein the thiol of the C-terminal Cys of said ankyrin repeat protein is further conjugated to a maleimide-coupled polyethylene glycol.

11. The binding protein of claim 10 wherein said maleimide-coupled polyethylene glycol is α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy-polyoxyethylene, and wherein the polyethylene glycol moiety has a molecular weight of at least 10 kDa.

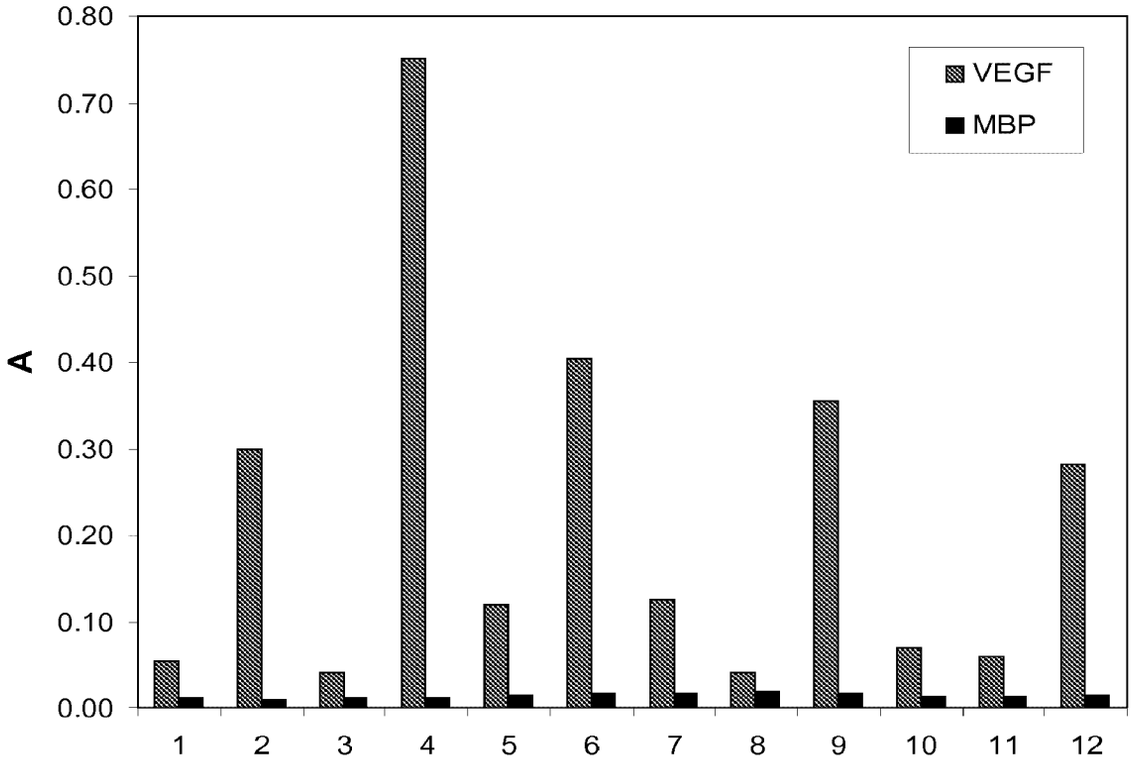
12. A pharmaceutical composition comprising the binding protein of any one of claims 1 to 11 and optionally a pharmaceutical acceptable carrier and/or diluent.

13. A pharmaceutical composition according to claim 12 for use in the treatment of an eye disease.

14. A pharmaceutical composition according to claim 12 for use in the treatment of an eye disease by intravitreal injection.

15. A method of treating pathological angiogenesis in a mammal including man, comprising administering to a patient in need thereof an effective amount of a compound according to any one of claims 1 to 11.

Fig. 1



Regeneron Exhibit 1002.0132

Fig. 2a

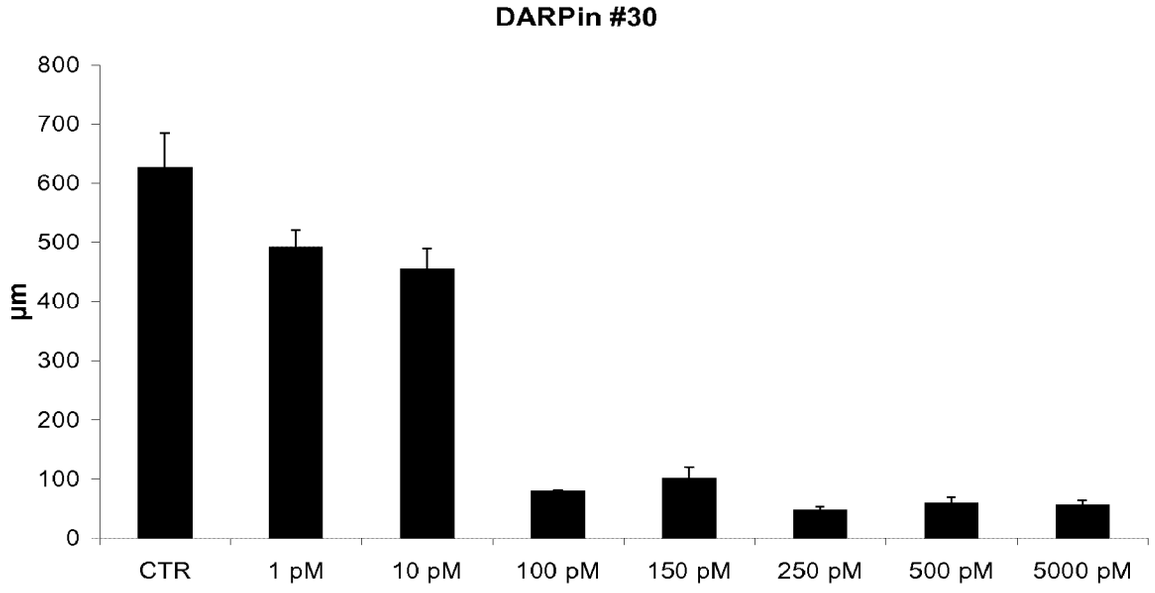
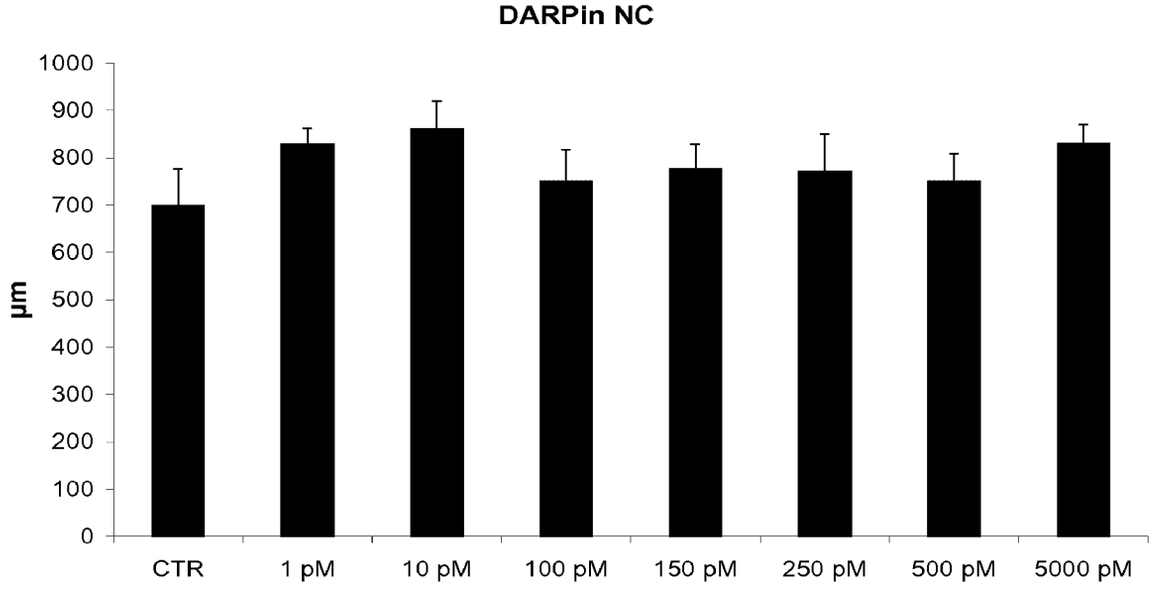


Fig. 2b



Regeneron Exhibit 1002.0133

Fig. 3a

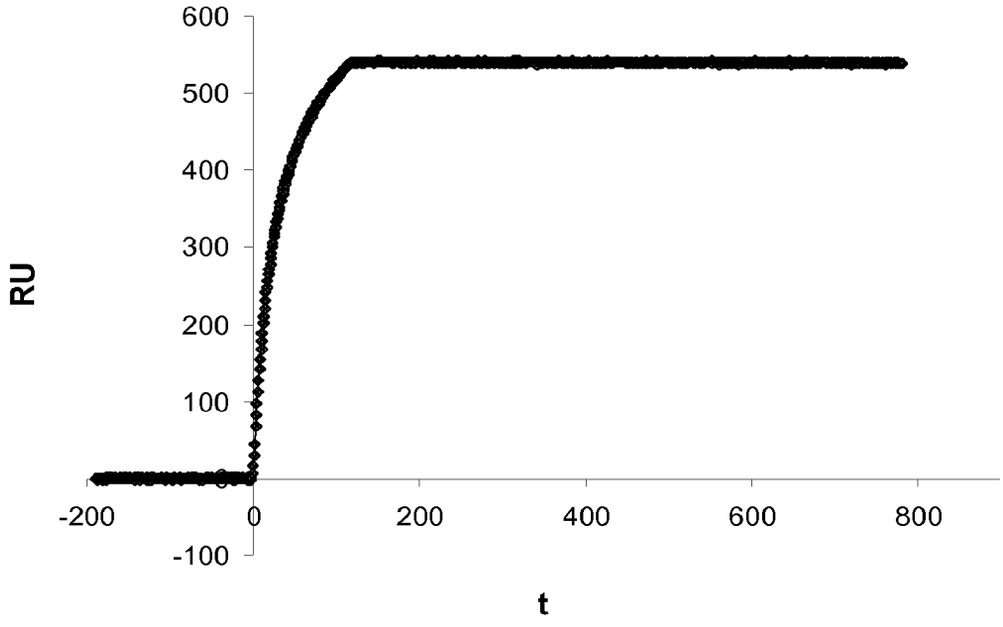
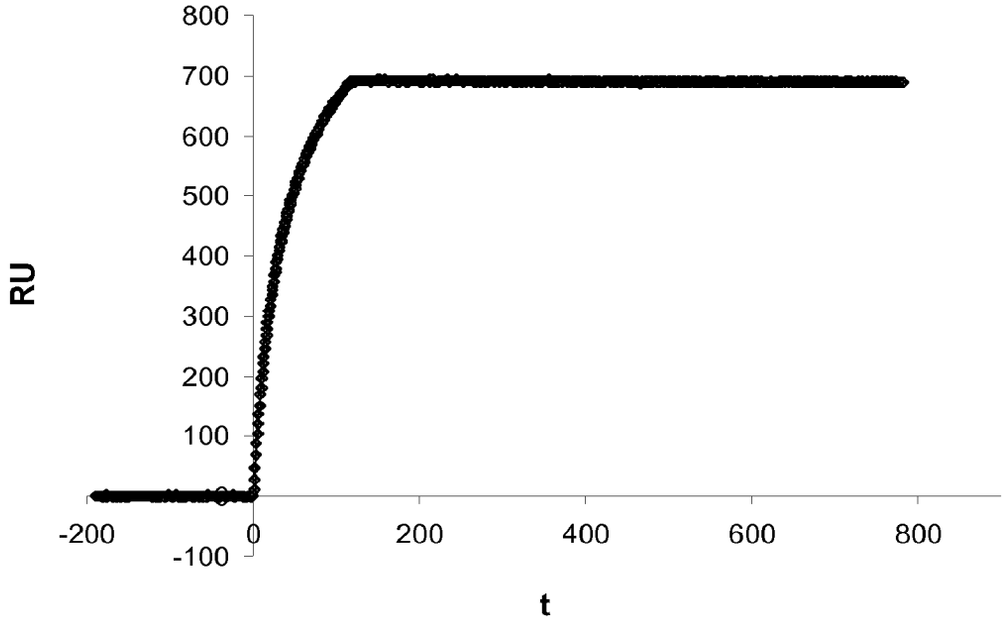
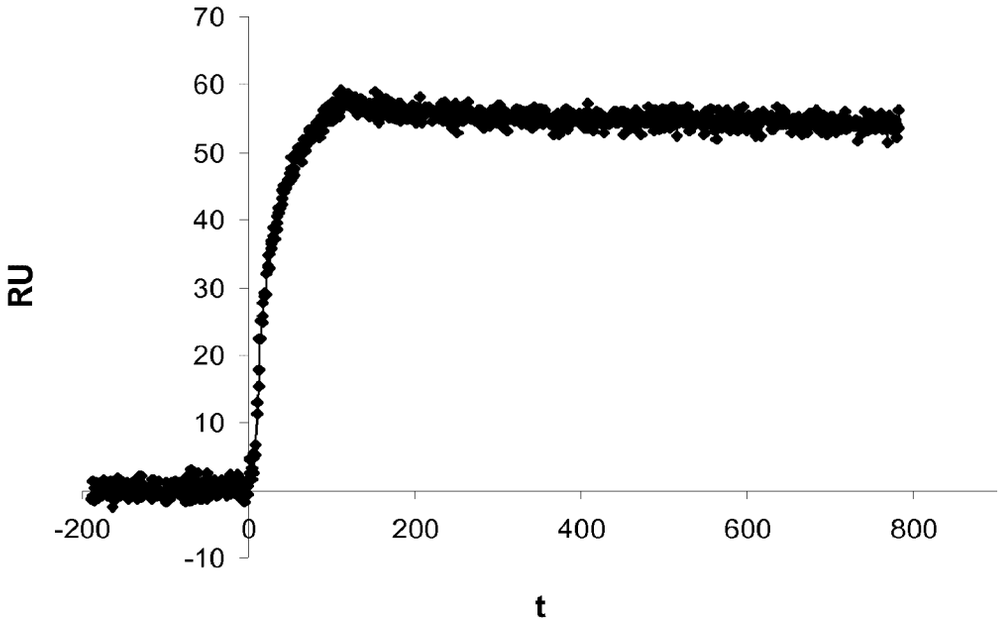


Fig. 3b



Regeneron Exhibit 1002.0134

Fig. 3c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60
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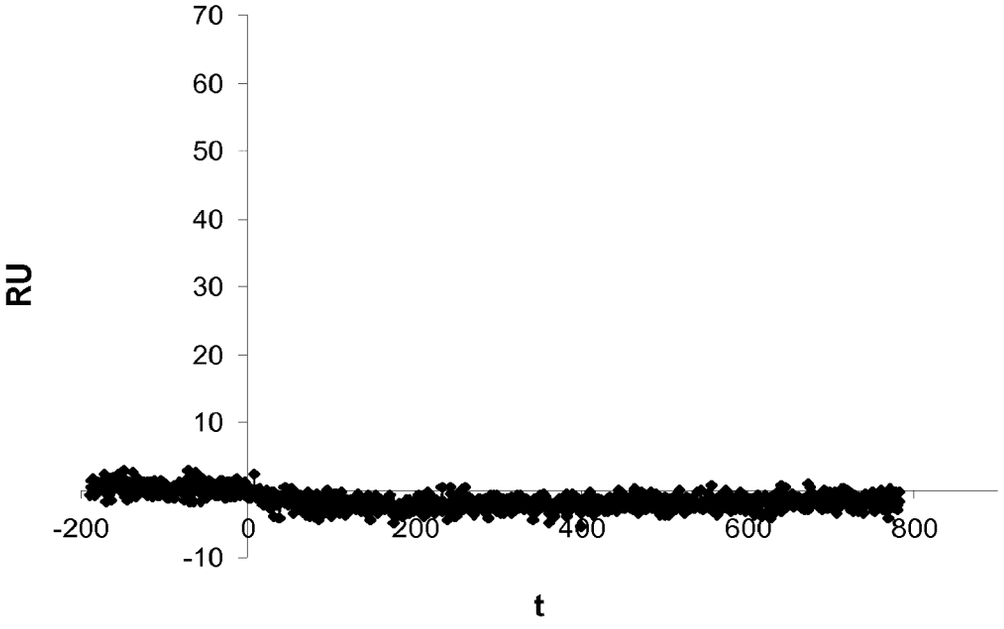
Fig. 3d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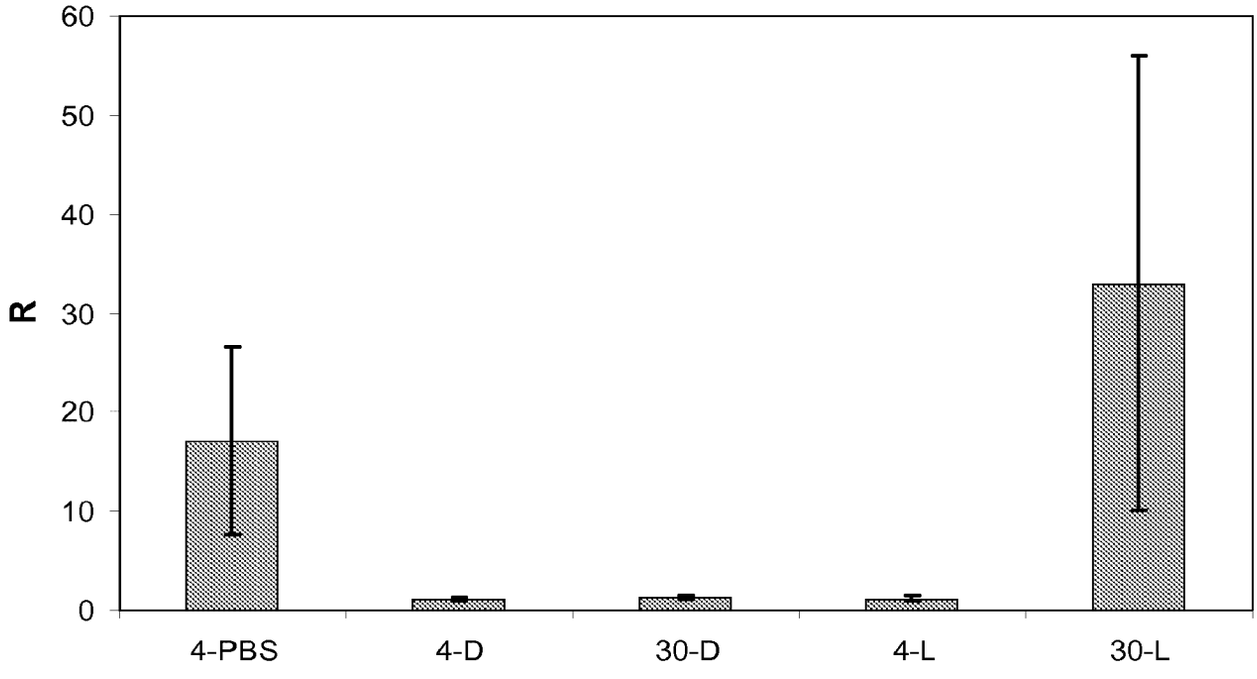
800
t

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/056824

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 A61P27/02 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2010/060748 A1 (MOLECULAR PARTNERS AG [CH]; BINZ HANS KASPAR [CH]; FORRER PATRIK [CH];) 3 June 2010 (2010-06-03) examples 1-4 page 15, line 17 - line 19 page 27, line 6 - line 20 sequences 18, 24, 36, 33, 22 ----- -/--	1-15

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2011	11/07/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Monami, Amélie

Form PCT/ISA/210 (second sheet) (April 2005)

Regeneron Exhibit 1002.0137

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NG EUGENE W M ET AL: "Anti-VEGF aptamer (pegaptanib) therapy for ocular vascular diseases", ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, NEW YORK ACADEMY OF SCIENCES, NEW YORK, NY, US LNKD- DOI:10.1196/ANNALS.1348.062, 1 January 2006 (2006-01-01), pages 151-171, XP008099093, ISSN: 0077-8923 abstract page 157, paragraph 1 - page 160, paragraph 1	1-15
A	WO 00/75319 A1 (REGENERON PHARMACEUTICALS, INC. [US]) 14 December 2000 (2000-12-14) cited in the application page 36, line 3 - line 4 examples 1-34	1-15
A	STUMPP M T ET AL: "DARPins: A new generation of protein therapeutics", DRUG DISCOVERY TODAY, ELSEVIER, RAHWAY, NJ, US LNKD- DOI:10.1016/J.DRUDIS.2008.04.013, vol. 13, no. 15-16, 1 August 2008 (2008-08-01), pages 695-701, XP023440383, ISSN: 1359-6446 [retrieved on 2008-07-11] page 698, column 1, paragraph 2 - column 2, paragraph 3	1-15
A	VANCE D ET AL: "The design of polyvalent scaffolds for targeted delivery", ADVANCED DRUG DELIVERY REVIEWS, ELSEVIER BV, AMSTERDAM, NL LNKD- DOI:10.1016/J.ADDR.2009.06.002, vol. 61, no. 11, 30 September 2009 (2009-09-30), pages 931-939, XP026666153, ISSN: 0169-409X [retrieved on 2009-07-24] page 935, column 1, paragraph 3 - page 937, column 1, paragraph 1	1-15

-/--

Regeneron Exhibit 1002.0138

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SASSA Y ET AL: "Antiangiogenic drugs in the management of ocular diseases: Focus on antivascular endothelial growth factor", CLINICAL OPHTHALMOLOGY 2010 DOVE MEDICAL PRESS NZL, vol. 4, no. 1, 26 April 2010 (2010-04-26), pages 275-283, XP002592518, abstract page 276, column 2, paragraph 3 - page 277, column 1, paragraph 2 page 277, column 2, paragraph 2 - page 278, column 2, paragraph 1 page 279, column 1, paragraph 3 - column 2, paragraph 1 -----	1-15

Regeneron Exhibit 1002.0139

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/056824

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010060748 A1	03-06-2010	AU 2009319204 A1	03-06-2010
WO 0075319 A1	14-12-2000	AT 293164 T	15-04-2005
		AT 417928 T	15-01-2009
		AU 779303 B2	13-01-2005
		AU 5040400 A	28-12-2000
		BR 0011407 A	02-04-2002
		CA 2376379 A1	14-12-2000
		CN 1369009 A	11-09-2002
		CN 101433715 A	20-05-2009
		CZ 20014387 A3	16-10-2002
		DE 60019415 D1	19-05-2005
		DE 60019415 T2	09-03-2006
		DK 1544299 T3	23-03-2009
		EP 1183353 A1	06-03-2002
		EP 1544299 A1	22-06-2005
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		ES 2319305 T3	06-05-2009
		HK 1043388 A1	10-06-2005
		HR 20010908 A2	30-04-2005
		HU 0201515 A2	28-08-2002
		IL 146890 A	08-07-2008
		JP 2003501089 A	14-01-2003
		JP 2011024595 A	10-02-2011
		MX PA01012630 A	22-07-2002
		NO 20016036 A	08-02-2002
		NZ 515913 A	30-01-2004
		PL 352246 A1	11-08-2003
		PT 1183353 E	30-06-2005
		PT 1544299 E	18-03-2009
		SK 17522001 A3	01-04-2003
		ZA 200110068 A	06-12-2002

Regeneron Exhibit 1002.0140

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	SYRINGE
First Named Inventor/Applicant Name:	Juergen Sigg
Filer:	Andrew K. Holmes/Andrea Jacquin
Attorney Docket Number:	55157-US0NP

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	390	390
Utility Search Fee	1111	1	620	620
Utility Examination Fee	1311	1	250	250
Pages:				
Claims:				
Claims in excess of 20	1202	12	62	744
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**2004**

Electronic Acknowledgement Receipt

EFS ID:	14795204
Application Number:	13750352
International Application Number:	
Confirmation Number:	5306
Title of Invention:	SYRINGE
First Named Inventor/Applicant Name:	Juergen Sigg
Customer Number:	1095
Filer:	Andrew K. Holmes/Andrea Jacquin
Filer Authorized By:	Andrew K. Holmes
Attorney Docket Number:	55157-US0NP
Receipt Date:	25-JAN-2013
Filing Date:	
Time Stamp:	15:29:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$2004
RAM confirmation Number	2300
Deposit Account	190134
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		51557-US-NP-FilingPaperwork_2013Jan25.pdf	17382287 e9a4ea6faf5e6290207dee7e0829a8b92a3e5cce	yes	47

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal of New Application	1	2
Specification	3	18
Claims	19	22
Abstract	23	23
Drawings-only black and white line drawings	24	24
Sequence Listing	25	30
Transmittal Letter	31	31
Information Disclosure Statement (IDS) Form (SB08)	32	32
Application Data Sheet	33	39
CRF Statement Paper and CRF are the same	40	40
Oath or Declaration filed	41	45
Power of Attorney	46	47

Warnings:

Information:

2	Sequence Listing (Text File)	PAT055157-EP-EPA03_ST25.txt	9954	no	0

Warnings:

Information:

3	Foreign Reference	09_WO10060748.pdf	2420035 1c3abaf9178fe7e6791149f3422b3577b590386b	no	55

Warnings:

Information:					
4	Foreign Reference	10_WO11135067.pdf	2051636 e3206c82e0248de09a52ca2a25d879cd4c3b1a3b	no	46
Warnings:					
Information:					
5	Non Patent Literature	01_Badkar_2011.pdf	193783 ca701fed672e75df876ad25e55c3ebe8459d0a91	no	7
Warnings:					
Information:					
6	Non Patent Literature	02_Schoenknecht_2007_.pdf	83296 83de5aeb7353727f6adfab685c49ac4e7e01f84c	no	1
Warnings:					
Information:					
7	Non Patent Literature	03_Holash_2002.pdf	316199 d0f356f173df69543416922817109273247126da	no	6
Warnings:					
Information:					
8	Non Patent Literature	04_RielyMiller_2007.pdf	206291 b9b59edd9b55ebc2de5710d663d2bc9ed08884ea	no	6
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Information:					
9	Non Patent Literature	05_Li_2011.pdf	2078229 90af21007a63e700c3342fdeedeb6f67bd821e02	no	7
Warnings:					
Information:					
10	Non Patent Literature	08_SmithWaterman_1981.pdf	333293 9e8395cd4879bb3cd371be78ae5fa292325c3649	no	8
Warnings:					
Information:					
11	Fee Worksheet (SB06)	fee-info.pdf	36400 995bd66ac722189c170e6d355cbe185ec7c9d7ae	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	25111403	

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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PTO/SB/08a (07-09)
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Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	Not yet known
Filing Date	Herewith
First Named Inventor	Sigg, Juergen et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT055157-US-NP

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number / Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document / Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		WO2010/060748	06-03-2010	Molecular Partners AG		☐
		WO2011/135067	11-03-2011	Molecular Partners AG		☐
						☐

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		Badkar et al., Analysis of Two Commercially Available Bortezomib Products : Differences in Assay of Active Agent and Impurity Profile » AAPS PharmaSciTech, Vol. 12, No. 2, pp. 564-572, (June 2011)	☐
		Schoenknecht, "Requirements on pre-fillable glass suringes", AAPS National Biotechnology Conference 2007 – Abstract no. NBC07-000488, 2007	☐
		Holash et al., "VEGF-Trap: A VEGF blocker with potent anitumor effects", PNAS USA, Vol. 99, No. 17, pp. 11393-11398, (August 20, 2002)	☐
		Riely & Miller, "Vascular Endothelial Growth Factor Trap in Non-Small Cell lung Cancer", Clin Cancer Res, 13:4623-7s, (August 1, 2007)	☐
		Li et al., "KH906, a recombinant human VEGF receptor fusion protein, is a new effective topical treatment for corneal neovascularization", Molecular Vision, 17:797-803,(March 25, 2011)	☐
		Smith & Waterman, " Comparison of Biosequences", Adv Appl. Math, 2:482-489, (1981)	☐
			☐

Examiner Signature		**Date Considered**	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Juergen		SIGG	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Loerrach	Country of Residence i	DE

Mailing Address of Inventor:

Address 1	Novartis Pharma AG		
Address 2	Postfach		
City	Basel	State/Province	
Postal Code	4002	Country i	CH

Inventor 2 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Christopher		Royer	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Munich	Country of Residence i	DE

Mailing Address of Inventor:

Address 1	Novartis Pharma AG		
Address 2	Postfach		
City	Basel	State/Province	
Postal Code	4002	Country i	CH

Inventor 3 — Remove

Legal Name

Regeneron Exhibit 1002.0148

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Andrew	Mark	BRYANT	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Reinach, Basel Land	Country of Residence i	CH

Mailing Address of Inventor:

Address 1	Novartis Pharma AG		
Address 2	Postfach		
City	Basel	**State/Province**	
Postal Code	4002	**Country** i	CH

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Heinrich	Martin	BUETTGEN	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Rheinfelden	Country of Residence i	CH

Mailing Address of Inventor:

Address 1	Novartis Pharma AG		
Address 2	Postfach		
City	Basel	**State/Province**	
Postal Code	4002	**Country** i	CH

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Marie		PICCI	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Ranspack-le-bas	Country of Residence i	FR

Mailing Address of Inventor:

Address 1	Novartis Pharma AG		
Address 2	Postfach		
City	Basel	**State/Province**	
Postal Code	4002	**Country** i	CH

Regeneron Exhibit 1002.0149

PTO/AIA/14 (08-12)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	01095		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	Syringe		
Attorney Docket Number	55157-US-NP	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		**Sub Class (if any)**	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		**Suggested Figure for Publication (if any)**	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	01095		

PTO/AIA/14 (08-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
12174860.2	EP	2012-07-03	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
12189649.2	EP	2012-10-23	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
202012011016.0	DE	2012-11-16	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
2012101677	AU	2012-11-16	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
2012101678	AU	2012-11-16	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
202012011260.0	DE	2012-11-23	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
202012011259.7	DE	2012-11-23	◉ Yes ○ No
			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
12195360.8	EP	2012-12-03	◉ Yes ○ No

PTO/AIA/14 (08-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
2013100071	AU	2013-01-23	(•) Yes ( ) No
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
2013100070	AU	2013-01-23	(•) Yes ( ) No
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
	DE	2013-01-23	(•) Yes ( ) No

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

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○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :			
If the Assignee is an Organization check here. ☒			
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Signature	/ Andrew Holmes /			Date (YYYY-MM-DD)	2013-01-25
First Name	Andrew	Last Name	Holmes	Registration Number	51813

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	55157-US-NP
	Application Number	
Title of Invention	Syringe	

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CASE PAT055157-US-NP

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Express Mail Label Number	Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Sigg, Juergen et al.
APPLICATION NO: Not Yet Known
FILED: Herewith
FOR: SYRINGE

MS: Sequence Listing
Commissioner for Patents
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Respectfully submitted,

/ Andrew Holmes /

Andrew Holmes
Agent for Applicant
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Date: January 25, 2013

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Sheet 1 of 1

Complete if Known	
Application Number	13/750352
Filing Date	January 25, 2013
First Named Inventor	Sigg, Juergen et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT055157-US-NP

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US 2006/293270 A1	2006-12-28	Adamis et al.	
		US 2011-257601 A1	2011-10-20	Furfine et al.	
		US 2010/0310309	2010-12-09	Abendroth et al.	
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		WO 2007/035621 A1	2007-03-29	Eyetech, Inc.		☐
		DE 10 2008 005938 A1 (equivalent to US 2010/0310309)	2009-07-30	Arzneimittel GmbH Apotheker Vetter & Co.		☐
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NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
		Ausubel et al., "Current Protocols in Molecular Biology", 7.7.18 of Current protocols in Molecular Biology, eds., supplement 30, (1987)	☐
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31-Oct-2012 08:19
PAT66157-EP-EPA
Entered by: sereich2

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

World Intellectual Property Organization
International Bureau

PCT

(43) International Publication Date
29 March 2007 (29.03.2007)

(10) International Publication Number
WO 2007/035621 A1

(51) International Patent Classification:
A61M 5/32 (2006.01) *A61F 9/00* (2006.01)

(21) International Application Number:
PCT/US2006/036260

(22) International Filing Date:
18 September 2006 (18.09.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/717,865 16 September 2005 (16.09.2005) US

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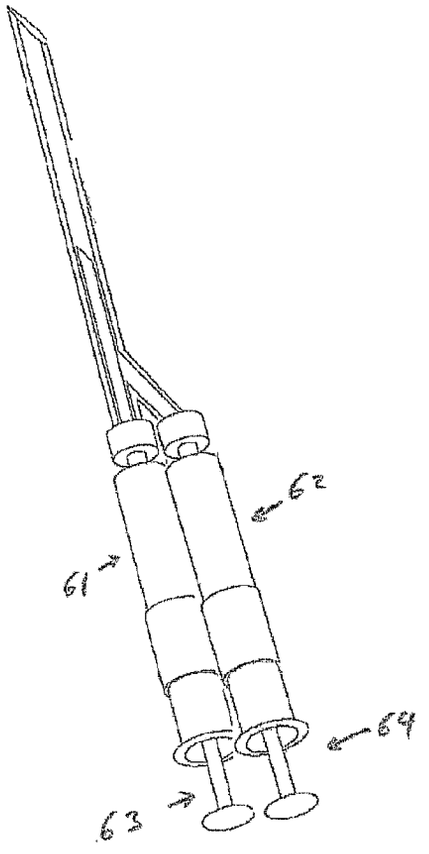
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ,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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: OPHTHALMIC SYRINGE



(57) Abstract: The present invention provides a device for use in ophthalmology. In particular, the present invention provides a device for use in intravitreous administration of ocular agents. The present invention also provides methods of delivering one or more drugs to a human eye and methods for treating an ophthalmic disease, disorder, or condition.

WO 2007/035621 A1

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Published:
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OPHTHALMIC SYRINGE

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial Number 60/717,865 filed September 16, 2005, Attorney Docket No. EYE-036P, which is hereby incorporated in its entirety by reference.

FIELD OF THE INVENTION

The present invention relates to methods of administering ophthalmic medicines and devices related thereto. In particular, the invention relates to intravitreous injection using an ophthalmic syringe and needle.

BACKGROUND OF THE INVENTION

Intravitreous (IVT) injection has been used in the treatment of human ocular disease for nearly a century beginning in 1911 as means to introduce air for retinal tamponade and repair of detachment (J. Ohm, *Albrecht von Graefes Arch Ophthalmol* 1911; 79:442–450). Over the past two decades, the use of intravitreous injection has gained increasing acceptance in the therapeutic management of many intraocular diseases, particularly disorders affecting the posterior segment of the eye (Jager *et al.*, *Retina* 24:676-698, 2004). IVT injection is increasingly being incorporated into management of ocular diseases and the number of approved products for IVT injection is anticipated to grow on the basis of promising results from ongoing clinical studies. Currently formivirsen sodium (Vitravene®, Novartis AG, Basel, Switzerland), ranibizumab injection (Lucentis™, Genentech, Inc., South San Francisco, CA) and pegaptanib sodium (Macugen®, (OSI) Eyetech, Inc. NY, NY) are three medicines approved by the Food and Drug Administration as IVT injections.

Advantages of IVT injection of medicines and diagnostics include the achievement of maximum vitreous concentrations while minimizing toxicity attributed to systemic administration. While these advantages are becoming widely appreciated, the ophthalmology community turns its focus to various complications potentially associated with IVT injection. Risks of IVT injection, some vision threatening, include endophthalmitis, retinal detachment, iritis/uveitis, inflammation, intraocular hemorrhage, ocular hypertension, hypotony,

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pneumatic retinopexy, and cataract (R.D. Jager *et al.*, *Retina* 24:676-698, 2004 and C.N. Ta, *Retina*, 24:699-705, 2004).

Endophthalmitis is a condition in which the tissues inside the eyeball become inflamed and is generally caused by bacterial infection. The most common sources of bacteria causing postoperative endophthalmitis are believed to be the patient's conjunctiva or eyelids. Unless treated effectively, endophthalmitis can rapidly lead to severe vision loss or blindness. The relative risks of developing postoperative endophthalmitis depend on a number of factors, including the presence of eyelid or conjunctival diseases, the patient's general health, the use of immunosuppressant medications, the type of intraocular surgery, and intraoperative complications. Of these factors, intraoperative complications, particularly breaks in the posterior capsule with vitreous loss, carry the greatest risk for the development of endophthalmitis.

Although intravitreous injection is a simple procedure with a small wound, it has been demonstrated that bacteria potentially introduced by the procedure are sufficient to induce endophthalmitis, which is likely due to the inability of the vitreous to clear the infectious microorganisms. Other equally plausible explanations for the apparent high risk of endophthalmitis after intravitreous injections may be the very limited sample size as well as publication bias. It is important, nevertheless, to minimize the risk of developing endophthalmitis by reducing or eliminating bacteria from the ocular surface at the time of the injection and to strictly adhere to aseptic technique. The use of topical antibiotics has been shown to reduce conjunctival and eyelid bacterial flora, which may in turn also decrease the risk of endophthalmitis.

Because transient increases in intra-ocular pressure (IOP) may cause mild discomfort and can be associated in rare instances with irreversible damage to retinal ganglion cells and/or retinal vascular occlusion, many investigators reported using prophylactic and/or therapeutic measures to prevent increases in IOP after IVT injection. These have included the use of aqueous paracentesis, preoperative treatment with pressure-lowering agents and digital massage or the use of a Honan IOP reducer.

Particulate contaminants present in a drug, in a syringe, or in or on materials used at the time of injection also may have the potential to induce detrimental effects when injected into the vitreous. This has been demonstrated in the case of glove lubricants, which are

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highly inflammatory when injected into the posterior ocular chamber (H.S. Park, *Korean J. Ophthalmol.* 1997; 11:51-59).

Other serious complications rarely occurred after IVT injection, making it difficult, in most instances, to determine whether these were truly injection-related or simply sporadic, unrelated comorbidities.

Serious adverse events are for the most part transient and/or treatable, and the risks of serious adverse events reported after IVT injection is low. Even so, there is a need for improved devices and methods for IVT injection. The risks and benefits of IVT injection will likely carry increased weight in patient and clinician treatment as more treatment options become available.

Guidelines for IVT injection are continuing to evolve (L.P. Aiello *et al., Retina*, 24:S3-S19, 2004). For example, povidone iodine and an antibiotic are administered prior to IVT injection. Also, IVT injections are generally performed with a sterile surgical drape and lid speculum in place and a 27 or 30 gauge needle is typically used with an injection site 3.5-4.0 mm posterior to the limbus.

As new treatment modalities for macular diseases become available, the number of intravitreous injections administered is expected to increase dramatically. For example, intravitreous injection of the vascular endothelial growth factor (VEGF) inhibitor, Macugen®, has become available for the treatment of age-related macular degeneration. Also, intravitreous injections of triamcinolone acetonide are now commonly used for the treatment of macular edema.

The prevalence of endophthalmitis after intravitreous injection of anti-VEGF agents is unknown. Due to the very limited data regarding the rate of endophthalmitis after intravitreous injections, it is difficult to speculate about the true prevalence of endophthalmitis after these types of procedures. The increased use of intravitreous injections for the delivery of these agents to the retina will provide data regarding the prevalence and risk factors for post-injection endophthalmitis and in the future define a more accurate rate of endophthalmitis.

Drug delivery into the eye is challenging because the anatomy, physiology and biochemistry of the eye includes several defensive barriers that render ocular tissues

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impervious to foreign substances. Techniques used for administering active agents into the eye include systemic routes, intraocular injections, injections around the eye, intraocular implants, and topical applications. Patient acceptance and safety are key issues that play a key role as to which treatments are used.

Ocular bioavailability of drugs applied topically in formulations such as eye drops is very poor. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of the eye, and by other concomitant factors, for example: drainage of the instilled solutions; lacrhymation, tear evaporation; non-productive absorption/adsorption such as conjunctival absorption, poor corneal permeability, binding by the lachrymal proteins, and metabolism.

Alternative approaches to delivery include *in situ* activated gel-forming systems, mucoadhesive formulations, ocular penetration enhancers and ophthalmic inserts. *In situ* activated gel-forming systems are liquid vehicles that undergo a viscosity increase upon instillation in the eye, thus favoring pre-corneal retention. Such a change in viscosity can be triggered by a change in temperature, pH or electrolyte composition. Mucoadhesive formulations are vehicles containing polymers that adhere via non-covalent bonds to conjunctival mucin, thus ensuring contact of the medication with the pre-corneal tissues until mucin turnover causes elimination of the polymer. Ocular penetration enhancers are mainly surface active agents that are applied to the cornea to enhance the permeability of superficial cells by destroying the cell membranes and causing cell lysis in a dose-dependent manner. Ophthalmic inserts are solid devices intended to be placed in the conjunctival sac and to deliver the drug at a comparatively slow rate. One such device is Ocusert®, by Alza Corporation, which is a diffusion unit consisting of a drug reservoir enclosed by two release-controlling membranes made of a copolymer. M.F. Saettone provides a review of continued endeavors devoted to ocular delivery. ("Progress and Problems in Ophthalmic Drug Delivery", *Business Briefing: Pharmatech, Future Drug Delivery*, 2002, 167-171).

Many types of ophthalmic surgeries such as cataract surgery require use of various fluids which are both delivered and removed from the eye over the course of the surgery. The simultaneous delivery of two or more therapeutics typically requires multiple separate needle penetrations. In areas where bacterial infection and/or structural damage are a concern, the risks associated with multiple injections may become unacceptable. Multiple injections may be circumvented by using a multi-compartment syringe or a double-barrel syringe.

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Administration of multiple viscoelastic solutions with a multi-compartment syringe is described in US Patent Application Publication No. 2004/0167480. A double-barrel syringe for ophthalmic surgeries is described in US Patent Application Publication No. 2004/0064102.

Such invasive intraocular administrations may not be favorable because they cause patient discomfort and sometimes fear, while risking permanent tissue damage. A device which allows the simultaneous or sequential delivery of a therapeutic while requiring a single needle penetration would significantly reduce any needle associated complications.

SUMMARY OF THE INVENTION

The present invention provides a device for use in ophthalmology. In particular, the present invention provides a device for use in intravitreous administration of ocular agents. The present invention also provides methods of delivering one or more drugs to a human eye.

In one aspect, the invention relates to ophthalmic drug delivery devices and features a device for delivery of a therapeutic agent to the eye of a mammal.

The invention features a drug delivery device for delivering a therapeutic compound to the eye and drug delivery methods related thereto. The invention also features a syringe for intravitreal delivery and methods of using the syringe to treat an ophthalmic disease, disorder, or condition.

Other features and advantages of the invention will be apparent from the following description, the drawings, and the claims.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a needle assembly comprising a luer hub, a cannula and a needle tip having a standard bevel.

Figure 2 is a schematic representation of a needle assembly comprising a luer hub, a cannula and a needle tip shield.

Figure 3 is a schematic representation of a syringe and needle assembly comprising a low dead space hub assembly.

Figure 4 shows drawings of a first embodiment of a double barrel syringe.

Figure 5 shows drawings of a first embodiment of a double barrel syringe.

Figure 6 is a schematic representation of a fluid exchange device.

Figure 7 is a schematic representation of a tandem syringe.

Figure 8 is a graph showing penetration force required by various needles.

DETAILED DESCRIPTION OF THE INVENTION

One aspect provides a syringe useful in ophthalmic applications for delivery of a material into the eye.

Needle

Any suitable needle may be used. Suitable needles provide facile penetration of the sclera with minimal injury. A needle typically includes an elongated tube with an outside surface, a proximal end, a distal end and an open bore therethrough. As seen in Figure 1, the needle assembly 20 may have a hub 23 attached to the proximal end of the needle 22 that is used to attach the needle to a syringe. In one embodiment the hub is a Luer hub.

The needle may be attached to the syringe permanently (e.g., staked) or may be attached to the syringe by a Luer fitting. The Luer fitting may be a standard Luer fitting, Luer slip fitting or a Luer lock fitting. The Luer fitting has either a tip (male) or hub (female)

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component, and provides the ability to insure leak-proof and mechanically secure connections to any other device having a mating Luer fitting. Luer connectors can comprise round and tapered male and matching female mating surfaces. Luer connectors can form a locking configuration by adding a threaded locking collar to the male luer connector, which mates with ears on the female luer connector, thereby providing a positive "locked" connection. Luer fittings have several advantages. Luer fittings provide compatibility among various medical devices, offering the clinician the benefits of choosing a preferred needle. In addition, Luer-lock connections insure against possibility of needle coming off of the syringe during the injection procedure. Standards for Luer fittings are described in American National Standard ANSI/HIMA MD 70.1-1983 and the International Standard ISO-594-1 and ISO-7886-1.

A non-standard Luer fitting may be used. Examples of non-standard Luer fittings include, but are not limited to, the Tru-Lok™ fluid transfer adaptor by Becton Dickinson. Other non-standard fittings include Tyco Health Care, Kendall Monoject® low dead space (LDS) needles featuring tri bevel, anti-coring, stainless steel needles. Examples of low waste space fittings are found in US Patent Nos. 6,840,291, 5,902,277 5,902,271, 5,902,270 5,902,269 5,782,803, the contents of each are hereby incorporated by reference in its entirety.

The needle may also be attached to the syringe via a ceramic coated tip (CCT) interface, i.e. 'press fit'.

In one embodiment, the needle is beveled and coated with a suitable silicone. In one embodiment, the needle is a PrecisionGlide® needle available from Becton-Dickenson. Suitable PrecisionGlide® needles include but are not limited to a ½ inch 30 gauge needle and a ½ inch 27 gauge needle. In one embodiment, the needle is a PrecisionGlide® shown in Figure 3. Referring to the figure, the needle comprises a polypropylene Luer hub 33 and a stainless steel cannula 34, lubricated with silicone, having a three-bevel point, attached to the hub via an epoxy joint.

The needle tip may have a standard bevel. In one embodiment, the needle may have more than one bevel. In one embodiment, the needle has three bevels. In one embodiment, the needle has five bevels. Examples of a five-bevel needle are described in US Patent No. 6,629,963, and 6,009,933, US patent Application publication Nos. 2044/0111066, 2004/0030303 and PCT Application No. 2005/016420

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In one embodiment, the needle is a coated needle. In one embodiment, the needle is a lubricated needle. The needle optionally includes a lubricious coating applied to and adherent to the outside surface of the tube, as described in US Patent No. 5,911,711.

In one embodiment, the coating is a silicone coating. Any suitable silicone coating may be used. Examples of suitable coatings include, but are not limited to, those available from SurModics, Eden Prairie, MN (see US Patent Nos. 6,706,408, 6,669,994, 6,254,634 and 6,121,027).

In one embodiment the coating is a medicated coating.

Preferably the needle is a 27 gauge needle or smaller. In one embodiment the needle is a 30 gauge needle.

In one embodiment, the needle has a length of less than 1 inch. In another embodiment, the needle has a length of about 0.5 inches.

Needle tip shield

As seen in Figure 2, the needle assembly 20 may comprises a needle tip shield 21 enclosing needle 22. Needle 22 is attached to luer hub 23 via epoxy joint 24. In one embodiment, the tip shield 21 is rigid. Examples of suitable rigid shields include but are not limited to those disclosed in US Patent No. 4,986,818. As depicted in Figure 2, the tip shield is not in contact with the needle tip. Needle tip shields in contact with the needle potentially dull the needle and wipe away any lubrication on the needle. In another embodiment, the tip shield comprises one or more apertures or is permeable to sterilizing gases. The apertures may facilitate sterilization by allowing sterilizing gasses or steam to access the interior of the needle shield. In a particular embodiment, the tip shield is synthetic isoprene, ethylene oxide (EtO) or hydrogen peroxide (H_2O_2) permeable. In another embodiment, the syringe barrels, stoppers and plunger rod components and assemblies can also be gamma irradiated. In one embodiment, the needle tip shield comprises a polypropylene. In another embodiment, the needle tip shield comprises a styrene block thermoplastic elastomer.

Penetration Force

The needles of the present invention are used for penetration of the scleral tissue for administration of the syringe contents into the vitreous. Preferably the needles require a low

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penetration force. Preferably the needles require a low penetration force with low variability. In one embodiment, the needles require a penetration force of less than 500 grams (g). In another embodiment, the needles require a penetration force of less than 100 grams (g). In another embodiment, the needles require a penetration force of less than 50 grams (g).

In one embodiment, the needles require a penetration force with a variability range of +/- 20 %. In one embodiment, the needles require a penetration force with a variability range of +/- 50 g. In another embodiment, the needles require a penetration force with a variability range of +/- 20 g

In one embodiment, the penetration force is reduced by reducing the needles coefficient of friction. In one embodiment the penetration force is reduced by using a lubricious coating on the needle.

Syringe

The syringe barrel is typically made of glass or a thermoplastic material. In one embodiment the syringe is a 1 mL Type I glass barrel syringe sealed with a bromobutyl rubber stopper. Examples of pre-filled syringes are found in US Patent No. 4,252,118. In one embodiment the syringe is a BD Hypak SCF® syringe. In a particular embodiment, the syringe is a single dose, pre-filled syringe. In one embodiment, the syringe barrel has a volume of 1 mL or less. In a particular embodiment, the syringe barrel has a microliter volume. The syringe barrels of the present invention may further be provided with graduations to assist in precision filling of the barrel.

In one embodiment, the syringe is a plastic syringe. In another embodiment, the syringe comprises a cyclic olefin copolymer (COC). In another embodiment the cyclic olefin copolymer is TopPac® (Schott).

In another embodiment, the final Luer formation is made using a platinum wire. In a particular embodiment, the syringe is substantially free of tungsten. Staked needle production requires a small hole and seat for gluing in the needle. The small hole requires a high temperature tungsten pin. Some of the tungsten pin material may shed into the glass during processing. Luer lock syringes are alternatively formed using a platinum pin material. The platinum may not leave a significant residue in the glass as compared to tungsten.

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Optimal particulate matter concentrations may be achieved primarily through strict control of the environment and material cleanliness.

Volume

The ophthalmic injection solutions of the present invention are useful as microliter (μL)-volume injections. Microliter (μL)-volume injections may also be referred to as "ultra-low volume injections". In one embodiment, the ophthalmic injection solution to be delivered has a volume of about 1.0 mL (1000 μL) or less. In another embodiment the ophthalmic injection solution to be delivered has a volume of about 200 μL or less. In another embodiment the ophthalmic injection solution to be delivered has a volume of about 100 μL or less. In another embodiment the ophthalmic injection solution to be delivered has a volume of about 90 μL. In another embodiment the ophthalmic injection solution to be delivered has a volume of about 50 μL.

Sub-Visible Particulate Matter

The ophthalmic injection solutions of the present invention, including solutions constituted from sterile solids intended for parenteral use, as used herein are substantially free from particles that can be observed on visual inspection. There are also strict controls on sub-visible particulate matter for ophthalmic injections. The ophthalmic injection solutions of the present invention can be tested by a light obscuration procedure or may be tested by a microscopic procedure as described in USP Chapter <788>. United States Pharmacopoeia (USP) Chapters <788> *Particulate Matter in Injections* and <789> *Particulate Matter in Ophthalmic Solutions* describe physical tests for the purpose of enumerating extraneous particles within specific size ranges. The United States Pharmacopoeia, 28th revision and the National Formulary, 23rd edition (USP28-NF23), The United States Pharmacopeial Convention, Inc (2005), is hereby incorporated by reference in its entirety.

In one embodiment, the ophthalmic solution contained within the syringe of the present invention has a 10μm-size or larger sub-visible particulate count of less than or equal to about 60 particles per mL, a 25μm-size or larger sub-visible particulate count of less than or equal to about 10 particles per mL, or a 50μm-size or larger sub-visible particulate count of less than or equal to about 5 particles per mL. In one particular embodiment, the concentration of sub-visible particulate matter is less than or equal to about 150 ppb.

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In one embodiment the ophthalmic solution contained within the syringe of the present invention is subject to the particulate matter limits set forth in USP <789> wherein the average number of particles present in the units tested does not exceed the values listed in Table 1.

Table 1.

	Diameter		
	≥ 10 μm	≥ 25 μm	≥ 50 μm
Number of particles	50 per mL	5 per mL	2 per mL

In one embodiment, the ophthalmic solution contained within the syringe of the present invention has a 10μm-size or larger sub-visible particulate count of less than or equal to about 20 particles per mL, a 25μm-size or larger sub-visible particulate count of less than or equal to about 5 particles per mL, or a 50μm-size or larger sub-visible particulate count of less than or equal to about 2 particles per mL. In one particular embodiment, the concentration of sub-visible particulate matter contained within the syringe of the present invention is less than or equal to about 150 ppb.

Waste volume

As represented in Figure 3, the syringe assembly has a low waste space, which is defined as the volume located in the syringe tip 31 of syringe barrel 32, needle hub 33 and needle cannula 34. The International Standard ISO-7886-1 identifies the maximum waste space for a 3ml syringe tip to be 0.07 mL.

In a particular embodiment, the needle/syringe combination of the present invention has a low waste space. Examples of low waste space fittings are found in US Patent Nos. 6,840,291, 5,902,277 5,902,271, 5,902,270 5,902,269 5,782,803, the contents of each is hereby incorporated by reference in its entirety. An example of a needle/syringe combination having a low waste space includes Tru-lok™ fluid transfer adaptors by Becton Dickinson

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(US Patent No. 6,840,291) and Monoject® low dead space (LDS) needles Tyco Health Care, Kendall (catalog Nos. 1188005058 and 1188001112) featuring tri bevel, anti-coring, stainless steel needles.

The needle/syringe combination of the present invention has a waste space of less than 0.1 mL. In one embodiment, the waste space is less than 0.05 mL. In another embodiment, the waste space is approx. 50-60 μL. In one embodiment, the waste space for the 1 mL Hypak Luer tip syringe is from about 0.040 to about 0.050 mL. In another embodiment, the waste space is less than 0.001 mL.

Syringe tip cap

The syringe assembly may comprise a syringe tip cap. The syringe tip cap is used to seal the barrel of a prefilled syringe. In one embodiment, the syringe tip cap is a plastic rigid tip cap. Examples of suitable syringe tip caps include, but are not limited to, those found in US Patent Nos. 6,190,364; 6,196,998; 6,520,935 and 5,833,653; US patent Application Publication No. 2004/0215148 and US design patent Nos. 457954S1 and 493526S1. In one embodiment, the rigid tip cap is an elastomeric formulation comprising an elastomer, reinforcement and a curing system. In another embodiment, the elastomer is a synthetic isoprene blend, the reinforcement is an inert material, and the curing system is a resin. In another embodiment, the syringe tip cap comprises a chloro/bromobutyl rubber stopper.

Multiple barrel syringe

Another aspect of the invention provides a syringe comprising more than one barrel. The multiple barrel syringe may permit simultaneous, selective or sequential delivery of one or more different materials.

In one embodiment the syringe comprises a first and second barrel positioned in side-by-side relationship including a first and second plunger for telescoping movement within their respective chambers (see US Patent Application Publication No. 2004/0064102, which is herby incorporated by reference in its entirety). The plungers are optionally connected to a common handle allowing for the dispensing of the materials from the two chambers simultaneously at the same rate, as disclosed, for example, in US Patent No. 5,792,103. In another embodiment, the plungers are detachably connected to the plunger stopper.

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Figures 4 and 5 show examples of dual barrel syringes for the simultaneous or sequential delivery of two or more therapeutic agents. The syringe includes a first barrel, a second barrel, and one or more needles. Each barrel contains a therapeutic agent dissolved or suspended in a liquid formulation. Referring now to the drawings, there is seen in Figures 4 and 5 first and second embodiments of the double barrel syringe which differ in the respect that the first embodiment (Figure 4) is configured for direct filling of the first and second materials inside their respective barrels while the second embodiment (Figures 5) is configured for insertion of pre-filled first and second carpules into the first and second barrels, respectively.

Referring to the first embodiment shown in Figure 4, the syringe comprises first and second barrels 41, 42 each having an internal chamber 43, 44 for holding a quantity of first and second, liquefied materials therein, respectively. The first and second barrels 41, 42 are arranged in side-by-side relationship with each other. First and second plungers 45, 46 are positioned for sliding within the first and second barrels 41, 42 for telescoping movement therein, respectively. The syringe tip 47 is located adjacent the distal ends of the first and second barrels 41, 42 and is in common, fluid communication therewith via exit orifices 48 and 49. The exit orifices 48 and 49 provide the pathway for the first and second materials, respectively, to tip 47 and thereby allowing passage of the first and second therethrough. Tip 47 may be in the form of a needle directly attached to the syringe body, or may be in the form of a cannula which is attached to the syringe body via a Luer lock.

Referring to the second embodiment shown in Figure 5, the syringe comprises first and second carpules 51, 52 removably insertable within said first and second barrels 53, 54, respectively. First and second syringe needles 55, 56 located in the first and second barrels 53, 54 adjacent the distal ends thereof. As such, upon fully inserting the first and second carpules 51, 52 in the first and second barrels 53, 54, the first and second syringe needles 55, 56 pierce the carpule plug provided at the respective carpule distal end. The first and second needles 55, 56 extend into the tip 57.

A single needle with two or more hollow bores may perform both injections or multiple needles may be used. When one needle is employed, two cannulas, one affixed to one of each barrel, may lead to the one needle. Alternatively, when one needle is employed, the hollow bores may be arranged in a concentric pattern. In such a concentric pattern, one bore is for introduction of a first fluid into the vitreous, and one bore is for introduction of

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second fluid into the vitreous. When two needles are employed, a second needle may be attached to the exterior of a first needle. Needles may be manufactured from standard materials, e.g., stainless steel, by methods known in the art.

PCT Publication No. WO 2004/073765 describes, in part, a Fluid Exchange System (FES) which is designed to remove a specific volume from a closed system and sequentially deliver a measured volume. By replacing the vacuum chamber with a housing that can accept a pre-filled syringe and removing the air vents (manifolds) an addition therapeutic may be delivered utilizing the original needle penetration. Additional syringe housings could also be added to allow for multiple sequential administrations. Figure 6 illustrates an apparatus comprising a first and second housing 61, 62 capable of accepting a first and second prefilled syringe 63, 64.

In one aspect, multiple medicaments can be administered using a tandem syringe. A tandem syringe typically comprises two or more compartments within one external barrel. Examples of tandem syringes include but are not limited to those found in US Patent Nos. 4,313,440; 4,715,854; 5,102,388; 5,298,024; 6,132,400; and US Patent Application Publication No. 2004/0167480, each of which is herby incorporated by reference in its entirety.

In one embodiment, the tandem syringe comprises an outer first compartment including a first sealing member and an inner second compartment in which the first sealing member functions as the plunger for the outer first compartment (see Figure 7). Referring to Figure 7, the outer first compartment 71 is filled with a first injection solution. The inner second compartment 72 is filled with a second injection solution. The inner second compartment comprises a first sealing member 73 and functions as the piston for the first compartment. When the first injection solution in completely administered, the first sealing member 73 is pierced using a piercing device 74 at the distal end of the outer first chamber. Once the first sealing member 73 is pierced, the second injection solution is administered by pushing stopper 75 with plunger 77 thereby forcing the second injection solution past stopper 73 and out through the needle 76.

Each compartment may be pre-filled with its injection solution separately providing for storage of the injection solutions without mixing or contact with each other.

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In one embodiment compartment 71 (inner compartment) contains the final solution to be injected. Compartment 71 is first loaded separately, then assembled with the main housing forming compartment 72. Compartment 72 in then loaded with the first solution to be injected.

Advantages

The syringes of the present invention have several advantages. Advantages include the benefits of ease of use, flexibility, cost effectiveness, patient comfort and safety. An advantage of using a non-fixed needle/syringe combination, such as one using a Luer fitting, as described herein is the allowance for a choice of application needle. For example, a practitioner may select either a 27 or 30 gauge disposable needle. A non-fixed needle is typically sharper than a fixed needle because the non-fixed needle will not be susceptible to dulling as a result of contact with the sheath needed for a fixed needle pre-filled syringe. Sharper needles reduce patient discomfort and reduce the risk of infection.

Definitions

The grammatically correct and preferred term "intravitreous" is used herein and in the art. The term "intravitreal" is used colloquially as an alternative to the term "intravitreous" for injections into the eye's vitreous humor between the lens and the retina.

"Particulate matter" includes mobile, randomly sourced, extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis because of the small amount of material they represent or because of their heterogeneous composition.

The portion of the device that is toward the practitioner is termed "proximal" and the portion of the device that is toward the patient is termed "distal."

"Penetration force" is the measure of force applied to the needle prior to the needle cutting the tissue. Penetration force is typically measured throughout the art in grams (g).

"Drag force" is a measure of force applied to the needle required to continue the penetration into the tissue.

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An "Injection" is a preparation intended for parenteral administration. Injections include, but are not limited to, liquid preparations that are drug substances or solutions/suspensions thereof.

By "substantially constant pressure" is meant pressure that is constant with minor, temporary variations due to filling, emptying, or a change in osmotic pressure of the surrounding liquid.

"Parenteral" articles are preparations intended for injection through the skin or other external boundary tissue, rather than through the alimentary canal, so that the active substances they contain are administered, using gravity or force, directly into a blood vessel, organ, tissue, or lesion. Parenteral articles are prepared by methods designed to ensure that they meet Pharmacopeial requirements for sterility, pyrogens, particulate matter, and other contaminants (USP Chapter 1).

The designation "Small-Volume Injection" applies to an injection that is packaged in containers labeled as containing 100 mL or less.

The designation "Microliter-volume Injection" or "Ultra-Low-Volume Injection" applies to an Injection that is packaged in containers labeled as containing 1.0 mL (1000 μL) or less.

As used herein, the term "dead space" or "Waste space" is the volume of injection solution within the syringe/needle assembly containing any residual injection solution present following an injection that does not get evacuated from the syringe during the injection.

By "therapeutic agent" is meant any compound or mixture of compounds that provide a therapeutic effect for one or more diseases, disorders, or conditions. Such compounds include, without limitation, small organic or inorganic molecules, proteins (e.g., antibodies), peptides, lipids (e.g., steroids) and nucleic acids (e.g., aptamers). Therapeutic agents are, for example, antibiotics, analgesics, anti-inflammatory compounds, or any other compound for the treatment of a disease, disorder, or condition.

By "treating" is meant the medical management of a patient with the intent that a cure, amelioration, or prevention of a disease, pathological condition, or disorder will result. This term includes active treatment, that is, treatment directed specifically toward improvement of a

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disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventive treatment, that is, treatment directed to prevention of the disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the disease, pathological condition, or disorder. The term "treating" also includes symptomatic treatment, that is, treatment directed toward constitutional symptoms of the disease, pathological condition, or disorder.

Ophthalmic solutions are sterile solutions, essentially free from foreign particles, suitably compounded and packaged for instillation or injection into the eye. Preparation of an ophthalmic solution requires careful consideration of such factors as the inherent toxicity of the drug itself, isotonicity value, the need for buffering agents, the need for a preservative (and, if needed, its selection), sterilization, and proper packaging.

While specific reference has been made to the use of the devices of the present invention to administer therapeutic agents to the eye, it is to be understood that the present invention can be used to deliver a therapeutic agent to any desired site, including, but not limited to, intraorbital, intraocular, intraaural, intratympanic, intrathecal, intracavitary, peritumoral, intratumoral, intraspinal, epidural, intracranial, and intracardial.

A device of the invention may be used in the treatment of any eye disease. A device of the invention may also be used to direct a therapeutic agent to a particular eye tissue, e.g., the retina or the choroid. The therapeutic agent or combination of agents will be chosen based on the disease, disorder, or condition being treated. In addition to a therapeutic agent for a particular condition, other compounds may be included for secondary effects, for example, an antibiotic to prevent microbial growth. The amount and frequency of the dosage will depend on the disease, disorder, or condition being treated and the therapeutic agent employed. One skilled in the art can make this determination.

Therapeutic agents that may be employed in the device of the invention include, without limitation, small molecules, hormones, proteins, peptides, aptamers, antibodies, lipids, glycolipids, DNA, RNA, PNA, enzymes, sugars, saccharides, glycoproteins, polymers,

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metalloproteases, transition metals, or chelators. In addition, nucleic acid vectors can also be delivered wherein the nucleic acid may be expressed to produce a protein that may have a variety of pharmacological, physiological or immunological activities. Macromolecules with a molecular weight of about 5 kDa to about 500 kDa may also be used in accordance with the invention.

For ophthalmic drug delivery applications, exemplary disease states include macular degeneration, diabetic retinopathy, glaucoma, optic disc neovascularization, iris neovascularization, retinal neovascularization, choroidal neovascularization, pannus, pterygium, macular edema, vascular retinopathy, retinal vein occlusion, histoplasmosis, ischemic retinal disease, retinal degeneration, uveitis, inflammatory diseases of the retina, keratitis, cytomegalovirus retinitis, an infection, conjunctivitis, cystoid macular edema, cancer, and proliferative vitreoretinopathy.

Classes of therapeutic agents include anti-infectives including, without limitation, antibiotics, antivirals, and antifungals; analgesics; antiallergenic agents; mast cell stabilizers; steroidal and non-steroidal anti-inflammatory agents; decongestants; anti-glaucoma agents including, without limitation, adrenergics, beta-adrenergic blocking agents, alpha-adrenergic blocking agonists, parasympathomimetic agents, cholinesterase inhibitors, carbonic anhydrase inhibitors, and protaglandins; antioxidants; nutritional supplements; angiogenesis inhibitors; antimetabolites; fibrinolytics; wound modulating agents; neuroprotective drugs; angiostatic steroids; mydriatics; cyclopegic mydriatics; miotics; vasoconstrictors; vasodilators; anticlotting agents; anticancer agents; immunomodulatory agents; VEGF antagonists; immunosuppresant agents; and combinations and prodrugs thereof.

Specific therapeutic agents include MACUGEN® (pegaptanib sodium injection) as described in U.S. Patent No. 6,051,698, herein incorporated in its entirety by reference. Pegaptanib sodium is also referred to as EYE001 or NX1838.

Pegaptanib sodium is a covalent conjugate of an oligonucleotide of twenty-eight nucleotides in length that terminates in a pentylamino linker, to which two 20-kilodalton (kDa) monomethoxypolyethylene glycol (PEG) units are covalently attached via the two amino groups on a lysine residue. The molecular formula for pegaptanib sodium is $C_{294}H_{342}F_{13}N_{107}Na_{28}O_{188}P_{28}(C_2H_4O)_n$ (where n is approximately 900) and the molecular weight is approximately 50 kDa.

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The chemical name for pegaptanib sodium is as follows: RNA, ((2'-deoxy-2'-fluoro)C-G_m-G_m-AA-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-A_m-G_m-(2'-deoxy-2′ -fluoro)U-G_m-A_m-A_m-(2'-deoxy-2'-fluoro)U-G_m-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)U-A_m-(2'-deoxy-2'-fluoro)U-A_m-(2'-deoxy-2'-fluoro)C-A_m-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)C-G_m-(3'→3')-dT), 5'-ester with α, α '-[4,12-dioxo-6-[[[5-(phosphoonoxy)pentyl]amino]carbonyl]-3,13-dioxa-5,11-diaza-1,15-pentadecanediyl]bis[ω-methoxypoly(oxy-1,2-ethanediyl)], sodium salt.

MACUGEN® (pegaptanib sodium injection) is a sterile, aqueous solution containing pegaptanib sodium for intravitreous injection. Macugen is supplied in a single-dose, pre-filled syringe and is formulated as a 3.47 mg/mL solution, measured as the free acid form of the oligonucleotide. The active ingredient is 0.3 mg of the free acid form of the oligonucleotide without polyethylene glycol, in a nominal volume of 90 μL. This dose is equivalent to 1.6 mg of pegaptanib sodium (PEGylated oligonucleotide) or 0.32 mg when expressed as the sodium salt form of the oligonucleotide moiety. The product is a sterile, clear, preservative-free solution containing sodium chloride, monobasic sodium phosphate monohydrate, dibasic sodium phosphate heptahydrate, hydrochloric acid, and/or sodium hydroxide to adjust the pH and water for injection. Macugen is formulated to have an osmolality of 280-360 mOsm/Kg, and a pH of 6-7.

Dosage levels of pegaptanib sodium on the order of about 1 μg/kg to 100 mg/kg of body weight per administration are useful in the treatment of neovascular disorders. Examples of formulations are found in WO 03/039404, which is hereby incorporated by reference in its entirety. In some embodiments, pegaptanib sodium is administered at a dosage of about 0.1 mg to about 1.0 mg locally into the eye, wherein the treatment is effective to treat occult, minimally classic, and predominantly classic forms of wet macular degeneration. When administered directly to the eye, the dosage range is about 0.3 mg to about 3 mg per eye, in some embodiments the dosage range is about 0.1 mg to about 1.0 mg per eye. In one embodiment, pegaptanib sodium is administered in a therapeutically effective amount of about 0.003 – 3.0 mg, 0.1 – 1.0 mg, or about 0.3 mg. In one embodiment, pegaptanib sodium is present in an ophthalmic injection solution formulation at a concentration ranging from 0.003 to 3.0 mg/mL. According to one embodiment, the carrier comprises sodium phosphate and sodium chloride. According to one specific embodiment the carrier comprises 10 mM sodium phosphate and 0.9% sodium chloride.

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According to one embodiment, the dose is effective to achieve a vitreous concentration of the anti-VEGF aptamer of about 10-30 ng/mL. According to another embodiment, the dose is effective to maintain a vitreous concentration of the anti-VEGF aptamer of about 10-30 ng/mL throughout a 6 week dosing interval.

In alternative embodiments, the anti-VEGF agent is an anti-VEGF aptamer and is administered at a dosage of less than 0.3 mg to about 0.003 mg locally into the eye. In some embodiments, the anti-VEGF aptamer is administered at a dosage less than about 0.30 mg. Examples of such formulations are found in US Patent Application Serial No. 60/692,727; which is hereby incorporated by reference in its entirety.

Specific therapeutic agents also include the anti-PDGF aptamer ARC-127 (Archemix Corp., Cambridge, MA), a PEGylated, anti-PDGF aptamer having the sequence CAGGCUACGN CGTAGAGCAU CANTGATCCU GT (SEQ ID NO: 10 from U.S. Patent No. 6,582,918, incorporated herein by reference in its entirety) having 2'-fluoro-2'-deoxyuridine at positions 6, 20 and 30, 2'-fluoro-2'-deoxycytidine at positions 8, 21, 28, and 29, 2'-O-Methyl-2'-deoxyguanosine at positions 9, 15, 17, and 31, 2'-O-Methyl-2'-deoxyadenosine at position 22, hexaethylene-glycol phosphoramidite at "N" in positions 10 and 23, and an inverted orientation T (i.e., 3'-3'-linked) at position 32.

A combination therapy for the treatment of ocular neovascular disorders using a VEGF antagonist and a PDGF antagonist is described in PCT Application No. WO 2005/020972, which is incorporated herein by reference in its entirety. An example of such a therapy comprises the administration of a combination of Macugen® and ARC127.

According to another embodiment, the present invention features a method for treating a patient suffering from an ocular disease, which method includes the following steps: (a) administering to the patient an effective amount of an anti- VEGF aptamer; and (b) providing the patient with phototherapy, such as photodynamic therapy or thermal laser photocoagulation as further described in PCT WO 03/039404, incorporated in its entirety by reference.

In one embodiment of the invention, the photodynamic therapy (PDT) includes the steps of: (i) delivering a photosensitizer to the eye tissue of a patient; and (ii) exposing the photosensitizer to light having a wavelength absorbed by the photosensitizer for a time and at an intensity sufficient to inhibit neovascularization in the patient's eye tissue. A variety of

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photosensitizers may be used, including but not limited to, benzoporphyrin derivatives (BPD), monoaspartyl chlorine, zinc phthalocyanine, tin etiopurpurin, tetrahydroxy tetraphenylporphyrin, and porfimer sodium (PHOTOFRIN), and green porphyrins.

Other therapeutic agents include 4,9(11)-pregnadien-17α,21-diol-3,20-dione, 4,9(11)-pregnadien-17α,21-diol-3,20-dione-21-acetate, combretastatin, timolol, betaxolol, atenolol, brimonidine, acetazolamide, methazolamide, dichlorphenamide, diamox, nimodipine, eliprodil, colchicine, vincristine, cytochalasin B, tetracycline, chlortetracycline, bacitracin, neomycin, polymyxin, gramicidin, oxytetracycline, chloramphenicol, gentamycin, erythromycin, sulfonamides, sulfacetamide, sulfamethizole, sulfisoxazole, fluconazole, nitrofurazone, amphotericin B, ketoconazole, trifluorothymidine, acyclovir, ganciclovir, didanosine, AZT, foscamet, vidarabine, idoxuridine, ribavirin, protease inhibitors, anti-cytomegalovirus agents, methapyriline; chlorpheniramine, pyrilamine pheniramine, hydrocortisone, dexamethasone, fluocinolone, prednisone, prednisolone, methylprednisolone, fluorometholone, betamethasone, triamcinolone, phenylephrine, naphazoline, tetrahydrozoline, pilocarpine, carbachol, diisopropylfluorophosphate, echothiophate iodide, demecarium bromide, atropine sulfate, cyclopentolate, homatropine, scopolamine, tropicamide, eucatropine, epinephrine, heparin, antifibrinogen, fibrinolysin, anti clotting activase, acetohexamide, chlorpropamide, glipizide, glyburide, tolazamide, tolbutamide, insulin, aldose reductase inhibitors, thalidomide, folic acid, 5-fluorouracil, adriamycin, asparaginase, azacytidine, azathioprine, bleomycin, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, cyclosporine, cytarabine, dacarbazine, dactinomycin, daunorubicin, estramustine, etoposide, etretinate, filgrastim, floxuridine, fludarabine, fluoxymesterone, flutamide, goserelin, hydroxyurea, ifosfamide, leuprolide, levamisole, lomustine, nitrogen mustard, melphalan, mercaptopurine, methotrexate, mitomycin, mitotane, pentostatin, pipobroman, plicamycin, procarbazine, sargramostim, streptozocin, tamoxifen, taxol, teniposide, thioguanine, uracil mustard, vinblastine, vindesine, pituitary hormones, , insulin-related growth factor, thyroid hormones, growth hormones, heat shock proteins, immunological response modifiers such as muramyl dipeptide, interferons (including α, β, and γ interferons), interleukin-2, cytokines, FK506, tumor necrosis factor, thymopentin, transforming factor beta2, erythropoietin; antineogenesis proteins, monoclonal antibodies, brain nerve growth factor (BNGF), celiary nerve growth factor (CNGF), vascular endothelial growth factor (VEGF), monoclonal antibodies or aptamers directed against growth factors, and combinations and prodrugs thereof.

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A therapeutic agent may be present in any suitable formulation for delivery to the eye. Methods well known in the art for making formulations are found, for example, in *Remington: The Science and Practice of Pharmacy* (20th ed., A.R. Gennaro ed., Lippincott: Philadelphia, 2000). Therapeutic agents may be administered to humans, domestic pets, livestock, or other animals with a pharmaceutically acceptable diluent, carrier, or excipient.

Therapeutic formulations may be liquid solutions, suspensions, or other formulations deliverable via a needle. Formulations may, for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes.

The therapeutic agent may be admixed with a pharmaceutically acceptable carrier adapted to provide sustained release of the therapeutic agent. Sustained release carriers include emulsions, suspensions, polymeric matrices, microspheres, microcapsules, microparticles, liposomes, multivesicular liposomes, lipospheres, hydrogels, salts, and polymers with the therapeutic agent reversibly bound electrostatically, chemically or by entrapment. Suitable sustained release formulations which may be used are known in the art and are disclosed in, for example, U.S. Patent Nos. 4,865,846, 4,115,544, 5,185,152, 4,078,052, 4,241,046, 4,853,224, 4,865,846, 6,309,669, 5,326,761, 6,071,534, 6,132,766 and 6,277,413 and PCTs WO 01/74400, WO 03/24420, WO 03/028765, WO 02/15888, WO 03/092665 and WO 03/070219, all of which are hereby incorporated in their entirety by reference.

Formulations of the drug may also include a transscleral diffusion promoting agent, such as dimethylsulfoxide, ethanol, dimethylformamide, propylene glycol, N-methylpyrolidone, oleic acid, isopropyl myristate, polar aprotic solvents, polar protic solvents, steroids, sugars, polymers, small molecules, charged small molecules, lipids, peptides, proteins, and surfactants.

A therapeutic agent may be optionally administered as a pharmaceutically acceptable salt, such as a non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as

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hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, or the like. Metal complexes include cations, such as divalent cations including calcium and magnesium, zinc, iron, and the like. In addition, a therapeutic agent may be optionally administered as a pharmaceutically acceptable prodrug, e.g., an ester or amide.

The chemical compounds for use in such therapies may be produced and isolated as described herein or by any standard technique known to those in the field of medicinal chemistry. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the identified compound to patients suffering from a disease, disorder, or condition of the eye. Administration may begin before, during, or after the patient is symptomatic.

Although the above process was described using the syringe of the invention, alternative methods of injection can be employed. Other variations on these configurations will be apparent to one skilled in the art.

EXAMPLES

The following examples serve to illustrate certain useful embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof. Alternative materials and methods can be utilized to obtain similar results.

Example 1

Macugen® Formulation

Macugen® ((OSI) Eyetech, Inc., NY, NY) is formulated at 0.3mg/90μL having a tungsten particulate count of less than 150 ppb. The solution is presented in USP Type I glass barrel syringes fitted with a Luer lock hub and sealed with a bromobutyl rubber plunger stopper. The syringe is fitted with a Luer lock 27-gauge, multi-beveled, silicone coated needle with a rigid plastic outer shield. The needle requires a penetration force of less than 100 g.

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Example 2

Macugen® Formulation

Macugen® (Eyetech Pharmaceuticals, NY, NY) is formulated at 0.3mg/90μL, 0.03mg/90μL or 0.003mg/90μL and presented in USP Type I glass barrel syringes sealed with a bromobutyl rubber plunger stopper. The syringe is fitted with a Luer lock 27-gauge needle with a rigid plastic outer shield. The stoppered syringe is packaged in a foil pouch. A plastic plunger rod and flange adapter are also supplied for administration purposes. These components are provided in a separate foil pouch. Use of the flange is optional and is not required to administer the injection. The drug product is preservative-free and intended for single use by intravitreous injection only. The product should not be used if cloudy or if particles are present.

Active Ingredient: Pegaptanib Sodium Injection formulated as:

- 0.0347mg/mL solution to deliver a dose of 0.003mg pegaptanib sodium injection
- 0.347mg/mL solution to deliver a dose of 0.03mg pegaptanib sodium injection
- 3.47mg/mL solution to deliver a dose of 0.3mg pegaptanib sodium injection

Excipients:

Sodium Chloride, USP
Sodium Phosphate Monobasic, Monohydrate, USP
Sodium Phosphate Dibasic, Heptahydrate, USP
Sodium Hydroxide, USP (as needed)
Hydrochloric acid, USP (as needed)
Water for injection, USP

Preparation

The drug product pegaptanib sodium is a ready-to-use sterile solution provided in a single-use glass syringe. Administration of the syringe contents involves attaching the

threaded plastic plunger rod to the rubber stopper inside the barrel of the syringe. The rubber end cap is then removed to allow administration of the product. An optional flange is provided for administrative purposes.

Example 3

Intravitreous Injection

1% Mydriacyl and 2.5% Phenylephrine are applied topically to the study eye to achieve adequate pupillary dilation. Two to three drops of 50% saline diluted 10% povidone-iodine (betadine) solution are instilled into the eye. In the event of allergy to iodine, a drop of topical antibiotic is placed on the conjunctiva in place of iodine. A subconjunctival injection of 0.5 ml 2% xylocaine without epinephrine is administered in the inferotemporal quadrant in all patients - 3.0 to 3.5 mm from the limbus in aphakic/pseudophakic patients, and 3.5 to 4.0 mm in phakic patients. Investigators are instructed to select one of two pre-injection procedures (Options A and B, below). For patients with iodine allergy, investigators are required follow Option A, instilling one additional drop of antibiotic instead of povidone-iodine.

A. Administer topical ofloxacin, levofloxacin, or an antibiotic drop with comparable antimicrobial coverage for three days prior to the treatment followed by three consecutive drops of antibiotic and several drops of 5% povidone-iodine immediately before the treatment

B. Administer three consecutive drops of antibiotic and a 5% povidone-iodine flush of the fornices and caruncle with at least 10 cc of solution just prior to treatment.

Prior to treatment, topical antibiotic drops are administered 3 times separated by at least 5 minutes within one hour prior to treatment.

For patients who are prepared under Option A, following the last dose of antibiotic, the investigator instills two or three drops of 5% povidone-iodine into the eye. Using sterile gloves and cotton-tip applicators soaked in 5% povidone iodine, the investigator scrubs the eyelids, the upper and lower eyelid margins, and the caruncle 3 times. In the event of allergy to iodine, one additional drop of antibiotic is instilled instead of povidone-iodine.

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For patients who are prepared under Option B, the investigator waits at least 5 minutes after the last dose of antibiotic to perform a 5% povidone-iodine flush, irrigating the fornices and the caruncle with at least 10 cc of 5% povidone-iodine using a forced stream from a syringe connected to an angio-catheter to effect mechanical debridement.

After changing gloves, the investigator isolates the ocular field with a drape, pinning the eyelashes to the eyelids, and places one or two drops of 5% povidone-iodine on the ocular surface at the intended treatment site. An eyelid speculum is used for all injections.

Example 4

Needle Penetration of Porcine Sclera

Data was recorded on a Universal Material Testing Machine (Instron Corporation, Norwood, MA) to mimic insertion of the needle into the eye 6 mm below the sclera. The data was then transferred to a MINITAB® statistical software (Minitab, Inc, State College, PA) for analysis.

The samples (n=15) were tested as follows:

Control: Becton Dickenson (BD) 1cc TB syringe paired with a 27Ga ½ inch PrecisionGlide™ needle.

Group 1: HYPAK 1 mL long syringe 27Ga five-bevel ½ inch needle

Group 2: HYPAK 1 mL long syringe 29Ga five-bevel ½ inch needle

Group 3: BD 1mL TB syringe paired with a 30Ga ½ inch precision glide needle

A porcine eye is fixed in test stand and pressurized to standard conditions for blade tests to simulate live conditions. The syringe is placed in the test position on the Instron device. The crosshead speed is set to 150 millimeters per minute. The needle is penetrated about ½ the way into the sclera. The penetration location is about 6 mm below the center of the eye pointing toward the center axis of the eye. A new eye is used for each test (60 eyes total). The penetration force resulting from various needles are shown in Table 2 and Figure 8.

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Table 2.

Needle	Mean penetration force (normalized to PrecisionGlide 27G)	Mean Force (grams)	Std Dev.	Range(grams)
PrecisionGlide 27G	1	68.01	19.67	32.93 – 104.10
PrecisionGlide 30G	0.88	59.96	22.92	23.26 – 119.48
HYPAK 27G	2.80	190.3	53.5	113.4 – 318.1
HYPAK 29G (Physiolis)	3.24	220.25	61.9	138.8 – 323.3

Incorporation by Reference

The patent and scientific literature referred to herein establishes knowledge that is available to those of skill in the art. All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

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CLAIMS

We claim:

1. An apparatus for intravitreal injection comprising:

a syringe comprising a barrel having a proximal and a distal end and a volume of 1 mL or less, said barrel adapted to contain an injection solution wherein said solution contains a sub-visible particulate count of less than 50 particles per mL when contained in the barrel;

a Luer lock tip attached to the distal end of the barrel;

a needle having a gauge of 27 or narrower, said needle comprising a cannula attached to a Luer lock hub for attachment to the Luer lock tip, wherein the needle requires a penetration force of less than 100 g to penetrate scleral tissue;

a syringe tip cap attached to the Luer lock tip for sealing a solution contained in the barrel; and

a needle tip shield adapted to attach to the Luer lock hub and enclose the needle.

2. The apparatus of claim 1, wherein the needle has a gauge of 29 or narrower.

3. The apparatus of claim 1, wherein the needle is a 30 gauge cannula.

4. The apparatus of claim 1, wherein the needle comprises a multi-bevel tip.

5. The apparatus of claim 1, wherein the needle comprises a 3-bevel tip.

6. The apparatus of claim 1, wherein the needle comprises a 5-bevel tip.

7. The apparatus of claim 1, wherein the needle comprises a silicon coating.

8. The apparatus of claim 1, wherein the needle requires a penetration force of less than 70 g to penetrate scleral tissue.

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9. The apparatus of claim 1, wherein the needle requires a penetration force of less than 50 g to penetrate scleral tissue.

10. The apparatus of claim 8, wherein the needle requires a penetration force having a variability of +/- 50 %.

11. The apparatus of claim 8, wherein the needle requires a penetration force having a variability of +/- 30 %.

12. The apparatus of claim 8, wherein the needle requires a penetration force having a variability of +/- 10 %.

13. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 10μm-size or larger sub-visible particulate count of less than or equal to 60 particles per mL.

14. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 25μm-size or larger sub-visible particulate count of less than or equal to 10 particles per mL.

15. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 50μm-size or larger sub-visible particulate count of less than or equal to 5 particles per mL.

16. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 10μm-size or larger sub-visible particulate count of less than or equal to 20 particles per mL.

17. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 25μm-size or larger sub-visible particulate count of less than or equal to 5 particles per mL

18. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 50μm-size or larger sub-visible particulate count of less than or equal to 2 particles per mL.

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19. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 50μm-size or larger sub-visible particulate concentration of less than 150 ppb.

20. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 25μm-size or larger sub-visible particulate concentration of less than 150 ppb.

21. The apparatus of claim 1, wherein the injection solution contained in the barrel comprises a 10μm-size or larger sub-visible particulate concentration of less than 150 ppb.

22. The apparatus of claim 1, wherein the injection solution contained in the barrel has a volume of about 200 μL.

23. The apparatus of claim 1, wherein the injection solution contained in the barrel has a volume of about 100 μL.

24. The apparatus of claim 1, wherein the injection solution contained in the barrel has a volume of about 90 μL.

25. The apparatus of claim 1, wherein the injection solution contained in the barrel has a volume of about 50 μL.

26. The apparatus of claim 1, wherein the apparatus comprises a waste space of less than 60 μL.

27. The apparatus of claim 1, wherein the apparatus comprises a waste space of less than 0.1 μL.

28. The apparatus of claim 1, wherein the apparatus comprises a waste space of less than 0.05 μL.

29. The apparatus of claim 1, wherein the apparatus comprises a waste space of less than 0.001 μL.

30. The apparatus of claim 1, wherein the syringe tip cap is plastic.

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31. The apparatus of claim 1, wherein the syringe tip cap comprises an elastomeric formulation.

32. The apparatus of claim 1, wherein the syringe tip cap comprises an isoprene blend.

33. The apparatus of claim 1, wherein the syringe tip cap comprises a chlorobutyl or a bromobutyl rubber stopper.

34. The apparatus of claim 1, wherein the needle tip shield is rigid.

35. The apparatus of claim 1, wherein the needle tip shield does not contact the cannula.

36. The apparatus of claim 1, wherein the needle tip shield comprises one or more apertures.

37. The apparatus of claim 1, wherein the needle tip shield is permeable to a sterilizing gas or vapor, or plasma.

38. The apparatus of claim 37, wherein the sterilizing gas or vapor is H_2O_2 or EtO or plasma generated from H_2O_2.

39. The apparatus of claim 1, wherein the needle tip shield comprises polypropylene or a styrene block thermoplastic elastomer.

40. The apparatus of claim 1, wherein injection solution contained in the barrel comprises a therapeutic agent.

41. The apparatus of claim 1, wherein the therapeutic agent is an anti-VEGF aptamer.

42. The apparatus of claim 1, wherein the therapeutic agent is pegaptanib sodium.

43. The apparatus of claim 1, wherein injection solution comprises about 0.003 mg to about 3.0 mg of pegaptanib sodium.

44. The apparatus of claim 1, wherein injection solution comprises about 0.3 mg of pegaptanib sodium.

45. The apparatus of claim 1, wherein the syringe comprises more than one barrel.

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46. The apparatus of claim 45, wherein the syringe comprises a first barrel having a proximal and a distal end and a second barrel having a proximal and a distal end, each of the first barrel and the second barrel independently comprises a first injection solution and a second injection solution respectively.

47. The apparatus of claim 46, wherein the first barrel and second barrel are set in a tandem arrangement.

48. The apparatus of claim 46, wherein the first barrel and second barrel are set in a side-by-side arrangement.

49. The apparatus of claim 46, wherein the first injection solution comprises pegaptanib sodium and the second injection solution comprises an anti-PDGF aptamer.

50. The apparatus of claim 45, wherein the syringe comprises:

an outer first compartment having a proximal and distal end and a first sealing member;

an inner second compartment, said inner second compartment is filled with a second injection solution, wherein the inner second compartment functions as the plunger for the outer first compartment;

a piercing device at the distal end of the outer first compartment for piercing the first sealing member;

a first injection solution contained in the outer first compartment; and

a second injection solution contained in the inner second compartment.

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Figure 1

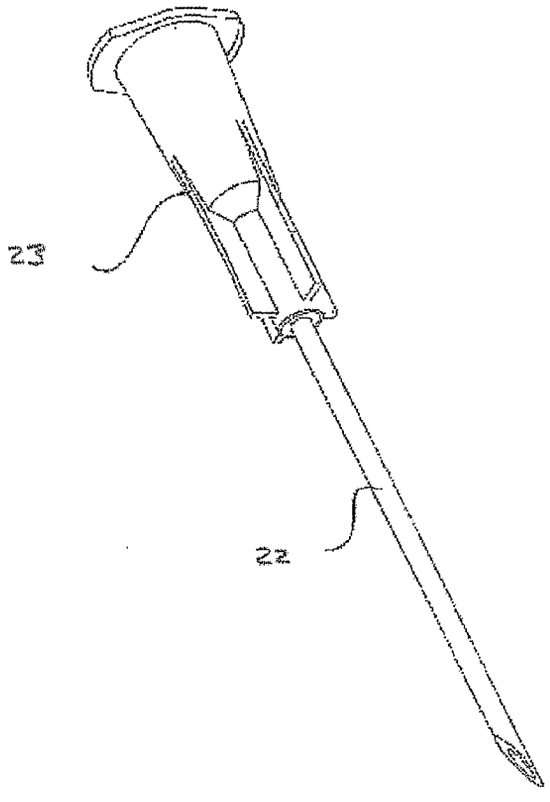

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Figure 2

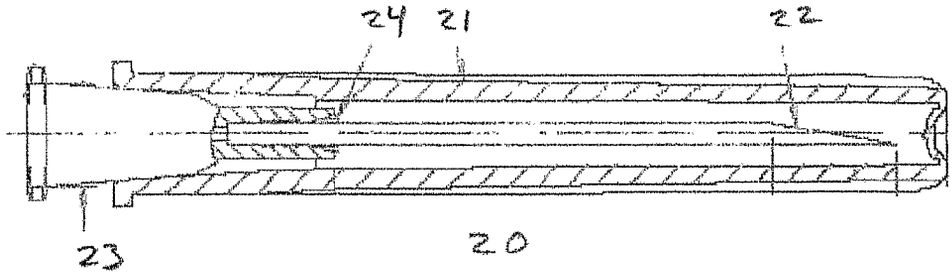

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Figure 3

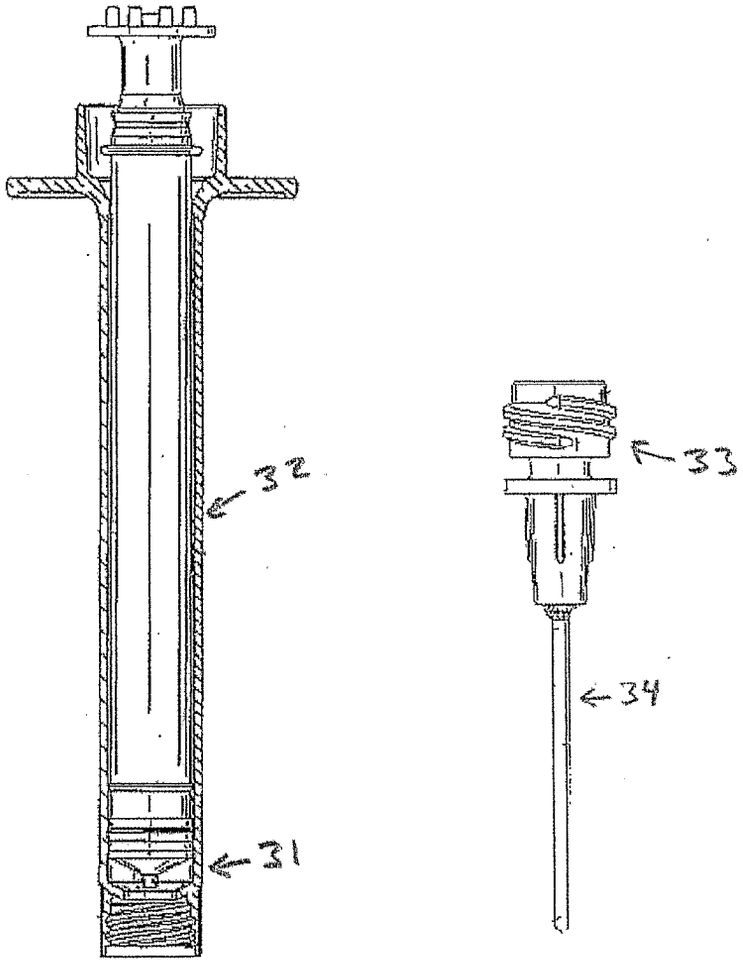

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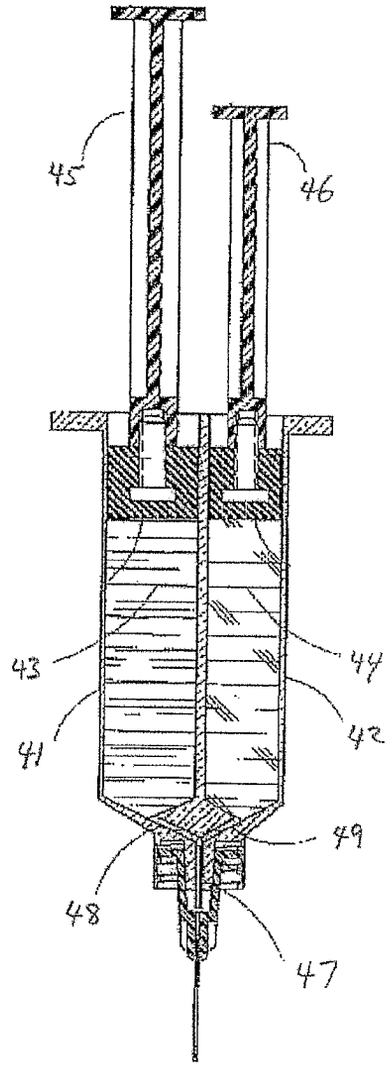
Figure 4



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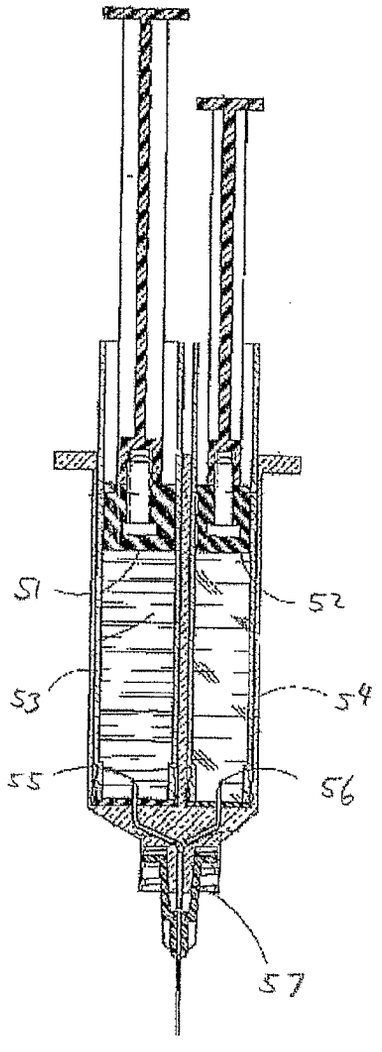

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Figure 6

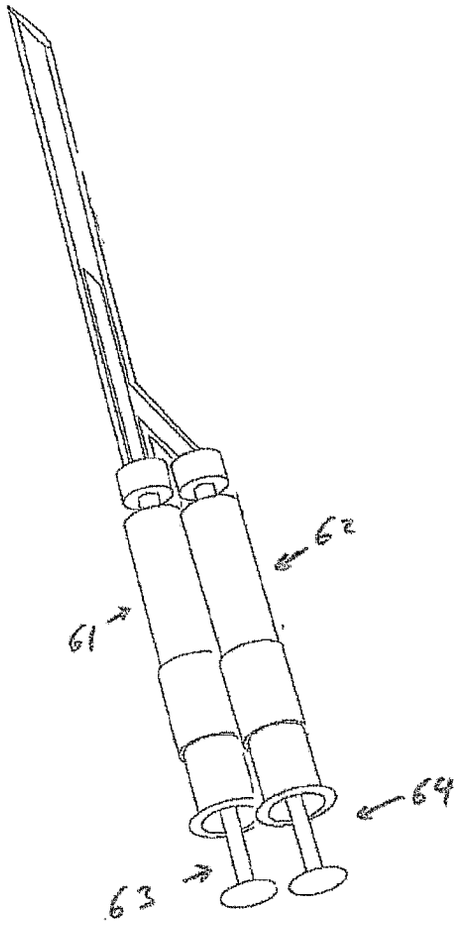

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Figure 7

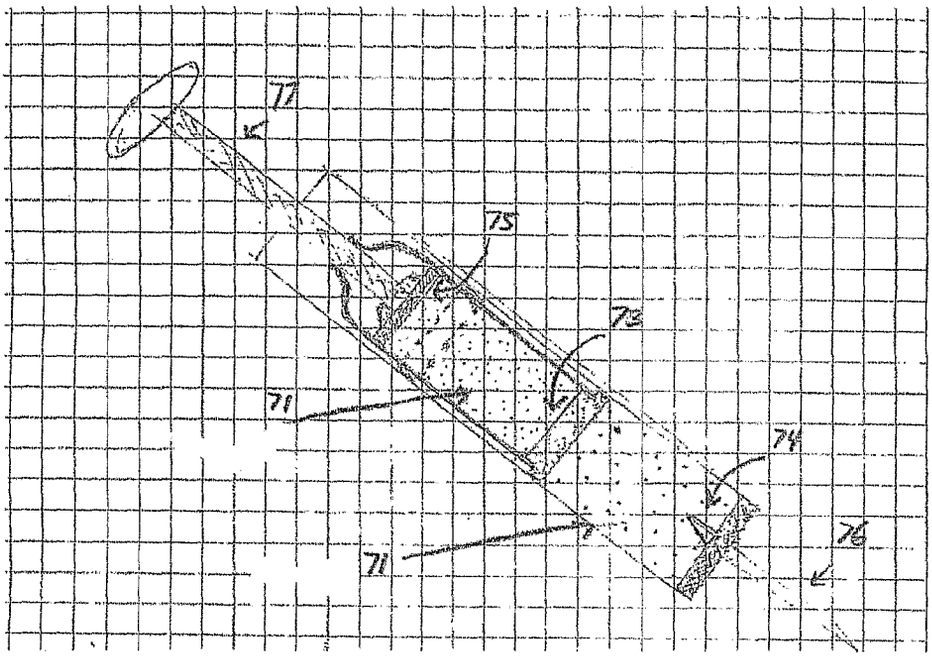

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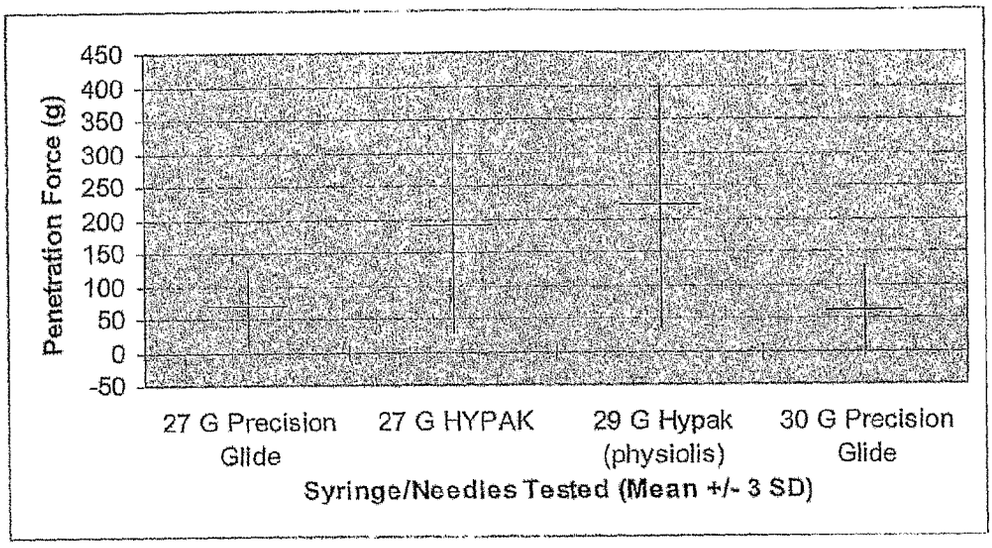
Figure 8



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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/036260

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61M5/32 A61F9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61F A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/148947 A1 (KADZIAUSKAS KENNETH E [US]) 7 July 2005 (2005-07-07) figures 1-8 paragraph [0022] paragraph [0002] paragraph [0031] - paragraph [0046]	1,30-36, 39-42
X	US 2003/120201 A1 (ABERGEL R PATRICK [US]) 26 June 2003 (2003-06-26) figures 1-10 paragraph [0028] - paragraph [0034]	1-3,7, 30-36, 39-50
X	US 5 792 099 A (DECAMP DENNIS [US] ET AL) 11 August 1998 (1998-08-11) figures 1-5 column 4, line 7 - column 6, line 61	1-3

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 January 2007	12/01/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Reinbold, Sylvie

2

Form PCT/ISA/210 (second sheet) (April 2005)

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/036260

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 03/089035 A (IMPRINT PHARM LTD [GB]; CROCKER PETER JOHN [GB]; LITTLE MERVYN AUBREY) 30 October 2003 (2003-10-30) the whole document	1-50

2

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

Regeneron Exhibit 1002.0200

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/036260

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005148947 A1	07-07-2005	WO 2005065753 A1	21-07-2005
US 2003120201 A1	26-06-2003	US 2003120217 A1	26-06-2003
US 5792099 A	11-08-1998	NONE	
WO 03089035 A	30-10-2003	AU 2003229908 A1	03-11-2003
		CA 2482047 A1	30-10-2003
		CN 1646183 A	27-07-2005
		CZ 20041044 A3	14-09-2005
		EP 1494741 A1	12-01-2005
		GB 2402345 A	08-12-2004
		JP 2005523082 T	04-08-2005
		US 2005177117 A1	11-08-2005

Regeneron Exhibit 1002.0201

Electronic Acknowledgement Receipt

EFS ID:	14823126
Application Number:	13750352
International Application Number:	
Confirmation Number:	5306
Title of Invention:	SYRINGE
First Named Inventor/Applicant Name:	Juergen Sigg
Customer Number:	1095
Filer:	Andrew K. Holmes/Andrea Jacquin
Filer Authorized By:	Andrew K. Holmes
Attorney Docket Number:	55157-US0NP
Receipt Date:	29-JAN-2013
Filing Date:	
Time Stamp:	17:08:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		_55157-US-NP_SuppIDS_2013Jan29.pdf	403890 f7c4476d6c580dad55fb1f88a8fe369d1c8a624a	yes	2

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	1
	Information Disclosure Statement (IDS) Form (SB08)	2	2

Warnings:					
Information:					
2	Foreign Reference	01_WO2007035621.pdf	4757333 7c4b087089bec546502e0881d55004ed6950de3e	no	45
Warnings:					
Information:					
3	Non Patent Literature	11_Ausubel_1987_.pdf	10020477 dd4dcccc6396a6fb0fa46148908980b31166d0df	no	26
Warnings:					
Information:					
		Total Files Size (in bytes):	15181700		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Case PAT055157-US-NP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Sigg, Juergen et al.
APPLICATION NO: 13/750352
FILED: January 25, 2013
FOR: SYRINGE

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ supplemental to the Information Disclosure Statement filed January 25, 2013.

☒ within three months of the filing date of the application. Therefore, no fees are required.

☒ before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required.

If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

/ Andrew Holmes /

Andrew Holmes
Agent for Applicant
Reg. No. 51,813

Novartis Pharmaceuticals Corporation
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Date: January 29, 2013

Regeneron Exhibit 1002.0204

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Durreshwar Anjum
Timestamp: [year=2013; month=2; day=1; hr=12; min=45; sec=22; ms=297;]
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Validated By CRFValidator v 1.0.4

Application No: 13750352 Version No: 1.0

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Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
65                  70                  75                  80


Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
                85                  90                  95


Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile
            100                 105                 110


Glu Leu Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr
        115                 120                 125
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Regeneron Exhibit 1002.0207

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Glu Leu Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys
    130                 135                 140

His Gln His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly
145                 150                 155                 160

Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr
                165                 170                 175

Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met
            180                 185                 190

Thr Lys Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Asp Lys Thr
        195                 200                 205

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
    210                 215                 220

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
225                 230                 235                 240

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
                245                 250                 255

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
            260                 265                 270

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
        275                 280                 285

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
    290                 295                 300

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
305                 310                 315                 320

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
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Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
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Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
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Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
385                 390                 395                 400

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
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Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
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Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu
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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
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Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
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Thr Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Val
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Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu Lys Leu Val
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Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp Phe Asn
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Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg
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Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr
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Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys
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Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr Phe Val Arg
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Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met Glu Ser Leu
225                 230                 235                 240

Val Glu Ala Thr Val Gly Glu Arg Val Arg Leu Pro Ala Lys Tyr Leu
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Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly Ile Pro Leu
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Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro
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Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
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Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
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Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            420                 425                 430

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
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Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
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Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
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Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
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Lys Ala Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
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Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
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Ser Leu Ser Leu Ser Pro Gly Lys
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            20                  25                  30


Ala Asp Ser Thr Gly Trp Thr Pro Leu His Leu Ala Val Pro Trp Gly
        35                  40                  45


His Leu Glu Ile Val Glu Val Leu Leu Lys Tyr Gly Ala Asp Val Asn
    50                  55                  60


Ala Lys Asp Phe Gln Gly Trp Thr Pro Leu His Leu Ala Ala Ala Ile
65                  70                  75                  80


Gly His Gln Glu Ile Val Glu Val Leu Leu Lys Asn Gly Ala Asp Val
                85                  90                  95


Asn Ala Gln Asp Lys Phe Gly Lys Thr Ala Phe Asp Ile Ser Ile Asp
            100                 105                 110


Asn Gly Asn Glu Asp Leu Ala Glu Ile Leu Gln Lys Ala Ala
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S.N-13750352

	Docket Number	PAT055157-US-NP
FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10		
Express Mail Label Number	Date of Deposit	

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

UTILITY PATENT APPLICATION TRANSMITTAL AND FEE SHEET

Transmitted herewith for filing under 37 CFR §1.53(b) is the utility patent application of

Applicant (or identifier): Sigg, Juergen et al.

Title: SYRINGE

Enclosed are:

1. ☒ Specification (Including Claims and Abstract) - 27 pages
2. ☒ Drawings - 1 sheets
3. ☐ Executed Declaration and Power of Attorney (original or copy)
4. ☐ Microfiche Computer Program (appendix)
5. Nucleotide and/or Amino Acid Sequence Submission
   - ☐ Computer Readable Copy
   - ☐ Paper Copy
   - ☐ Statement Verifying Identity of Above Copies
6. ☐ Preliminary Amendment
7. ☐ Assignment Papers (Cover Sheet & Document(s))
8. ☐ English Translation of
9. ☒ Information Disclosure Statement
10. ☐ Certified Copy of Priority Document(s)
11. ☒ Return Receipt Postcard
12. ☒ Application Data Sheet
13. ☒ Other: a) Submission of Sequence Listing Including Statement of Verification (1 Sheet)
    b) Unexecuted Declaration for Utility Application using an Application Data Sheet (5 Sheets)
    c) Transmittal for Power of Attorney to one or more registered Practitioners and Power of Attorney by Applicant (2 sheets)

Filing fee calculation:

☐ Before calculating the filing fee, please enter the enclosed Preliminary Amendment.
☐ Before calculating the filing fee, please cancel claims

02/06/2013 MTEKLEMI 00000036 190134 13750352
01 FC:1051 130.00 DA

Basic Filing Fee								$ 380
Search Fee								$ 620
Examination Fee								$ 250
Multiple Dependent Claim Fee ($)								$ 0
Foreign Language Surcharge ($)								$ 0
	For	Number Filed		Number Extra		Rate		
Extra Claims	Total Claims	32	-20	12	x	$ 60	=	$ 720
	Independent Claims	1	-3	0	x	$ 250	=	$ 0
Application Size Fee								
Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof (rounded up to a whole number)		Rate		
28	- 100	0	/50	0	x	$ 310	=	$ 0
						TOTAL FILING FEE		$ 1970

☒ Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $1970. An additional copy of this paper is enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.16 and §1.17 which may be required in connection with this application, or credit any overpayment, to Deposit Account No. 19-0134 in the name of Novartis.

Please address all correspondence to the address associated with Customer No. 001095, which is currently:

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936

Please direct all telephone calls to the undersigned at the number given below, and all telefaxes to +1 9737818265.

Respectfully submitted,

/ Andrew Holmes /

Andrew Holmes
Agent for Applicant
Reg. No. 51,813

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 8627785816

Date: January 25, 2013

Regeneron Exhibit 1002.0214

CASE PAT055157-US-NP

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10	
Express Mail Label Number	Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Sigg, Juergen et al.
APPLICATION NO: 13/750352
FILED: January 25, 2013
FOR: SYRINGE

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL APPLICATION DATA SHEET

The Application Data Sheet filed in the above-identified application needs the Foreign Priority Information updated to add the application number for the German application filed on January 23, 2013. Please add the Application No. in the blank space, which is the last foreign priority application listed, which is for the German application filed on January 23, 2013 as follows:

Foreign Priority Information:

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
202013000688.9	DE	2013-01-23	* Yes

Respectfully submitted,

/ Andrew Holmes /

Andrew Holmes
Agent for Applicant
Reg. No. 51,813

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 8627785816

Date: February 7, 2013

Regeneron Exhibit 1002.0215

Electronic Acknowledgement Receipt

EFS ID:	14898687
Application Number:	13750352
International Application Number:	
Confirmation Number:	5306
Title of Invention:	SYRINGE
First Named Inventor/Applicant Name:	Juergen Sigg
Customer Number:	1095
Filer:	Andrew K. Holmes/Andrea Jacquin
Filer Authorized By:	Andrew K. Holmes
Attorney Docket Number:	55157-US-NP
Receipt Date:	07-FEB-2013
Filing Date:	25-JAN-2013
Time Stamp:	10:51:34
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	55157-US-NP-SupplementalApplicationDataSheetLetter_2013Feb7.pdf	229807 78c0153c5ee22f1c6e8580c63e1826e1ed2ff4c1	no	1

Warnings:	
Information:	

Regeneron Exhibit 1002.0216

Total Files Size (in bytes):	229807

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/750,352

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	390
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	620
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	250
TOTAL CLAIMS (37 CFR 1.16(i))	32 minus 20 =	* 12			OR	x 62 =	744
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 250 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2004

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/750,352	01/25/2013	Juergen Sigg	55157-US-NP

CONFIRMATION NO. 5306

1095
NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

NOTICE

OC000000059155996

Date Mailed: 02/12/2013

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  All
  Applicant may submit the inventor's oath or declaration at any time before the Notice of Allowance and Fee(s) Due, PTOL-85, is mailed.

Regeneron Exhibit 1002.0219

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/750,352	01/25/2013	3763	2134	55157-US-NP	32	2

CONFIRMATION NO. 5306

1095
NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

FILING RECEIPT

OC000000059155995

Date Mailed: 02/12/2013

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Juergen Sigg, Loerrach, GERMANY;
Christopher Royer, Munich, GERMANY;
Andrew Mark Bryant, Reinach, SWITZERLAND;
Heinrich Martin Buettgen, Rheinfelden, SWITZERLAND;
Marie Picci, Ranspack-le-bas, FRANCE;

Applicant(s)

Novartis AG, Basel, SWITZERLAND

Assignment For Published Patent Application

Novartis AG, Basel, SWITZERLAND

Power of Attorney: The patent practitioners associated with Customer Number 01095

Domestic Applications for which benefit is claimed - None.
A proper domestic benefit claim must be provided in an Application Data Sheet in order to constitute a claim for domestic benefit. See 37 CFR 1.76 and 1.78.

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 12174860.2 07/03/2012
EUROPEAN PATENT OFFICE (EPO) 12189649.2 10/23/2012
GERMANY 202012011016.0 11/16/2012
AUSTRALIA 2012101677 11/16/2012
AUSTRALIA 2012101678 11/16/2012
GERMANY 202012011260.0 11/23/2012
GERMANY 202012011259.7 11/23/2012
EUROPEAN PATENT OFFICE (EPO) 12195360.8 12/03/2012

Regeneron Exhibit 1002.0220

AUSTRALIA 2013100071 01/23/2013
AUSTRALIA 2013100070 01/23/2013

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 02/05/2013

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/750,352**

Projected Publication Date: 01/09/2014

Non-Publication Request: No

Early Publication Request: No

Title

SYRINGE

Preliminary Class

604

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign

Regeneron Exhibit 1002.0221

patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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Regeneron Exhibit 1002.0222

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Regeneron Exhibit 1002.0223

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/750,352	01/25/2013	Juergen Sigg	55157-US-NP

CONFIRMATION NO. 5306

1095
NOVARTIS PHARMACEUTICAL CORPORATION
INTELLECTUAL PROPERTY DEPARTMENT
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

POA ACCEPTANCE LETTER

OC000000059156059

Date Mailed: 02/12/2013

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 01/25/2013.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/snguyen/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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Bescheinigung	Certificate	Attestation
Die angehefteten Unterlagen stimmen mit der als ursprünglich eingereicht geltenden Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.	The attached documents are exact copies of the text in which the European patent application described on the following page is deemed to have been filed.	Les documents joints à la présente attestation sont conformes au texte, considéré comme initialement déposé, de la demande de brevet européen qui est spécifiée à la page suivante.

Patentanmeldung Nr.	**Patent application No.**	**Demande de brevet n°**
	12174860.2 / EP12174860	

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP12174860.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

U. Ingmann

MV22832

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Anmeldung Nr:
Application no.: 12174860.2
Demande no :

Anmeldetag:
Date of filing: 03.07.12
Date de dépôt :

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Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:
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Syringe

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Am Anmeldetag benannte Vertragstaaten / Contracting States designated at date of filing / Etats contractants désignées lors du dépôt:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

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PAT055157-EP-EPA

SYRINGE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion,

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the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone free, or substantially silicone free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being

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dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals

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defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm and less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

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In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone droplets being injected into the eye. Furthermore, silicone can cause proteins to aggregate. A typical 1ml syringe comprises

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100-800μg silicone in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800μg (i.e. about less than about 500μg, less than about 300μg, less than about 200μg, less than about 100μg, less than about 75μg, less than about 50μg, less than about 25μg, less than about 15μg, less than about 10μg) silicone in the barrel. Methods for measuring the amount of silicone in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. In one embodiment the glide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper.

In one embodiment the syringe barrel has an internal coating of silicone that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone inside a syringe barrel.

In one embodiment, the syringe is silicone free, or substantially silicone free. Such low silicone levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles $\geq$50μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles $\geq$25μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 50 particles $\geq$10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles $\geq$50μm in diameter per ml, no more than 5 particles $\geq$25μm in diameter per ml and no more than 50 particles $\geq$10μm in diameter per ml. In one embodiment,

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a syringe according to the invention meets USP789. In one embodiment the syringe has low levels of silicone sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011

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Molecular Vision 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDT
LIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVL
TIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
ATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLKYGADVNAKDFQGW
TPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNEDLAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not

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present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms),

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macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide or a hydrogen peroxide sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for

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up to 6 months, 9 months, 12 months, 15 months, 18 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes ≤10% (preferably ≤5%, ≤3%, ≤1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm EtO residue.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

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Figure 5 shows a stopper

MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the

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bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist.

2. A pre-filled syringe according to claim 1, wherein the syringe has a nominal maximum fill volume of between about 0.1ml and about 1.5ml.

3. A pre-filled syringe according to claim 1 or claim 2, wherein the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml.

4. A pre-filled syringe according to any previous claim, wherein the syringe is filled with between about 0.01ml and about 1.5ml of a VEGF antagonist solution.

5. A pre-filled syringe according to any previous claim, wherein the syringe is filled with between about 0.15ml and about 0.175ml of a VEGF antagonist solution.

6. A pre-filled syringe according to any previous claim, wherein the syringe is filled with dosage volume of between about 0.03ml and about 0.05ml of a VEGF antagonist solution.

7. A pre-filled syringe according to any previous claim, wherein the syringe is filled with dosage volume of about 0.05ml of a VEGF antagonist solution.

8. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of silicone that has an average thickness of about 450nm or less.

8. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of less than about 500µg silicone.

9. A pre-filled syringe according to any previous claim, wherein the syringe is silicone free.

10. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution comprises one or more of (i) no more than 2 particles ≥50µm in diameter per ml, (ii) no more

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than 5 particles ≥25μm in diameter per ml, and (iii) no more than 50 particles ≥10μm in diameter per ml.

11. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution meets USP789.

12. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist is an anti-VEGF antibody.

13. A pre-filled syringe according to claim 12, wherein the anti-VEGF antibody is ranibizumab.

14. A pre-filled syringe according to claim 11, wherein the VEGF antagonist is a non-antibody VEGF antagonist.

15. A pre-filled syringe according to claim 14, wherein the non-antibody VEGF antagonist is aflibercept or conbercept.

16. A pre-filled syringe according to claim 15, wherein the non-antibody VEGF antagonist is aflibercept at a concentration of 40mg/ml.

17. A pre-filled syringe according to claim 16, wherein:

(i) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

(ii) the syringe is filled with between about 0.15ml and about 0.175ml of aflibercept,

(iii) the syringe is filled with dosage volume of about 0.05ml,

(iv) the syringe barrel has an internal coating of less than about 500μg silicone, and

(v) the VEGF antagonist solution comprises no more than 2 particles ≥50μm in diameter per ml.

18. A blister pack comprising a pre-filled syringe according to any previous claim, wherein the syringe has been sterilised using H_2O_2 or EtO.

19. A blister pack comprising a pre-filled syringe according to claim 18, wherein the outer surface of the syringe has ≤1ppm EtO residue.

20. A blister pack comprising a pre-filled syringe according to claim 18 or claim 19, wherein ≤5% of the VEGF antagonist is alkylated.

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21. A blister pack comprising a pre-filled syringe according to any of claims 18-21, wherein the syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}.

22. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-17, or a blister pack comprising a pre-filled syringe according to any one of claims 18-21, (ii) a needle, and optionally (iii) instructions for administration.

23. A kit according to claim 22, wherein the needle is a 30-gauge x ½ inch needle.

24. A pre-filled syringe according to any one of claims 1-17 for use in therapy.

25. A pre-filled syringe according to any one of claims 1-17 for use in the treatment of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

26. A method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention.

27. The method of claim 26, further comprising an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

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ABSTRACT

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

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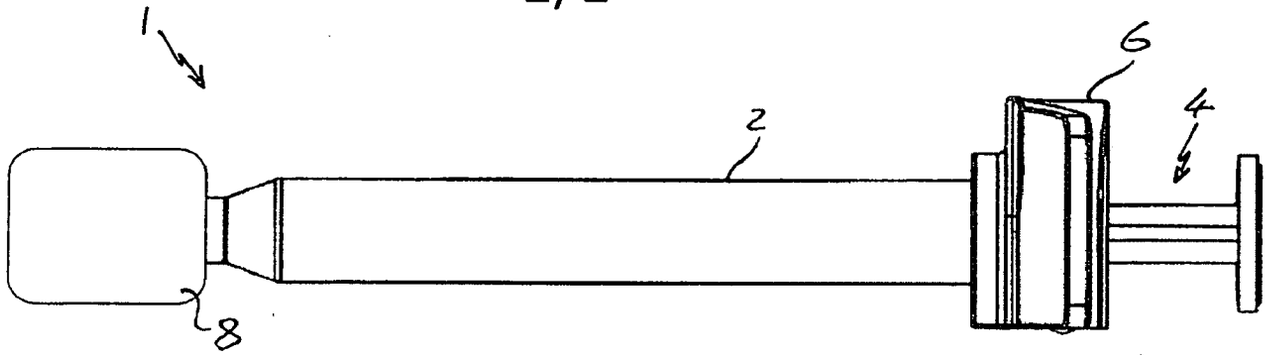

Fig 1

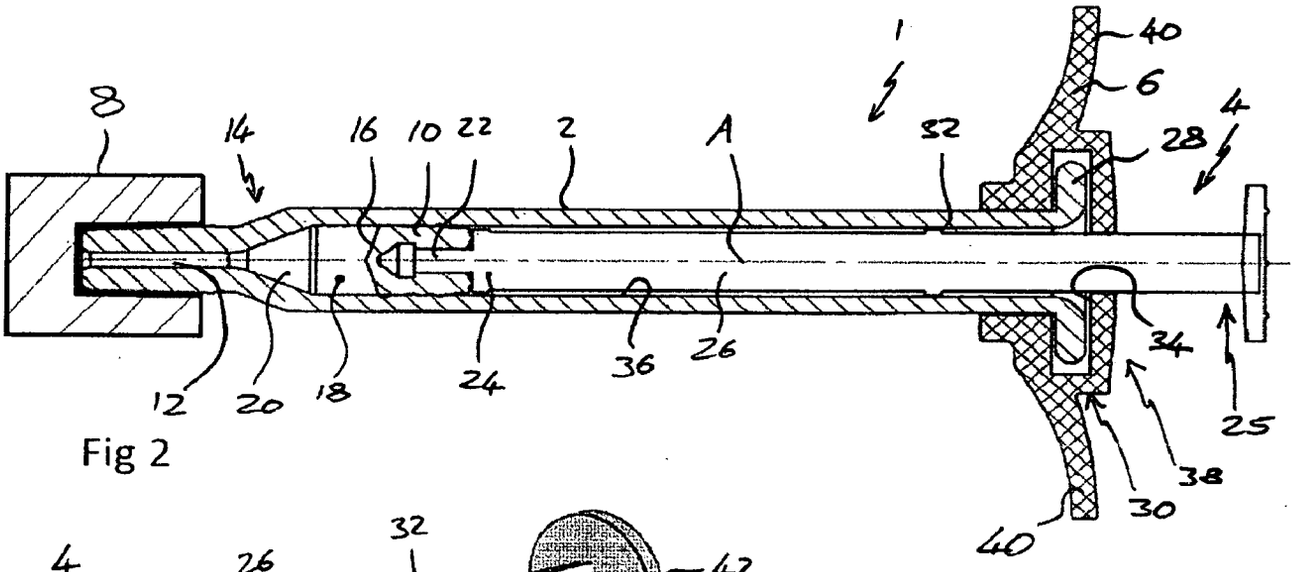

Fig 2

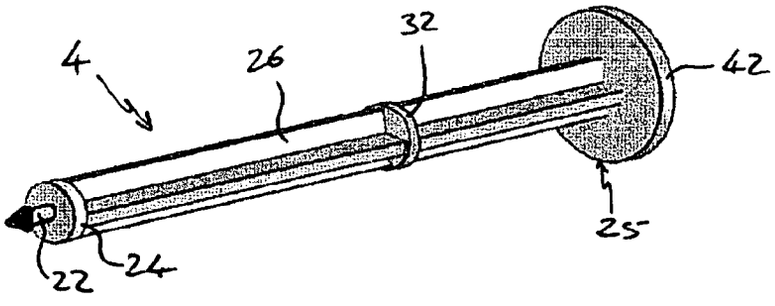

Fig 3

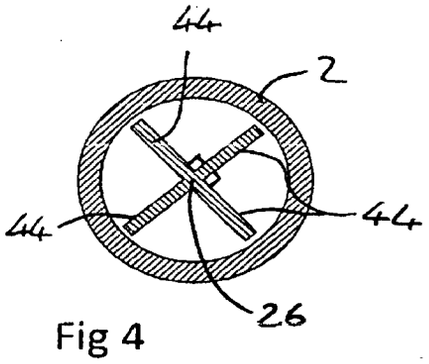

Fig 4

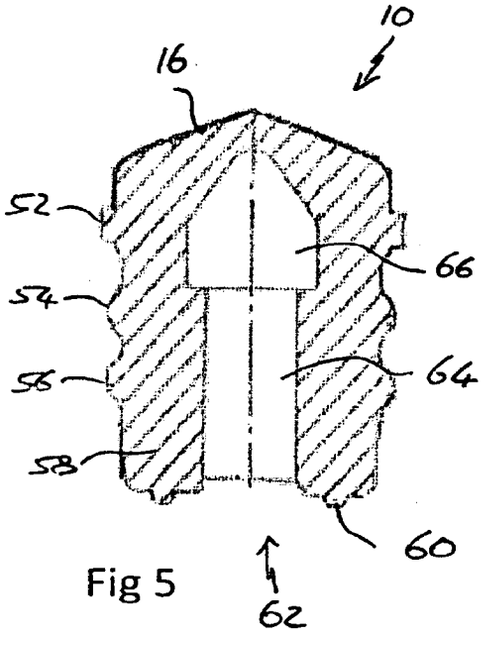

Fig 5

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Bescheinigung	Certificate	Attestation
Die angehefteten Unterlagen stimmen mit der als ursprünglich eingereicht geltenden Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.	The attached documents are exact copies of the text in which the European patent application described on the following page is deemed to have been filed.	Les documents joints à la présente attestation sont conformes au texte, considéré comme initialement déposé, de la demande de brevet européen qui est spécifiée à la page suivante.

Patentanmeldung Nr.	**Patent application No.**	**Demande de brevet n°**
	12189649.2 / EP12189649	

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP12189649.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

U. Ingmann

MV03101

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Anmeldung Nr:
Application no.: 12189649.2
Demande no :

Anmeldetag:
Date of filing: 23.10.12
Date de dépôt :

Anmelder / Applicant(s) / Demandeur(s):

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Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, oder falls die Anmeldung in einer Nicht-Amtssprache des EPA eingereicht wurde, siehe Beschreibung bezüglich ursprünglicher Bezeichnung.
If no title is shown, or if the application has been filed in a non-EPO language, please refer to the description for the original title.
Si aucun titre n'est indiqué, ou si la demande a été déposée dans une langue autre qu'une langue officielle de l'OEB, se référer à la description pour le titre original.)

Syringe

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Am Anmeldetag benannte Vertragstaaten / Contracting States designated at date of filing / Etats contractants désignées lors du dépôt:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

Regeneron Exhibit 1002.0246

PAT055157-EP-EPA

SYRINGE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion,

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the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being

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dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals

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defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

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In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe

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comprises 100-800µg silicone oil in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100µg-about 800µg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100µg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

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In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles ≥25μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789. In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

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SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDT
LIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVL
TIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
ATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

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GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLKYGADVNAKDFQGW
TPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNEDLAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in

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conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

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Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide or a hydrogen peroxide sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes $\leq$10% (preferably $\leq$5%, $\leq$3%, $\leq$1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has $\leq$1ppm, preferably $\leq$0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised

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using hydrogen peroxide, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

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MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the

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bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm.

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		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist.

2. A pre-filled syringe according to claim 1, wherein the syringe has a nominal maximum fill volume of between about 0.1ml and about 1.5ml.

3. A pre-filled syringe according to claim 1 or claim 2, wherein the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml.

4. A pre-filled syringe according to any previous claim, wherein the syringe is filled with between about 0.01ml and about 1.5ml of a VEGF antagonist solution.

5. A pre-filled syringe according to any previous claim, wherein the syringe is filled with between about 0.15ml and about 0.175ml of a VEGF antagonist solution.

6. A pre-filled syringe according to any previous claim, wherein the syringe is filled with a dosage volume of between about 0.03ml and about 0.05ml of a VEGF antagonist solution.

7. A pre-filled syringe according to any previous claim, wherein the syringe is filled with dosage volume of about 0.05ml of a VEGF antagonist solution.

8. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less.

9. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of less than about 500µg silicone oil, preferably less than about 50µg silicone oil, preferably less than about 25µg silicone oil.

10. A pre-filled syringe according to any previous claim, wherein the silicone oil is DC365 emulsion.

11. A pre-filled syringe according to any previous claim, wherein the syringe is silicone oil free.

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12. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution comprises one or more of (i) no more than 2 particles ≥50μm in diameter per ml, (ii) no more than 5 particles ≥25μm in diameter per ml, and (iii) no more than 50 particles ≥10μm in diameter per ml.

13. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution meets USP789.

14. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist is an anti-VEGF antibody.

15. A pre-filled syringe according to claim 14, wherein the anti-VEGF antibody is ranibizumab.

16. A pre-filled syringe according to any one of claims 1-13, wherein the VEGF antagonist is a non-antibody VEGF antagonist.

17. A pre-filled syringe according to claim 16, wherein the non-antibody VEGF antagonist is aflibercept or conbercept.

18. A pre-filled syringe according to claim 17, wherein the non-antibody VEGF antagonist is aflibercept at a concentration of 40mg/ml.

19. A pre-filled syringe according to claim 18, wherein:

(i) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

(ii) the syringe is filled with between about 0.15ml and about 0.175ml of aflibercept,

(iii) the syringe is filled with dosage volume of about 0.05ml,

(iv) the syringe barrel has an internal coating of less than about 500μg silicone oil, and

(v) the VEGF antagonist solution comprises no more than 2 particles ≥50μm in diameter per ml.

20. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper break loose force of less than about 11N.

21. A pre-filled syringe according to claim 20, wherein the syringe has a stopper break loose force of less than about 5N.

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22. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper slide force of less than about 11N.

23. A pre-filled syringe according to claim 22, wherein the syringe has a stopper slide force of less than about 5N.

24. A blister pack comprising a pre-filled syringe according to any previous claim, wherein the syringe has been sterilised using H_2O_2 or EtO.

25. A blister pack comprising a pre-filled syringe according to claim 24, wherein the outer surface of the syringe has $\leq$1ppm EtO or hydrogen peroxide residue.

26. A blister pack comprising a pre-filled syringe according to claim 24, wherein the syringe has been sterilised using EtO or hydrogen peroxide and the total EtO or hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

27. A blister pack comprising a pre-filled syringe according to any one of claims 24-26, wherein $\leq$5% of the VEGF antagonist is alkylated.

28. A blister pack comprising a pre-filled syringe according to any of claims 24-27, wherein the syringe has been sterilised using EtO or hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}.

29. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-23, or a blister pack comprising a pre-filled syringe according to any one of claims 24-28, (ii) a needle, and optionally (iii) instructions for administration.

30. A kit according to claim 29, wherein the needle is a 30-gauge x ½ inch needle.

31. A pre-filled syringe according to any one of claims 1-23 for use in therapy.

32. A pre-filled syringe according to any one of claims 1-23 for use in the treatment of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

33. A method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal

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neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe according to any one of claims 1-23.

34. The method of claim 33, further comprising an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

35. A method according to claim 33 or 34, wherein the VEGF antagonist administered is a non-antibody VEGF antagonist and wherein the patient has previously received treatment with an antibody VEGF antagonist.

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ABSTRACT

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

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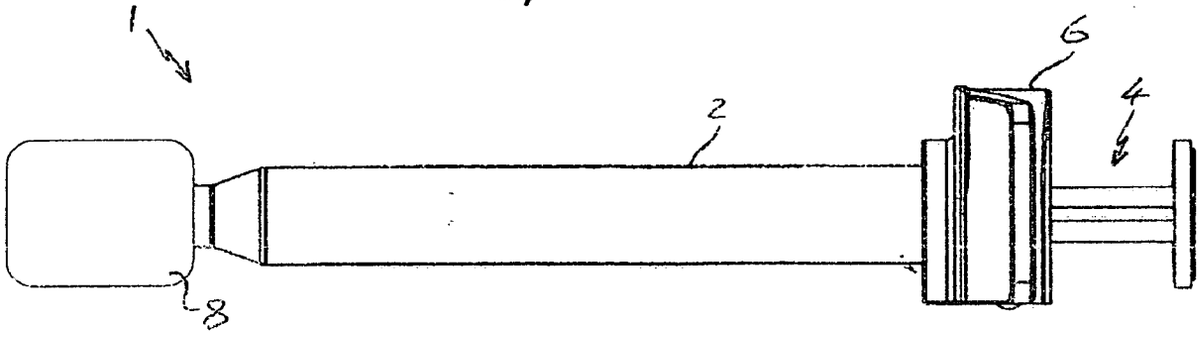

Fig 1

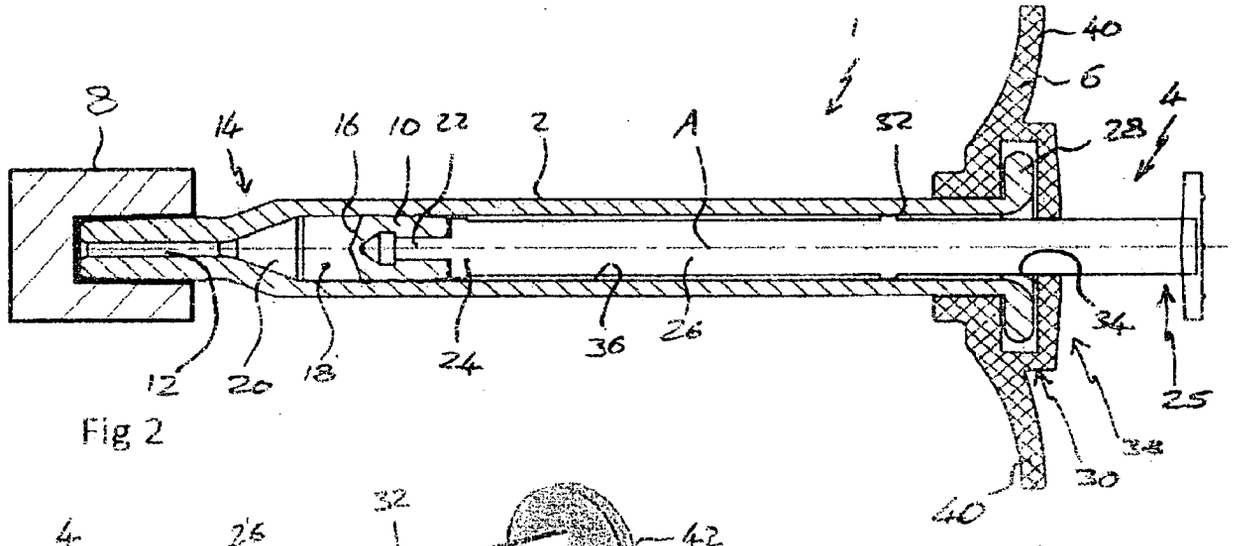

Fig 2

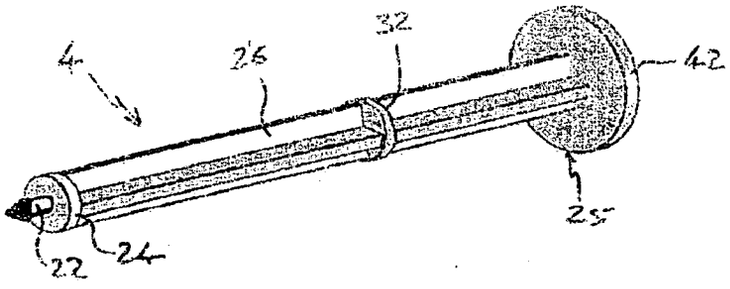

Fig 3

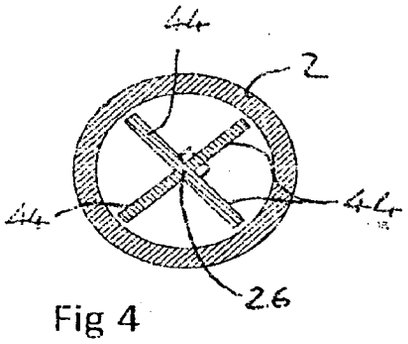

Fig 4

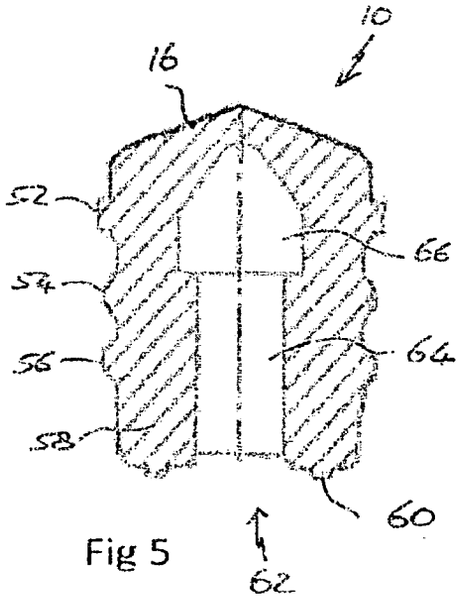

Fig 5

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Bescheinigung	Certificate	Attestation
Die angehefteten Unterlagen stimmen mit der als ursprünglich eingereicht geltenden Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.	The attached documents are exact copies of the text in which the European patent application described on the following page is deemed to have been filed.	Les documents joints à la présente attestation sont conformes au texte, considéré comme initialement déposé, de la demande de brevet européen qui est spécifiée à la page suivante.

Patentanmeldung Nr.	**Patent application No.**	**Demande de brevet n°**
	12195360.8 / EP12195360	

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP12195360.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

U. Ingmann

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Anmeldung Nr:
Application no.: 12195360.8
Demande no :

Anmeldetag:
Date of filing: 03.12.12
Date de dépôt :

Anmelder / Applicant(s) / Demandeur(s):

Novartis AG
Lichtstrasse 35
4056 Basel/CH

Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, oder falls die Anmeldung in einer Nicht-Amtssprache des EPA eingereicht wurde, siehe Beschreibung bezüglich ursprünglicher Bezeichnung.
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SYRINGE

In Anspruch genommene Priorität(en) / Priority(Priorities) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Am Anmeldetag benannte Vertragstaaten / Contracting States designated at date of filing / Etats contractants désignées lors du dépôt:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

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SYRINGE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised. Of course, the syringe must also remain easy to use, in that the force required to depress the plunger to administer the medicament must not be too high.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber

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from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant. In one embodiment, despite the low silicone oil level, the stopper break loose and slide force is less than 20N.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) or plunger with the mark, thus expelling excess ophthalmic solution and any air bubbles from the

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syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes

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where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

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In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution. Although unapproved for ophthalmic indications, bevacizumab is used off-label in such ophthalmic indications at a concentration of 25mg/ml; typically at a dosage volume of 0.05ml (1.25mg).

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as

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is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. With multiple injections, the amount of silicone droplets can build up in the eye, causing potential adverse effects. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel, though a survey of manufacturers reported that 500-1000µg was typically used in pre-filled syringes (Badkar *et al.* 2011, AAPS PharmaSciTech, 12(2):564-572). Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was surprisingly found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase (see e.g. Schoenknecht, AAPS National Biotechnology Conference 2007 – Abstract no. NBC07-000488, which indicates that while 400µg silicone oil is acceptable, usability improves when increased to 800µg). Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Smooth administration is particularly important in sensitive tissues such as the eye, where movement of the syringe during administration could cause local tissue damage. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about

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100μg-about 800μg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the forces are measured with a 30G x 0.5 inch needle attached to the syringe. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100μg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line. A further way to reduce silicone oil and inorganic silica levels in a pre-filled syringe is to avoid the use of silicone tubing in filling lines, for example between storage tanks and pumps.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles ≥25μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789 (United States Pharmacopoeia: Particulate Matter in Ophthalmic Solutions). In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

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VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different

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glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDT
LIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVL
TIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
ATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLKYGADVNAKDFQGW
TPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNEDLAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

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Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO)

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and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

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Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide (EtO) or a hydrogen peroxide (H_2O_2) sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months, 24 months or longer. Thus, in one embodiment, a syringe according to the invention (whilst in its blister pack) may have a shelf life of up to 6 months, 9 months, 12 months, 15 months, 18 months, 24 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes $\leq$10% (preferably $\leq$5%, $\leq$3%, $\leq$1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has $\leq$1ppm, preferably $\leq$0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, but the outer surface of the syringe has $\leq$1ppm, preferably $\leq$0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

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The term "about" in relation to a numerical value x means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF

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antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

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Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper movement forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm. Stopper design 2 had a 45% increase in the distance between the front circumferential rib and rear circumferential rib.

		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

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For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist wherein:

(a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

(b) the syringe is filled with between about 0.15ml and about 0.175ml of said VEGF antagonist solution which comprises a dosage volume of between about 0.03ml and about 0.05ml of said VEGF antagonist solution,

(c) the syringe barrel comprises less than about 500μg silicone oil, and

(d) the VEGF antagonist solution comprises no more than 2 particles ≥50μm in diameter per ml.

2. A pre-filled syringe according to any previous claim, wherein the syringe is filled with dosage volume of about 0.05ml of a VEGF antagonist solution.

3. A pre-filled syringe according to any previous claim, in which the dosage volume is determined by the volume of the variable volume chamber when a predetermined part of the stopper is aligned with a priming mark on the syringe.

4. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less.

5. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of less than about 500μg silicone oil, preferably less than about 50μg silicone oil, preferably less than about 25μg silicone oil, preferably less than about 10μg silicone oil.

6. A pre-filled syringe according to any previous claim, wherein the silicone oil is DC365 emulsion.

7. A pre-filled syringe according to any previous claim, wherein the syringe is silicone oil free.

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8. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution further comprises one or more of (i) no more than 5 particles ≥25μm in diameter per ml, and (ii) no more than 50 particles ≥10μm in diameter per ml.

9. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution meets USP789.

10. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist is an anti-VEGF antibody.

11. A pre-filled syringe according to claim 10, wherein the anti-VEGF antibody is ranibizumab.

12. A pre-filled syringe according to claim 11, wherein the ranibizumab is at a concentration of 10mg/ml.

13. A pre-filled syringe according to any one of claims 1-9, wherein the VEGF antagonist is a non-antibody VEGF antagonist.

14. A pre-filled syringe according to claim 13, wherein the non-antibody VEGF antagonist is aflibercept or conbercept.

15. A pre-filled syringe according to claim 14, wherein the non-antibody VEGF antagonist is aflibercept at a concentration of 40mg/ml.

16. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper break loose force of less than about 11N.

17. A pre-filled syringe according to claim 16, wherein the syringe has a stopper break loose force of less than about 5N.

18. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper slide force of less than about 11N.

19. A pre-filled syringe according to claim 18, wherein the syringe has a stopper slide force of less than about 5N.

20. A pre-filled syringe according to any of claims 16-19, wherein the stopper break loose force or stopper slide force is measured using a filled syringe, at a stopper travelling speed of 190mm/min, with a 30G x 0.5 inch needle attached to the syringe.

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21. A blister pack comprising a pre-filled syringe according to any previous claim, wherein the syringe has been sterilised using H_2O_2 or EtO.

22. A blister pack comprising a pre-filled syringe according to claim 21, wherein the outer surface of the syringe has $\leq$1ppm EtO or H_2O_2 residue.

23. A blister pack comprising a pre-filled syringe according to claim 21, wherein the syringe has been sterilised using EtO or H_2O_2 and the total EtO or H_2O_2 residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

24. A blister pack comprising a pre-filled syringe according to any one of claims 21-23, wherein $\leq$5% of the VEGF antagonist is alkylated.

25. A blister pack comprising a pre-filled syringe according to any of claims 21-24, wherein the syringe has been sterilised using EtO or H_2O_2 with a Sterility Assurance Level of at least 10^{-6}.

26. A blister pack according to any of claims 21-25, wherein the pre-filled syringe has a shelf life of up to 6 months, 9 months, 12 months, 15 months, 18 months, 24 months or longer.

27. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-20, or a blister pack comprising a pre-filled syringe according to any one of claims 21-26, (ii) a needle, and optionally (iii) instructions for administration.

28. A kit according to claim 27, wherein the needle is a 30-gauge x ½ inch needle.

29. A pre-filled syringe according to any one of claims 1-20 for use in therapy.

30. A pre-filled syringe according to any one of claims 1-20 for use in the treatment of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

31. A method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe according to any one of claims 1-20.

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32. The method of claim 31, further comprising an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

33. A method according to claim 31 or 32, wherein the VEGF antagonist administered is a non-antibody VEGF antagonist and wherein the patient has previously received treatment with an antibody VEGF antagonist.

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ABSTRACT

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

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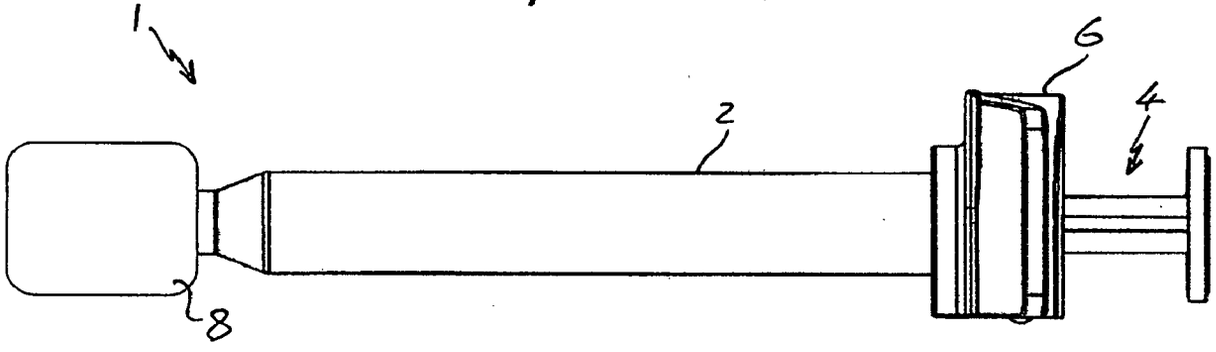

Fig 1

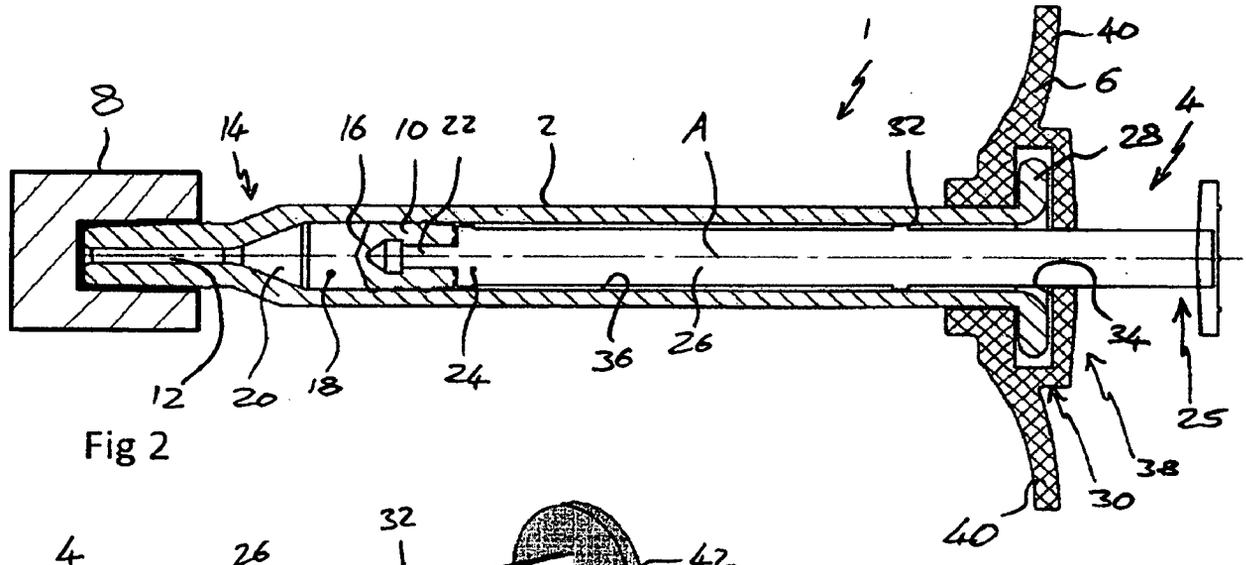

Fig 2

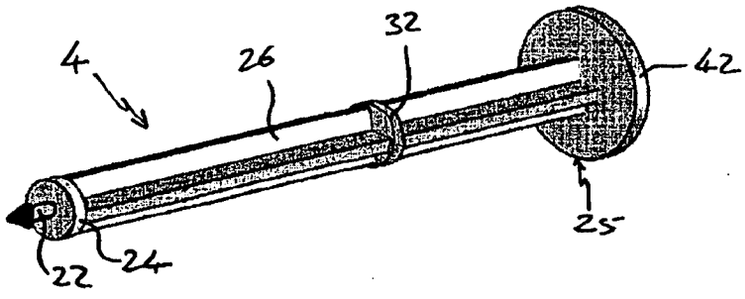

Fig 3

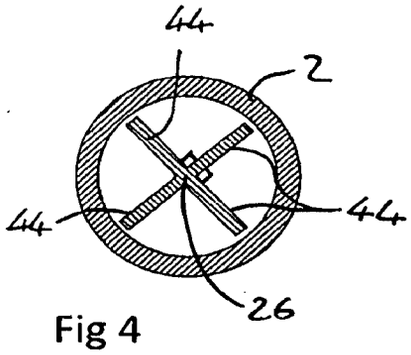

Fig 4

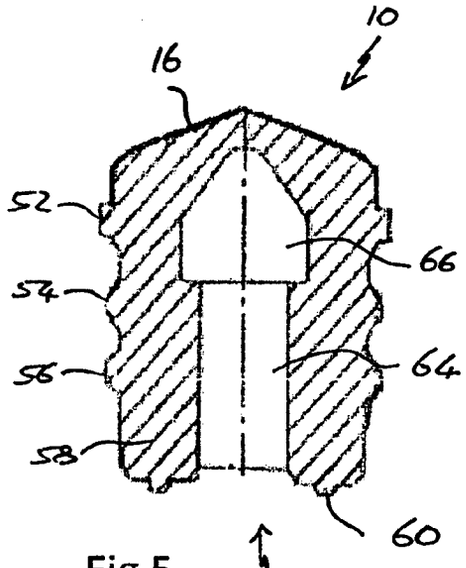

Fig 5

Regeneron Exhibit 1002.0291

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: **13750352** Filing Date: **03/15/2013**

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BUNDESREPUBLIK DEUTSCHLAND

Prioritätsbescheinigung
DE 20 2012 011 016.0
über die Einreichung einer Gebrauchsmusteranmeldung

Aktenzeichen:	20 2012 011 016.0
Anmeldetag:	16. November 2012
Anmelder/Inhaber:	Novartis AG, Basel, CH
Bezeichnung:	Syringe
Priorität:	23. Oktober 2012; EP; 12189649 03. Juli 2012; EP; 12174860
IPC:	A61M 5/178; A61K 38/02

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der Teile der am 16. November 2012 eingereichten Unterlagen dieser Gebrauchsmusteranmeldung unabhängig von gegebenenfalls durch das Kopierverfahren bedingten Farbabweichungen.

München, den 29. Januar 2013
Deutsches Patent- und Markenamt
Die Präsidentin
Im Auftrag

Bauernfeind

A 9161(Pat1)/1.12

16_11_2012-01-00003-00000005

Description

SYRINGE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber,

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characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear

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surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs

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may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

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In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or

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DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100µg-about 800µg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100µg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles $\geq$50µm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles $\geq$25µm in diameter per ml. In one embodiment, the ophthalmic

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solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789. In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLY
TCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

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Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNIT
VTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNT
IIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSG
SEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVE
ATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTN
PISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLK
YGADVNAKDFQGWTPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNED
LAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

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The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

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Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton

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containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide or a hydrogen peroxide sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes ≤10% (preferably ≤5%, ≤3%, ≤1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, *x*±10%.

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References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of

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the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

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Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm.

		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

16_11_2012-01-00003-00000019

Claims

1. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist, wherein

   (a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

   (b) the syringe is filled with between about 0.15ml and about 0.175ml of said VEGF antagonist solution which comprises a dosage volume of about 0.05ml of said VEGF antagonist solution,

   (c) the syringe barrel comprises less than about 500µg silicone oil,

   (d) the VEGF antagonist solution comprises no more than 2 particles $\geq$50µm in diameter per ml.

   and

   (e) the VEGF antagonist is the non-antibody VEGF antagonist aflibercept at a concentration of 40mg/ml.

2. A pre-filled syringe according to claim 1, wherein the syringe is filled with about 0.165ml of said VEGF antagonist solution.

3. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less.

4. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of less than about 500 µg silicone oil, preferably less than about 100µg silicone oil, preferably less than about 50µg silicone oil, preferably less than about 25µg silicone oil.

5. A pre-filled syringe according to any previous claim, wherein the silicone oil is DC365 emulsion.

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6. A pre-filled syringe according to any previous claim, wherein the syringe is silicone oil free.

7. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution further comprises one or more of (i) no more than 5 particles ≥25μm in diameter per ml, and (ii) no more than 50 particles ≥10μm in diameter per ml.

8. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution meets USP789.

9. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper break loose force of less than about 11N.

10. A pre-filled syringe according to claim 9, wherein the syringe has a stopper break loose force of less than about 5N.

11. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper slide force of less than about 11N.

12. A pre-filled syringe according to claim 11, wherein the syringe has a stopper slide force of less than about 5N.

13. A pre-filled syringe according to any previous claim, in which the dosage volume is determined by volume of the variable volume chamber when a predetermined part of the stopper or plunger is aligned with a priming mark on the syringe

14. A blister pack comprising a pre-filled syringe according to any previous claim, wherein the syringe has been sterilised using H_2O_2 or EtO.

15. A blister pack comprising a pre-filled syringe according to claim 14, wherein the outer surface of the syringe has ≤1ppm EtO or H_2O_2 residue.

16. A blister pack comprising a pre-filled syringe according to claim 14, wherein the syringe has been sterilised using EtO or H_2O_2 and the total EtO or H_2O_2 residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg.

17. A blister pack comprising a pre-filled syringe according to any one of claims 14-16, wherein ≤5% of the VEGF antagonist is alkylated.

18. A blister pack comprising a pre-filled syringe according to any of claims 14-16, wherein the syringe has been sterilised using EtO or hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}.

19. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-13, or a blister pack comprising a pre-filled syringe according to any one of claims 13-17, (ii) a needle, and optionally (iii) instructions for administration.

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20. A kit according to claim 19, wherein the needle is a 30-gauge x ½ inch needle.

21. A pre-filled syringe according to any one of claims 1-13 for use in therapy.

22. A pre-filled syringe according to any one of claims 1-13 for use in the treatment of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

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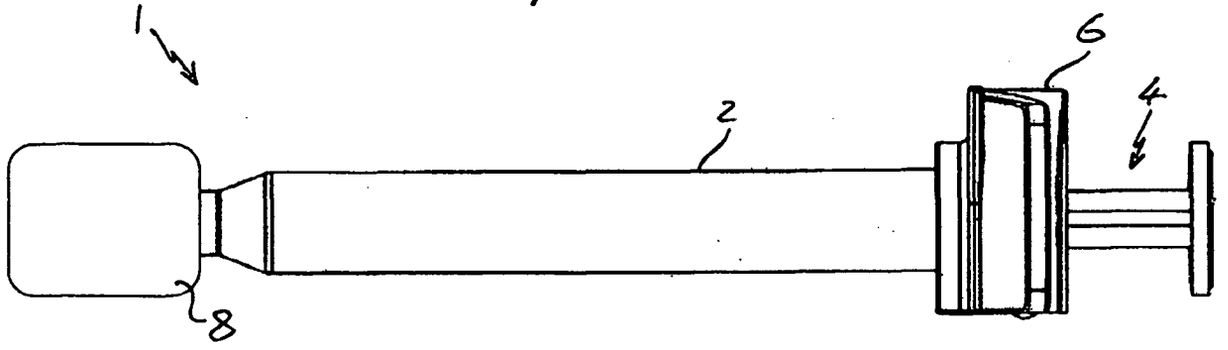

Fig 1

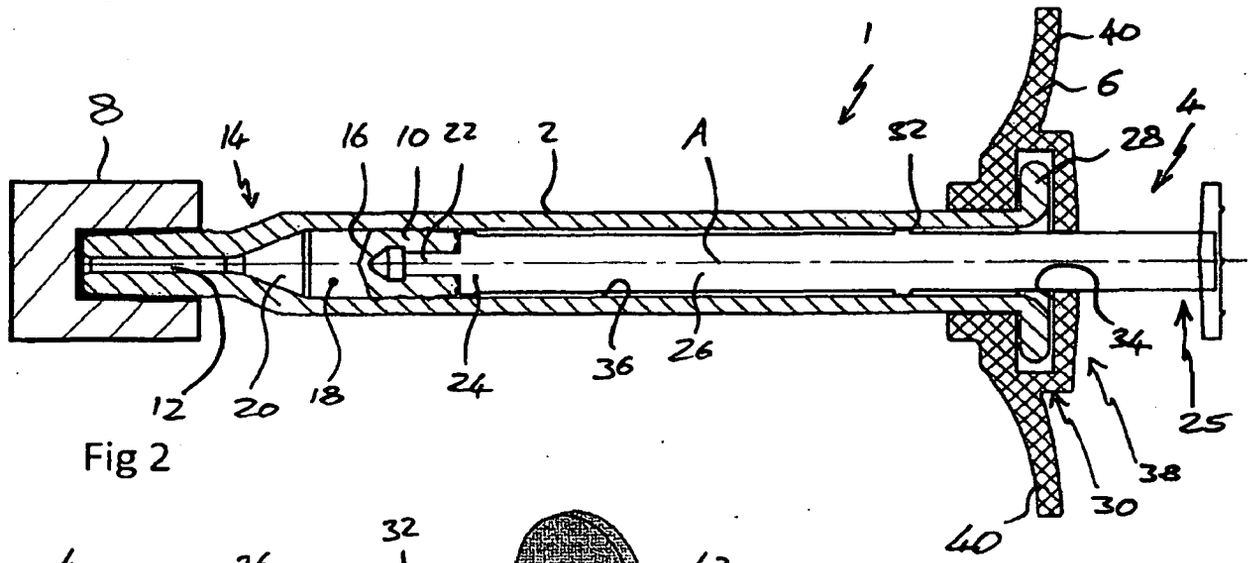

Fig 2

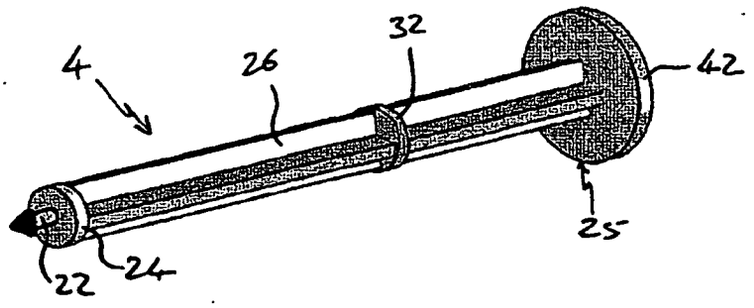

Fig 3

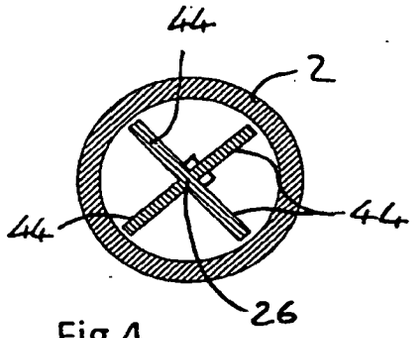

Fig 4

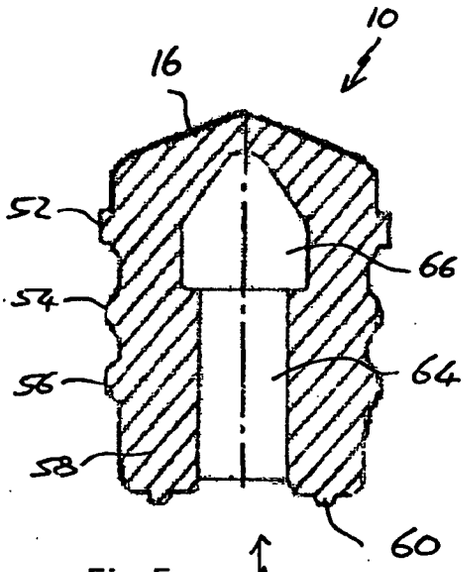

Fig 5

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: **13750352** Filing Date: **03/15/2013**

The presence of this form in the IFW record indicates that the following document is stored in the SCORE database

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I, MICHAEL SHEEHAN, PATENT AND PLANT BREEDERS RIGHTS ADMINISTRATION(PPBRA) hereby certify that annexed is a true copy of the Complete specification in connection with Innovation Patent No. 2012101677 for a patent by NOVARTIS AG as filed on 16 November 2012.

WITNESS my hand this
Twentieth day of December 2012

MJSheehan
MICHAEL SHEEHAN
PATENT AND PLANT BREEDERS
RIGHTS ADMINISTRATION(PPBRA)

COMMONWEALTH OF AUSTRALIA
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Regeneron Exhibit 1002.0313

2012101677 16 Nov 2012

PAT055157-AU-Innovation1

DEVICE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion,

Regeneron Exhibit 1002.0314

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the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being

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dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals

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defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

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In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution. In one embodiment, the extractable volume from the syringe (that is the amount of product obtainable from the syringe following filling, taking into account loss due to dead space in the syringe and needle) is about 0.09ml.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for

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ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100µg-about 800µg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100µg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less; 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil

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in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles ≥25μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789. In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as

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a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDT
LIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVL
TIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
ATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a

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molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLKYGADVNAKDFQGW
TPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNEDLAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-

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determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-

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antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide or a hydrogen peroxide sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes $\leq$10%

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(preferably ≤5%, ≤3%, ≤1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, *x*±10%.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

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MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the

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bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm.

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		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist, wherein:

(a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

(b) the syringe is filled with between about 0.15ml and about 0.175ml of said VEGF antagonist solution which comprises a dosage volume of about 0.05ml of said VEGF antagonist solution,

(c) the syringe barrel comprises less than about 500µg silicone oil,

(d) the VEGF antagonist solution comprises no more than 2 particles ≥50µm in diameter per ml.

and

(e) the VEGF antagonist is the non-antibody VEGF antagonist aflibercept at a concentration of 40mg/ml.

2. A pre-filled syringe according to claim 1, wherein the syringe barrel comprises less than about 100µg silicone oil.

3. A pre-filled syringe according to claim 1 or 2, wherein the syringe has a stopper break loose force of less than about 11N.

4. A pre-filled syringe according to any one of the previous claims, wherein the VEGF antagonist solution further comprises (i) no more than 5 particles ≥25µm in diameter per ml, (ii) no more than 50 particles ≥10µm in diameter per ml, or a combination of both (i) and (ii).

5. A blister pack comprising a pre-filled syringe according to any one of the previous claims, wherein the syringe has been sterilised using H_2O_2 to a Sterility Assurance Level of at least 10^{-6}.

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ABSTRACT

The present invention relates to a device and in particular a syringe, more particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

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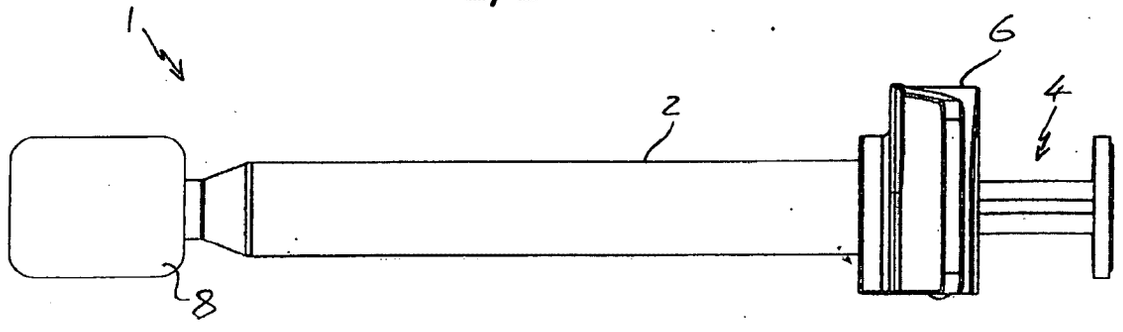

Fig 1

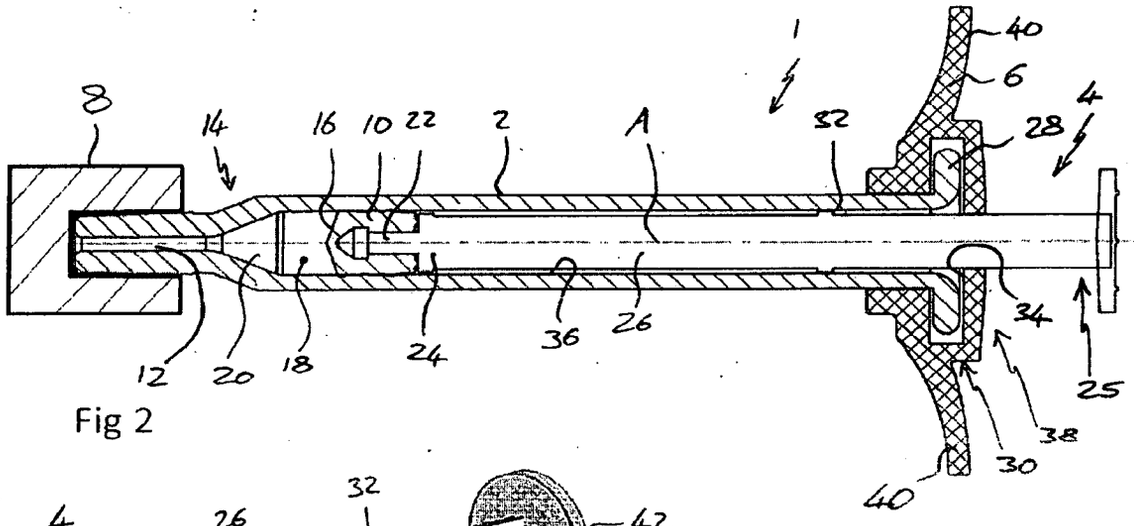

Fig 2

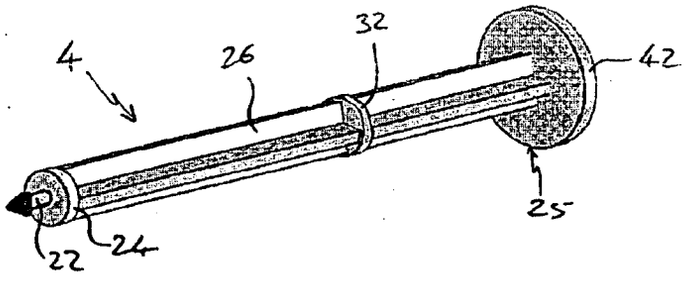

Fig 3

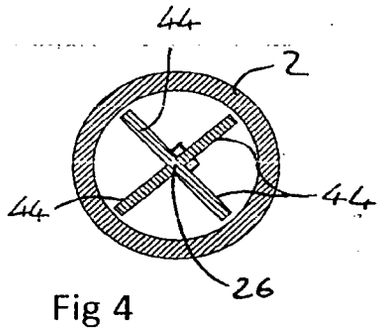

Fig 4

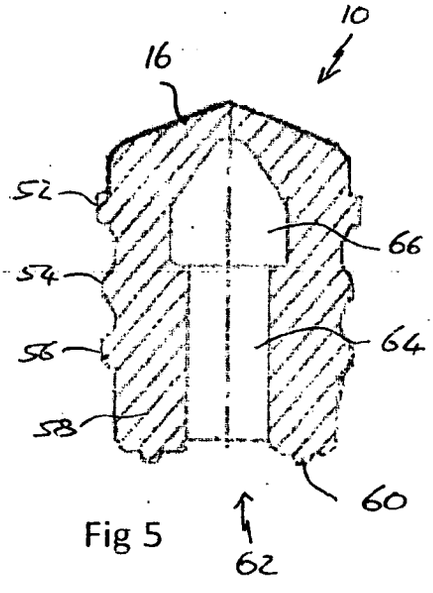

Fig 5

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DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: **13750352** Filing Date: **03/15/2013**

The presence of this form in the IFW record indicates that the following document is stored in the SCORE database

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Patent Office
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I, MICHAEL SHEEHAN, PATENT AND PLANT BREEDERS RIGHTS ADMINISTRATION(PPBRA) hereby certify that annexed is a true copy of the Complete specification in connection with Innovation Patent No. 2012101678 for a patent by NOVARTIS AG as filed on 16 November 2012.

WITNESS my hand this
Twentieth day of December 2012

MJSheehan
MICHAEL SHEEHAN
PATENT AND PLANT BREEDERS
RIGHTS ADMINISTRATION(PPBRA)

COMMONWEALTH OF AUSTRALIA
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PATENT OFFICE

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PAT055157-AU-Innovation2

USE OF DEVICE

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion,

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the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being

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dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals

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defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

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In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution. In one embodiment, the extractable volume from the syringe (that is the amount of product obtainable from the syringe following filling, taking into account loss due to dead space in the syringe and needle) is about 0.09ml.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for

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ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100µg-about 800µg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100µg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil

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in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles ≥25μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789. In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as

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a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATY
KEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDT
LIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEK
LVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVL
TIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
ATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a

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molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLKYGADVNAKDFQGW
TPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNEDLAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-

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determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-

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antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide or a hydrogen peroxide sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes $\leq$10%

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(preferably ≤5%, ≤3%, ≤1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, but the outer surface of the syringe has ≤1ppm, preferably ≤0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is ≤0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, *x*±10%.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

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MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the

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bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm.

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		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. Use of a pre-filled syringe in the treatment of wet age-related macular degeneration, wherein the syringe comprises a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist, wherein:

(a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

(b) the syringe is filled with between about 0.15ml and about 0.175ml of said VEGF antagonist solution which comprises a dosage volume of about 0.05ml of said VEGF antagonist solution,

(c) the syringe barrel comprises less than about 500µg silicone oil,

(d) the VEGF antagonist solution comprises no more than 2 particles ≥50µm in diameter per ml,

and

(e) the VEGF antagonist is the non-antibody VEGF antagonist aflibercept at a concentration of 40mg/ml.

2. A method of treating a patient suffering from wet age-related macular degeneration, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe as defined in claim 1.

3. The method of claim 2, further comprising an initial priming step in which a user depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with a priming mark.

4. A method according to claim 3, wherein the patient has previously received treatment with an antibody VEGF antagonist.

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5. The use according to claim 1, or method according to any one of claims 2 to 4, wherein the VEGF antagonist solution further comprises (i) no more than 5 particles ≥25μm in diameter per ml, (ii) no more than 50 particles ≥10μm in diameter per ml; or a combination of both (i) and (ii).

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ABSTRACT

The present invention relates to a device and in particular a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections. The present invention also relates to uses of the device and methods.

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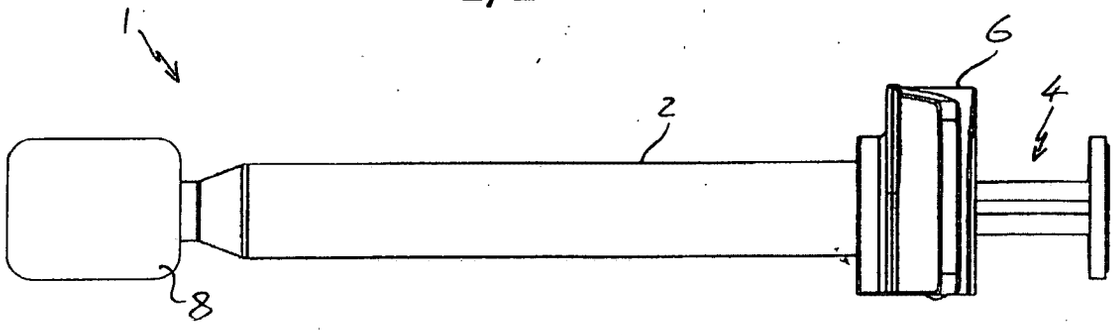

Fig 1

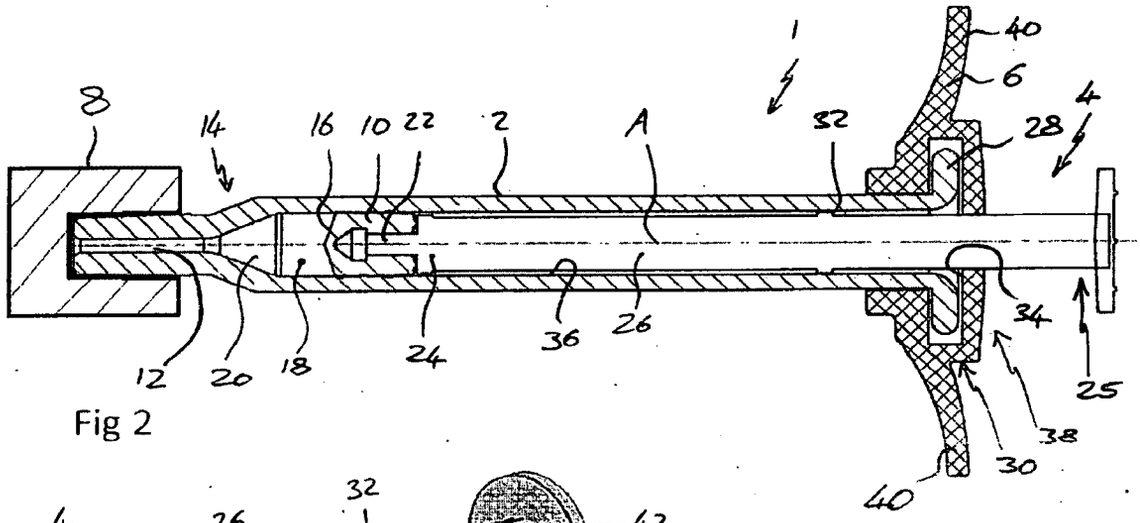

Fig 2

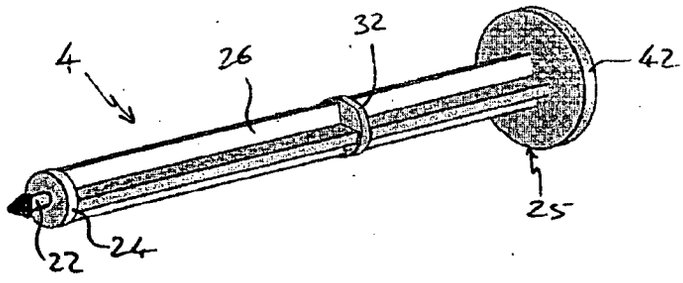

Fig 3

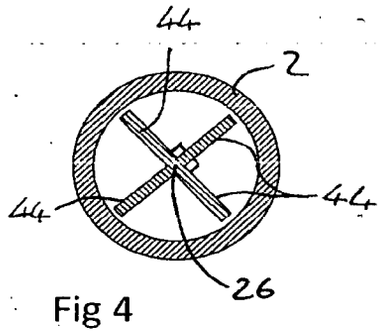

Fig 4

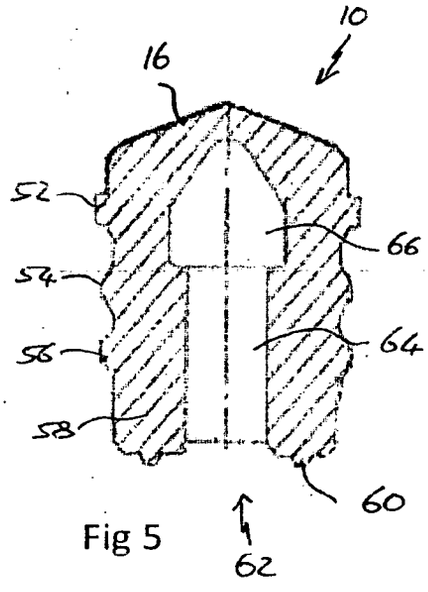

Fig 5

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DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: **13750352** Filing Date: **03/15/2013**

The presence of this form in the IFW record indicates that the following document is stored in the SCORE database

- Certified Copy of Foreign Application with Color Illustrations

To access the documents in the SCORE database, refer to instructions below.

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BUNDESREPUBLIK DEUTSCHLAND

Prioritätsbescheinigung
DE 20 2012 011 260.0
über die Einreichung einer Gebrauchsmusteranmeldung

Aktenzeichen:	20 2012 011 260.0
Anmeldetag:	23. November 2012
Anmelder/Inhaber:	Novartis AG, Basel, CH
Bezeichnung:	Ranibizumab-syringe
Priorität:	23. Oktober 2012; EP; 12189649 03. Juli 2012; EP; 12174860
IPC:	A61M 5/31; A61M 5/178; A61K 39/395

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der Teile der am 23. November 2012 eingereichten Unterlagen dieser Gebrauchsmusteranmeldung unabhängig von gegebenenfalls durch das Kopierverfahren bedingten Farbabweichungen.

München, den 8. Februar 2013
Deutsches Patent- und Markenamt
Die Präsidentin
Im Auftrag

Rüschenschmidt

A 9161(Pat1)/1.

23_11_2012-01-00003-00000123

Description

Ranibizumab-Syringe

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber,

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characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear

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surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs

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may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

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In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or

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DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100µg-about 800µg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100µg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles $\geq$50µm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles $\geq$25µm in diameter per ml. In one embodiment, the ophthalmic

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solution comprises no more than 50 particles ≥10μm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50μm in diameter per ml, no more than 5 particles ≥25μm in diameter per ml and no more than 50 particles ≥10μm in diameter per ml. In one embodiment, a syringe according to the invention meets USP789. In one embodiment the syringe has low levels of silicone oil sufficient for the syringe to meet USP789.

VEGF Antagonists

Antibody VEGF antagonists

VEGF is a well-characterised signal protein which stimulates angiogenesis. Two antibody VEGF antagonists have been approved for human use, namely ranibizumab (Lucentis®) and bevacizumab (Avastin®).

Non-Antibody VEGF antagonists

In one aspect of the invention, the non-antibody VEGF antagonist is an immunoadhesin. One such immuoadhesin is aflibercept (Eylea®), which has recently been approved for human use and is also known as VEGF-trap (Holash *et al.* (2002) *PNAS USA* 99:11393-98; Riely & Miller (2007) *Clin Cancer Res* 13:4623-7s). Aflibercept is the preferred non-antibody VEGF antagonist for use with the invention. Aflibercept is a recombinant human soluble VEGF receptor fusion protein consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1. It is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. It is conveniently produced as a glycoprotein by expression in recombinant CHO K1 cells. Each monomer can have the following amino acid sequence (SEQ ID NO: 1):

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLY
TCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG

and disulfide bridges can be formed between residues 30-79, 124-185, 246-306 and 352-410 within each monomer, and between residues 211-211 and 214-214 between the monomers.

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Another non-antibody VEGF antagonist immunoadhesin currently in pre-clinical development is a recombinant human soluble VEGF receptor fusion protein similar to VEGF-trap containing extracellular ligand-binding domains 3 and 4 from VEGFR2/KDR, and domain 2 from VEGFR1/Flt-1; these domains are fused to a human IgG Fc protein fragment (Li et al., 2011 *Molecular Vision* 17:797-803). This antagonist binds to isoforms VEGF-A, VEGF-B and VEGF-C. The molecule is prepared using two different production processes resulting in different glycosylation patterns on the final proteins. The two glycoforms are referred to as KH902 (conbercept) and KH906. The fusion protein can have the following amino acid sequence (SEQ ID NO:2):

MVSYWDTGVLLCALLSCLLLTGSSSGGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNIT
VTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNT
IIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSG
SEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVE
ATVGERVRLPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTN
PISKEKQSHVVSLVVYVPPGPGDKTHTCPLCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

and, like VEGF-trap, can be present as a dimer. This fusion protein and related molecules are further characterized in EP1767546.

Other non-antibody VEGF antagonists include antibody mimetics (*e.g.* Affibody® molecules, affilins, affitins, anticalins, avimers, Kunitz domain peptides, and monobodies) with VEGF antagonist activity. This includes recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2. One example for such a molecule is DARPin® MP0112. The ankyrin binding domain may have the following amino acid sequence (SEQ ID NO: 3):

GSDLGKKLLEAARAGQDDEVRILMANGADVNTADSTGWTPLHLAVPWGHLEIVEVLLK
YGADVNAKDFQGWTPLHLAAAIGHQEIVEVLLKNGADVNAQDKFGKTAFDISIDNGNED
LAEILQKAA

Recombinant binding proteins comprising an ankyrin repeat domain that binds VEGF-A and prevents it from binding to VEGFR-2 are described in more detail in WO2010/060748 and WO2011/135067.

Further specific antibody mimetics with VEGF antagonist activity are the 40 kD pegylated anticalin PRS-050 and the monobody angiocept (CT-322).

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The afore-mentioned non-antibody VEGF antagonist may be modified to further improve their pharmacokinetic properties or bioavailability. For example, a non-antibody VEGF antagonist may be chemically modified (e.g., pegylated) to extend its *in vivo* half-life. Alternatively or in addition, it may be modified by glycosylation or the addition of further glycosylation sites not present in the protein sequence of the natural protein from which the VEGF antagonist was derived.

Variants of the above-specified VEGF antagonists that have improved characteristics for the desired application may be produced by the addition or deletion of amino acids. Ordinarily, these amino acid sequence variants will have an amino acid sequence having at least 60% amino acid sequence identity with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

Preferably, the non-antibody VEGF antagonist of the invention binds to VEGF via one or more protein domain(s) that are not derived from the antigen-binding domain of an antibody. The non-antibody VEGF antagonist of the invention are preferably proteinaceous, but may include modifications that are non-proteinaceous (e.g., pegylation, glycosylation).

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Therapy

The syringe of the invention may be used to treat an ocular disease, including but not limited to choroidal neovascularisation, age-related macular degeneration (both wet and dry forms), macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

Thus the invention provides a method of treating a patient suffering from of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising the step of administering an ophthalmic solution to the patient using a pre-filled syringe of the invention. This method preferably further comprises an initial priming step in which the physician depresses the plunger of the pre-filled syringe to align the pre-determined part of the stopper with the priming mark.

In one embodiment, the invention provides a method of treating an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy, comprising administering a non-antibody VEGF antagonist with a pre-filled syringe of the invention, wherein the patient has previously received treatment with an antibody VEGF antagonist.

Kits

Also provided are kits comprising the pre-filled syringes of the invention. In one embodiment, such a kit comprises a pre-filled syringe of the invention in a blister pack. The blister pack may itself be sterile on the inside. In one embodiment, syringes according to the invention may be placed inside such blister packs prior to undergoing sterilisation, for example terminal sterilisation.

Such a kit may further comprise a needle for administration of the VEGF antagonist. If the VEGF antagonist is to be administered intravitreally, it is typical to use a 30-gauge x ½ inch needle, though 31-gauge and 32-gauge needles may be used. For intravitreal administration, 33-gauge or 34-gauge needles could alternatively be used. Such kits may further comprise instructions for use. In one embodiment, the invention provides a carton

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containing a pre-filled syringe according to the invention contained within a blister pack, a needle and optionally instructions for administration.

Sterilisation

As noted above, a terminal sterilisation process may be used to sterilise the syringe and such a process may use a known process such as an ethylene oxide or a hydrogen peroxide sterilisation process. Needles to be used with the syringe may be sterilised by the same method, as may kits according to the invention.

The package is exposed to the sterilising gas until the outside of the syringe is sterile. Following such a process, the outer surface of the syringe may remain sterile (whilst in its blister pack) for up to 6 months, 9 months, 12 months, 15 months, 18 months or longer. In one embodiment, less than one syringe in a million has detectable microbial presence on the outside of the syringe after 18 months of storage. In one embodiment, the pre-filled syringe has been sterilised using EtO with a Sterility Assurance Level of at least 10^{-6}. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}. Of course, it is a requirement that significant amounts of the sterilising gas should not enter the variable volume chamber of the syringe. The term "significant amounts" as used herein refers to an amount of gas that would cause unacceptable modification of the ophthalmic solution within the variable volume chamber. In one embodiment, the sterilisation process causes $\leq$10% (preferably $\leq$5%, $\leq$3%, $\leq$1%) alkylation of the VEGF antagonist. In one embodiment, the pre-filled syringe has been sterilised using EtO, but the outer surface of the syringe has $\leq$1ppm, preferably $\leq$0.2ppm EtO residue. In one embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, but the outer surface of the syringe has $\leq$1ppm, preferably $\leq$0.2ppm hydrogen peroxide residue. In another embodiment, the pre-filled syringe has been sterilised using EtO, and the total EtO residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg. In another embodiment, the pre-filled syringe has been sterilised using hydrogen peroxide, and the total hydrogen peroxide residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

General

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x\pm$10%.

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References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a side view of a syringe

Figure 2 shows a cross section of a top down view of a syringe

Figure 3 shows a view of a plunger

Figure 4 shows a cross section though a plunger

Figure 5 shows a stopper

MODES FOR CARRYING OUT THE INVENTION

The invention will now be further described, by way of example only, with reference to the drawings.

Figure 1 shows a view from a side of a syringe 1 comprising a body 2, plunger 4, backstop 6 and a sealing device 8.

Figure 2 shows a cross section through the syringe 1 of Figure 1 from above. The syringe 1 is suitable for use in an ophthalmic injection. The syringe 1 comprises a body 2, a stopper 10 and a plunger 4. The syringe 1 extends along a first axis A. The body 2 comprises an outlet 12 at an outlet end 14 and the stopper 10 is arranged within the body 2 such that a front surface 16 of the stopper 10 and the body 2 define a variable volume chamber 18. The variable volume chamber 18 contains an injectable medicament 20 comprising an ophthalmic solution comprising a VEGF antagonist such as ranibizumab. The injectable fluid 20 can be expelled though the outlet 12 by movement of the stopper 10 towards the outlet end 14 thereby reducing the volume of the variable volume chamber 18. The plunger 4 comprises a plunger contact surface 22 at a first end 24 and a rod 26 extending between the plunger contact surface 22 and a rear portion 25. The plunger contact surface 22 is arranged to contact the stopper 10, such that the plunger 4 can be used to move the stopper 10 towards the outlet end 14 of

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the body 2. Such movement reduces the volume of the variable volume chamber 18 and causes fluid therein to be expelled though the outlet.

The backstop 6 is attached to the body 2 by coupling to a terminal flange 28 of the body 2. The backstop 6 includes sandwich portion 30 which is adapted to substantially sandwich at least some of the terminal flange 28 of the body 2. The backstop 6 is adapted to be coupled to the body 2 from the side by leaving one side of the backstop 6 open so that the backstop 6 can be fitted to the syringe 2.

The body 2 defines a substantially cylindrical bore 36 which has a bore radius. The rod 26 comprises a rod shoulder 32 directed away from the outlet end 14. The rod shoulder 32 extends from to a rod shoulder radius from the first axis A which is such that it is slightly less than the bore radius so that the shoulder fits within the bore 36. The backstop 6 includes a backstop shoulder 34 directed towards the outlet end 14. The shoulders 32, 34 are configured to cooperate to substantially prevent movement of the rod 26 away from the outlet end 14 when the backstop shoulder 34 and rod shoulder 32 are in contact. The backstop shoulder 34 extends from outside the bore radius to a radius less than the rod shoulder radius so that the rod shoulder 32 cannot pass the backstop shoulder 34 by moving along the first axis A. In this case the rod shoulder 32 is substantially disc, or ring, shaped and the backstop shoulder 34 includes an arc around a rear end 38 of the body 2.

The backstop 6 also includes two finger projections 40 which extend in opposite directions away from the body 2 substantially perpendicular to the first axis A to facilitate manual handling of the syringe 1 during use.

In this example the syringe comprises a 0.5ml body 2 filled with between about 0.1 and 0.3 ml of an injectable medicament 20 comprising a 10mg/ml injectable solution comprising ranibizumab. The syringe body 2 has an internal diameter of about between about 4.5mm and 4.8mm, a length of between about 45mm and 50mm.

The plunger 4 and stopper 10 will be described in more detail with reference to later figures.

Figure 3 shows a perspective view of the plunger 4 of Figure 1 showing the plunger contact surface 22 at the first end 24 of the plunger 4. The rod 26 extends from the first end 24 to the rear portion 25. The rear portion 25 includes a disc shaped flange 42 to facilitate user handling of the device. The flange 42 provides a larger surface area for contact by the user than a bare end of the rod 26.

Figure 4 shows a cross section though a syringe body 2 and rod 26. The rod 26 includes four longitudinal ribs 44 and the angle between the ribs is 90°.

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Figure 5 shows a detailed view of a stopper 10 showing a conical shaped front surface 16 and three circumferential ribs 52,54,56 around a substantially cylindrical body 58. The axial gap between the first rib 52 and the last rib 56 is about 3mm. The rear surface 60 of the stopper 10 includes a substantially central recess 62. The central recess 62 includes an initial bore 64 having a first diameter. The initial bore 64 leading from the rear surface 60 into the stopper 10 to an inner recess 66 having a second diameter, the second diameter being larger than the first diameter.

Stopper forces

0.5ml syringes siliconised with <100µg silicone oil, filled with Lucentis, comprising one of two different stopper designs were tested for maximal and average break out and slide force. Prior to testing, 30G x 0.5" needles were attached to the syringes. The testing was carried out at a stopper speed of 190mm/min over a travel length of 10.9mm.

		Stopper design 1			Stopper design 2	
		Batch A	Batch B	Batch C	Batch D	Batch E
Break loose force of syringes	Average of 10 syringes	2.2N	2.3N	1.9N	2.1N	2.5N
	Max individual value	2.5N	2.5N	2.3N	2.6N	2.7N
Sliding force	Average of 10 syringes	3.1N	3.2N	3.1N	4.1N	4.6N
	Max individual value	3.5N	3.5N	3.6N	4.7N	4.8N

For both stopper designs, average and maximum break out force remained below 3N. For both stopper designs, average and maximum sliding force remained below 5N.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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Claims

1. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist, wherein

   (a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

   (b) the syringe is filled with between about 0.15ml and about 0.175ml of said VEGF antagonist solution which comprises a dosage volume of about 0.05ml of said VEGF antagonist solution,

   (c) the syringe barrel comprises less than about 500µg silicone oil,

   (d) the VEGF antagonist solution comprises no more than 2 particles $\geq$50µm in diameter per ml.

   and

   (e) the VEGF antagonist is the antibody VEGF antagonist ranibizumab.

2. A pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber, characterised in that the fluid is an ophthalmic solution which comprises a VEGF-antagonist, wherein

   (a) the syringe has a nominal maximum fill volume of between about 0.5ml and about 1ml,

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(b) the syringe is filled with between about 0.15ml and about 0.175ml of said VEGF antagonist solution which comprises a dosage volume of about 0.05ml of said VEGF antagonist solution,

(c) the syringe barrel comprises less than about 500µg silicone oil,

(d) the VEGF antagonist solution comprises no more than 2 particles ≥50µm in diameter per ml, and

(e) the VEGF antagonist is the antibody VEGF antagonist ranibizumab at a concentration of 10 mg/ml.

3. A pre-filled syringe according to claim 1 or 2, wherein the syringe is filled with about 0.165ml of said VEGF antagonist solution.

4. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less.

5. A pre-filled syringe according to any previous claim, wherein the syringe barrel has an internal coating of less than about 500µg silicone oil, preferably less than about 100µg silicone oil, preferably less than about 50µg silicone oil, preferably less than about 25µg silicone oil.

6. A pre-filled syringe according to any previous claim, wherein the silicone oil is DC365 emulsion.

7. A pre-filled syringe according to any previous claim, wherein the syringe is silicone oil free.

8. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution further comprises one or more of (i) no more than 5 particles ≥25µm in diameter per ml, and (ii) no more than 50 particles ≥10µm in diameter per ml.

9. A pre-filled syringe according to any previous claim, wherein the VEGF antagonist solution meets USP789.

10. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper break loose force of less than about 11N.

11. A pre-filled syringe according to claim 10, wherein the syringe has a stopper break loose force of less than about 5N.

12. A pre-filled syringe according to any previous claim, wherein the syringe has a stopper slide force of less than about 11N.

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13. A pre-filled syringe according to claim 12, wherein the syringe has a stopper slide force of less than about 5N.

14. A pre-filled syringe according to any previous claim, in which the dosage volume is determined by volume of the variable volume chamber when a predetermined part of the stopper or plunger is aligned with a priming mark on the syringe

15. A blister pack comprising a pre-filled syringe according to any previous claim, wherein the syringe has been sterilised using H_2O_2 or EtO.

16. A blister pack comprising a pre-filled syringe according to claim 15, wherein the outer surface of the syringe has $\leq$1ppm EtO or H_2O_2 residue.

17. A blister pack comprising a pre-filled syringe according to claim 15, wherein the syringe has been sterilised using EtO or H_2O_2 and the total EtO or H_2O_2 residue found on the outside of the syringe and inside of the blister pack is $\leq$0.1mg.

18. A blister pack comprising a pre-filled syringe according to any one of claims 15-17, wherein $\leq$5% of the VEGF antagonist is alkylated.

19. A blister pack comprising a pre-filled syringe according to any of claims 15-18, wherein the syringe has been sterilised using EtO or hydrogen peroxide with a Sterility Assurance Level of at least 10^{-6}.

20. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-14, or a blister pack comprising a pre-filled syringe according to any one of claims 15-19, (ii) a needle, and optionally (iii) instructions for administration.

21. A kit according to claim 20, wherein the needle is a 30-gauge x ½ inch needle.

22. A pre-filled syringe according to any one of claims 1-14 for use in therapy.

23. A pre-filled syringe according to any one of claims 1-14 for use in the treatment of an ocular disease selected from choroidal neovascularisation, wet age-related macular degeneration, macular edema secondary to retinal vein occlusion (RVO) including both branch RVO (bRVO) and central RVO (cRVO), choroidal neovascularisation secondary to pathologic myopia (PM), diabetic macular edema (DME), diabetic retinopathy, and proliferative retinopathy.

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1/1

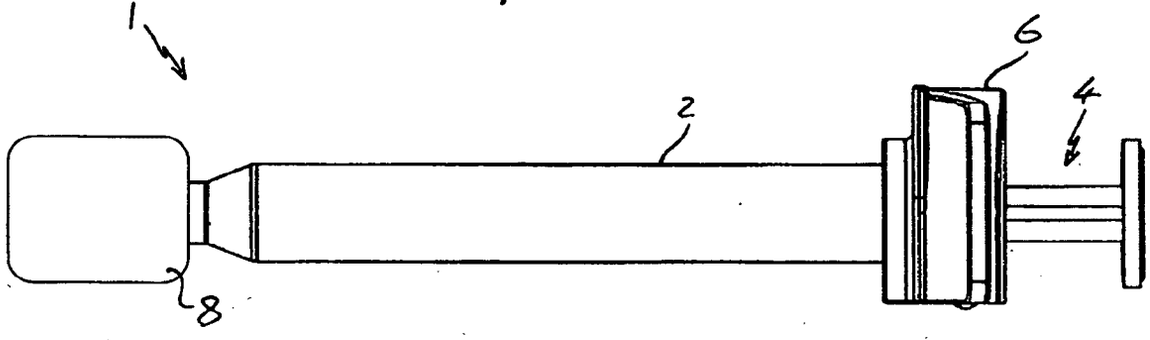

Fig 1

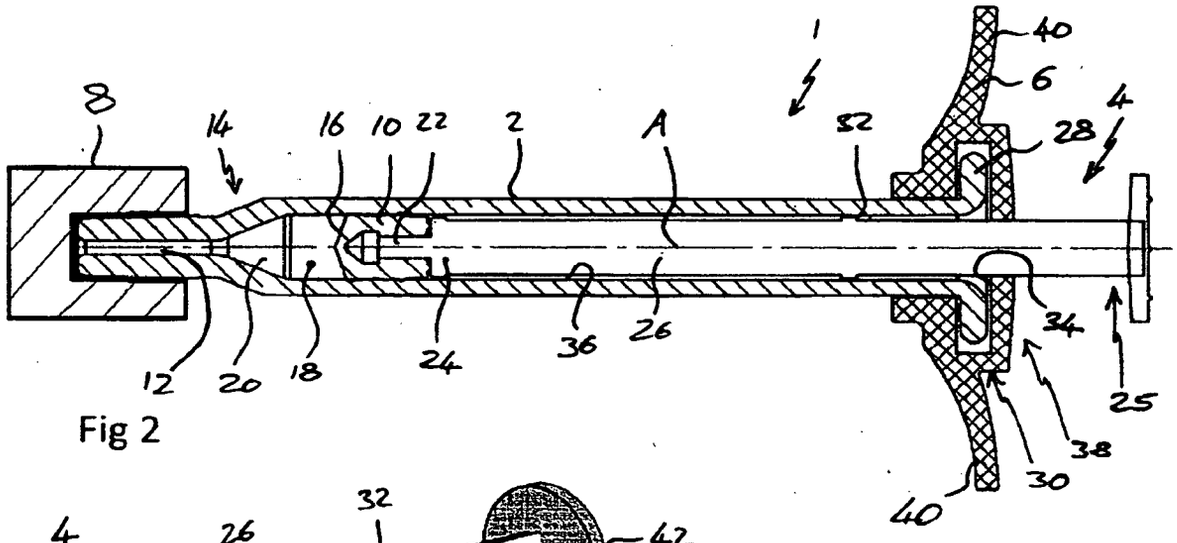

Fig 2

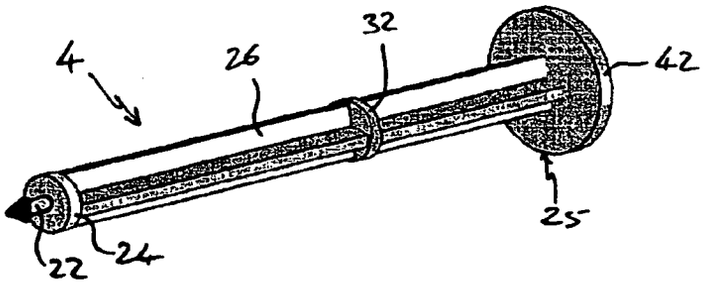

Fig 3

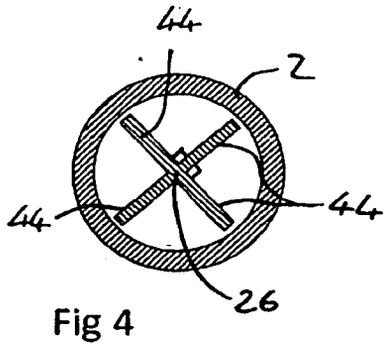

Fig 4

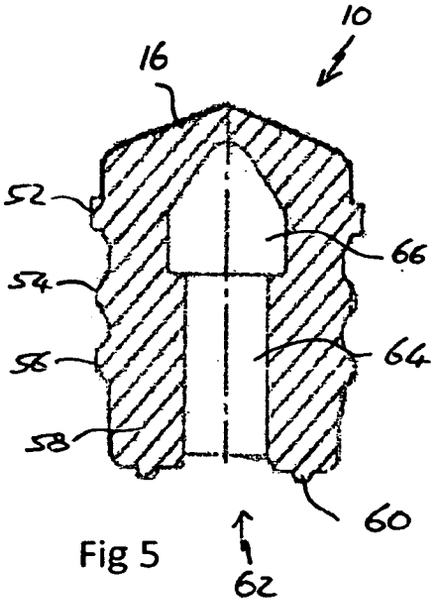

Fig 5

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

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BUNDESREPUBLIK DEUTSCHLAND

Prioritätsbescheinigung
DE 20 2012 011 259.7
über die Einreichung einer Gebrauchsmusteranmeldung

Aktenzeichen:	20 2012 011 259.7
Anmeldetag:	23. November 2012
Anmelder/Inhaber:	Novartis AG, Basel, CH
Bezeichnung:	Bevacizumab-syringe
Priorität:	03. Juli 2012; EP; 12174860 23. Oktober 2012; EP; 12189649
IPC:	A61M 5/31; A61M 5/178; A61K 39/395

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der Teile der am 23. November 2012 eingereichten Unterlagen dieser Gebrauchsmusteranmeldung unabhängig von gegebenenfalls durch das Kopierverfahren bedingten Farbabweichungen.

München, den 19. Februar 2013
Deutsches Patent- und Markenamt
Die Präsidentin
Im Auftrag

Weiss

A 9161(Pat

23_11_2012-01-00003-00000024

Description

Bevacizumab-Syringe

TECHNICAL FIELD

The present invention relates to a syringe, particularly to a small volume syringe such as a syringe suitable for ophthalmic injections.

BACKGROUND ART

Many medicaments are delivered to a patient in a syringe from which the user can dispense the medicament. If medicament is delivered to a patient in a syringe it is often to enable the patient, or a caregiver, to inject the medicament. It is important for patient safety and medicament integrity that the syringe and the contents of that syringe are sufficiently sterile to avoid infection, or other, risks for patients. Sterilisation can be achieved by terminal sterilisation in which the assembled product, typically already in its associated packaging, is sterilised using heat or a sterilising gas.

For small volume syringes, for example those for injections into the eye in which it is intended that about 0.1ml or less of liquid is to be injected the sterilisation can pose difficulties that are not necessarily associated with larger syringes. Changes in pressure, internal or external to the syringe, can cause parts of the syringe to move unpredictably, which may alter sealing characteristics and potentially compromise sterility. Incorrect handling of the syringe can also pose risks to product sterility.

Furthermore, certain therapeutics such as biologic molecules are particularly sensitive to sterilisation, be it cold gas sterilisation, thermal sterilisation, or irradiation. Thus, a careful balancing act is required to ensure that while a suitable level of sterilisation is carried out, the syringe remains suitably sealed, such that the therapeutic is not compromised.

There is therefore a need for a new syringe construct which provides a robust seal for its content, but which maintains ease of use.

DISCLOSURE OF THE INVENTION

The present invention provides a pre-filled syringe, the syringe comprising a body, a stopper and a plunger, the body comprising an outlet at an outlet end and the stopper being arranged within the body such that a front surface of the stopper and the body define a variable volume chamber from which a fluid can be expelled though the outlet, the plunger comprising a plunger contact surface at a first end and a rod extending between the plunger contact surface and a rear portion, the plunger contact surface arranged to contact the stopper, such that the plunger can be used to force the stopper towards the outlet end of the body, reducing the volume of the variable volume chamber,

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characterised in that the fluid comprises an ophthalmic solution. In one embodiment, the ophthalmic solution comprises a VEGF-antagonist.

In one embodiment, the syringe is suitable for ophthalmic injections, more particularly intravitreal injections, and as such has a suitably small volume. The syringe may also be silicone oil free, or substantially silicone oil free, or may comprise a low level of silicone oil as lubricant.

For ophthalmic injections, it is particularly important for the ophthalmic solution to have particularly low particle content. In one embodiment, the syringe meets US Pharmacopeia standard 789 (USP789).

Syringe

The body of the syringe may be a substantially cylindrical shell, or may include a substantially cylindrical bore with a non circular outer shape. The outlet end of the body includes an outlet through which a fluid housed within the variable volume chamber can be expelled as the volume of said chamber is reduced. The outlet may comprise a projection from the outlet end through which extends a channel having a smaller diameter than that of the variable volume chamber. The outlet may be adapted, for example via a luer lock type connection, for connection to a needle or other accessory such as a sealing device which is able to seal the variable volume chamber, but can be operated, or removed, to unseal the variable volume chamber and allow connection of the syringe to another accessory, such as a needle. Such a connection may be made directly between the syringe and accessory, or via the sealing device. The body extends along a first axis from the outlet end to a rear end.

The body may be made from a plastic material (e.g. a cyclic olefin polymer) or from glass and may include indicia on a surface thereof to act as an injection guide. In one embodiment the body may comprise a priming mark. This allows the physician to align a pre-determined part of the stopper (such as the tip of the front surface or one of the circumferential ribs, discussed later) with the mark, thus expelling excess ophthalmic solution and any air bubbles from the syringe. The priming process ensures that an exact, pre-determined dosage is administered to the patient.

The stopper may be made from rubber, silicone or other suitable resiliently deformable material. The stopper may be substantially cylindrical and the stopper may include one or more circumferential ribs around an outer surface of the stopper, the stopper and ribs being dimensioned such that the ribs form a substantially fluid tight seal with an internal surface of the syringe body. The front surface of the stopper may be any suitable shape, for example substantially planar, substantially conical or of a domed shape. The rear

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surface of the stopper may include a substantially central recess. Such a central recess could be used to connect a plunger to the stopper using a snap fit feature or thread connection in a known manner. The stopper may be substantially rotationally symmetric about an axis through the stopper.

The plunger comprises a plunger contact surface and extending from that a rod extends from the plunger contact surface to a rear portion. The rear portion may include a user contact portion adapted to be contacted by a user during an injection event. The user contact portion may comprise a substantially disc shaped portion, the radius of the disc extending substantially perpendicular to the axis along which the rod extends. The user contact portion could be any suitable shape. The axis along which the rod extends may be the first axis, or may be substantially parallel with the first axis.

The syringe may include a backstop arranged at a rear portion of the body. The backstop may be removable from the syringe. If the syringe body includes terminal flanges at the end opposite the outlet end the backstop may be configured to substantially sandwich terminal flanges of the body as this prevent movement of the backstop in a direction parallel to the first axis.

The rod may comprise at least one rod shoulder directed away from the outlet end and the backstop may include a backstop shoulder directed towards the outlet end to cooperate with the rod shoulder to substantially prevent movement of the rod away from the outlet end when the backstop shoulder and rod shoulder are in contact. Restriction of the movement of the rod away from the outlet end can help to maintain sterility during terminal sterilisation operations, or other operations in which the pressure within the variable volume chamber or outside the chamber may change. During such operations any gas trapped within the variable volume chamber, or bubbles that may form in a liquid therein, may change in volume and thereby cause the stopper to move. Movement of the stopper away from the outlet could result in the breaching of a sterility zone created by the stopper. This is particularly important for low volume syringes where there are much lower tolerances in the component sizes and less flexibility in the stopper. The term sterility zone as used herein is used to refer to the area within the syringe that is sealed by the stopper from access from either end of the syringe. This may be the area between a seal of the stopper, for example a circumferential rib, closest to the outlet and a seal of the stopper, for example a circumferential rib, furthest from the outlet. The distance between these two seals defines the sterility zone of the stopper since the stopper is installed into the syringe barrel in a sterile environment.

To further assist in maintaining sterility during the operations noted above the stopper may comprise at a front circumferential rib and a rear circumferential rib and those ribs

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may be separated in a direction along the first axis by at least 3mm, by at least 3.5 mm, by at least 3.75mm or by 4mm or more. One or more additional ribs (for example 2, 3, 4 or 5 additional ribs, or between 1-10, 2-8, 3-6 or 4-5 additional ribs) may be arranged between the front and rear ribs. In one embodiment there are a total of three circumferential ribs.

A stopper with such an enhanced sterility zone can also provide protection for the injectable medicament during a terminal sterilisation process. More ribs on the stopper, or a greater distance between the front and rear ribs can reduce the potential exposure of the medicament to the sterilising agent. However, increasing the number of ribs can increase the friction between the stopper and syringe body, reducing ease of use. While this may be overcome by increasing the siliconisation of the syringe, such an increase in silicone oil levels is particularly undesirable for syringes for ophthalmic use.

The rod shoulder may be arranged within the external diameter of the rod, or may be arranged outside the external diameter of the rod. By providing a shoulder that extends beyond the external diameter of the rod, but still fits within the body, the shoulder can help to stabilise the movement of the rod within the body by reducing movement of the rod perpendicular to the first axis. The rod shoulder may comprise any suitable shoulder forming elements on the rod, but in one embodiment the rod shoulder comprises a substantially disc shaped portion on the rod.

In one embodiment of the syringe, when arranged with the plunger contact surface in contact with the stopper and the variable volume chamber is at its intended maximum volume there is a clearance of no more than about 2mm between the rod shoulder and backstop shoulder. In some embodiments there is a clearance of less than about 1.5 mm and in some less than about 1mm. This distance is selected to substantially limit or prevent excessive rearward (away from the outlet end) movement of the stopper.

In one embodiment the variable volume chamber has an internal diameter greater than 5mm or 6mm, or less than 3mm or 4mm. The internal diameter may be between 3mm and 6mm, or between 4mm and 5mm.

In another embodiment the syringe is dimensioned so as to have a nominal maximum fill volume of between about 0.1ml and about 1.5ml. In certain embodiments the nominal maximum fill volume is between about 0.5ml and about 1ml. In certain embodiments the nominal maximum fill volume is about 0.5ml or about 1ml, or about 1.5ml.

The length of the body of the syringe may be less than 70mm, less than 60mm or less than 50mm. In one embodiment the length of the syringe body is between 45mm and 50mm.

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In one embodiment, the syringe is filled with between about 0.01ml and about 1.5ml (for example between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml, between about 0.15ml and about 0.175ml) of a VEGF antagonist solution. In one embodiment, the syringe is filled with 0.165ml of a VEGF antagonist solution. Of course, typically a syringe is filled with more than the desired dose to be administered to the patient, to take into account wastage due to "dead space" within the syringe and needle. There may also be a certain amount of wastage when the syringe is primed by the physician, so that it is ready to inject the patient.

Thus, in one embodiment, the syringe is filled with a dosage volume (i.e. the volume of medicament intended for delivery to the patent) of between about 0.01ml and about 1.5ml (e.g. between about 0.05ml and about 1ml, between about 0.1ml and about 0.5ml) of a VEGF antagonist solution. In one embodiment, the dosage volume is between about 0.03ml and about 0.05ml. For example, for Lucentis, the dosage volume is 0.05ml or 0.03ml (0.5mg or 0.3mg) of a 10mg/ml injectable medicament solution; for Eylea, the dosage volume is 0.05ml of a 40mg/ml injectable medicament solution.

In one embodiment the length of the syringe body is between about 45mm and about 50mm, the internal diameter is between about 4mm and about 5mm, the fill volume is between about 0.12 and about 0.3ml and the dosage volume is between about 0.03ml and about 0.05ml.

As the syringe contains a medicament solution, the outlet may be reversibly sealed to maintain sterility of the medicament. This sealing may be achieved through the use of a sealing device as is known in the art. For example the OVS™ system which is available from Vetter Pharma International GmbH.

It is typical to siliconise the syringe in order to allow ease of use, i.e. to apply silicone oil to the inside of the barrel, which decreases the force required to move the stopper. However, for ophthalmic use, it is desirable to decrease the likelihood of silicone oil droplets being injected into the eye. Furthermore, silicone oil can cause proteins to aggregate. A typical 1ml syringe comprises 100-800µg silicone oil in the barrel. Thus, in one embodiment, a syringe according to the invention comprises less than about 800µg (i.e. about less than about 500µg, less than about 300µg, less than about 200µg, less than about 100µg, less than about 75µg, less than about 50µg, less than about 25µg, less than about 15µg, less than about 10µg) silicone oil in the barrel. Methods for measuring the amount of silicone oil in such a syringe barrel are known in the art and include, for example, differential weighing methods and quantitation by infrared-spectroscopy of the oil diluted in a suitable solvent. Various types of silicone oil are available, but typically either DC360 (Dow Corning®; with a viscosity of 1000cP) or

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DC365 emulsion (Dow Corning®; DC360 oil with a viscosity of 350cP) are used for syringe siliconisation. In one embodiment, the pre-filled syringe of the invention comprises DC365 emulsion.

During testing it was found that, for syringes having small dimensions, such as those discussed above, and particularly those described in conjunction with the Figures below, the break loose and sliding forces for the stopper within the syringe are substantially unaffected by reducing the siliconisation levels far below the current standard to the levels discussed here. This is in contrast to conventional thinking that would suggest that if you decrease the silicone oil level, the forces required would increase. Having too great a force required to move the stopper can cause problems during use for some users, for example accurate dose setting or smooth dose delivery may be made more difficult if significant strength is required to move, and/or keep in motion, the stopper. Break loose and slide forces for pre-filled syringes known in the art are typically in the region of less than 20N, but where the pre-filled syringes contain about 100µg-about 800µg silicone oil. In one embodiment the glide/slide force for the stopper within the pre-filled syringe is less than about 11N or less than 9N, less than 7N, less than 5N or between about 3N to 5N. In one embodiment, the break loose force is less than about 11N or less than 9N, less than 7N, less than 5N or between about 2N to 5N. Note that such measurements are for a filled syringe, rather than an empty syringe. The forces are typically measured at a stopper travelling speed of 190mm/min. In one embodiment, the syringe has a nominal maximal fill volume of between about 0.5ml and 1ml, contains less than about 100µg silicone oil and has a break loose force between about 2N to 5N.

In one embodiment the syringe barrel has an internal coating of silicone oil that has an average thickness of about 450nm or less (i.e. 400nm or less, 350nm or less, 300nm or less, 200nm or less, 100nm or less, 50nm or less, 20nm or less). Methods to measure the thickness of silicone oil in a syringe are known in the art and include the rap.ID Layer Explorer® Application, which can also be used to measure the mass of silicone oil inside a syringe barrel.

In one embodiment, the syringe is silicone oil free, or substantially silicone oil free. Such low silicone oil levels can be achieved by using uncoated syringe barrels and/or by avoiding the use of silicone oil as a lubricant for product contacting machine parts, or pumps in the syringe assembly and fill line.

The syringe according to the invention may also meet certain requirements for particulate content. In one embodiment, the ophthalmic solution comprises no more than 2 particles ≥50µm in diameter per ml. In one embodiment, the ophthalmic solution comprises no more than 5 particles ≥25µm in diameter per ml. In one embodiment, the ophthalmic