and/or fostering development of a more favorable environment in the host organism (Kotwal, GJ, *Immunology Today*, 21(5), 242-248, 2000). VCCPs are among these proteins. Poxvirus complement control proteins are members of the complement control protein (CCP) superfamily and typically contain 4 SCR modules. These proteins possess features that make them particularly advantageous for treatment and prevention of macular degeneration related conditions and for treatment and prevention of choroidal neovascularization.

[00180] Thus in certain embodiments of the invention one or both of the therapeutic agents is a poxvirus complement control protein (PVCCP). The PVCCP can comprise a sequence encoded by, e.g., vaccinia virus, variola major virus, variola minor virus, cowpox virus, monkeypox virus, ectromelia virus, rabbitpox virus, myxoma virus, Yaba-like disease virus, or swinepox virus. In other embodiments the VCCP is a herpesvirus complement control protein (HVCCP). The HVCCP can comprise a sequence encoded by a *Macaca fuscata* rhadinovirus, cercopithecine herpesvirus 17, or human herpes virus 8. In other embodiments the HVCCP comprises a sequence encoded by herpes simplex virus saimiri ORF 4 or ORF 15 (Albrecht, JC. & Fleckenstein, B., J. Virol., 66, 3937-3940, 1992; Albrecht, J., et al., Virology, 190, 527-530, 1992).

[00181] The VCCP may inhibit the classical complement pathway, the alternate complement pathway, the lectin pathway, or any combination of these. In certain embodiments of the invention the VCCP, e.g., a PVCCP, binds to C3b, C4b, or both. In certain embodiments of the invention the PVCCP comprises one or more putative heparin binding sites (K/R-X-K/R) and/or possesses an overall positive charge. Preferably the PVCCP comprises at least 3 SCR modules (e.g., modules 1-3), preferably 4 SCR modules. The PVCCP protein can be a precursor of a mature PVCCP (i.e., can include a signal sequence that is normally cleaved off when the protein is expressed in virus-infected cells) or can be a mature form (i.e., lacking the signal sequence).

[00182] Vaccinia complement control protein (VCP) is a virus-encoded protein secreted from vaccinia infected cells. VCP is 244 amino acids in length, contains 4 SCRs, and is naturally produced by intracellular cleavage of a 263 amino acid precursor. VCP runs as an ~35 kD protein in a 12% SDS/polyacrylamide gel under reducing conditions and has a predicted molecular mass of about 28.6 kD. VCP is described in U.S. Patent Nos. 5,157,110 and 6,140,472, and in Kotwal, GK, et al., *Nature*, 355, 176-178, 1988. Figures 3A and 3B show the sequence of the precursor and mature VCP proteins, respectively. VCP has been shown to inhibit the classical pathway of complement activation via its ability to bind to C3 and C4 and act as a cofactor for factor I mediated cleavage of these components as well as promoting decay of existing convertase (Kotwal, GK, et al., *Science*, 250, 827-830, 1990; McKenzie et al., *J.*

Infect. Dis., 1566, 1245-1250, 1992). It has also been shown to inhibit the alternative pathway by causing cleavage of C3b into iC3b and thereby preventing formation of the alternative pathway C3 convertase (Sahu, A, et al., *J. Immunol.*, 160, 5596-5604, 1998). VCP thus blocks complement activation at multiple steps and reduces levels of the proinflammatory chemotactic factors C3a, C4a, and C5a.

[00183] VCP also possesses the ability to strongly bind heparin in addition to heparan sulfate proteoglycans. VCP contains two putative heparin binding sites located in modules 1 and 4 (Jha, P and Kotwal, GJ, and references therein). VCP is able to bind to the surface of endothelial cells, possibly via interaction with heparin and/or heparan sulfate at the cell surface, resulting in decreased antibody binding (Smith, SA, et al., *J. Virol.*, 74(12), 5659-5666, 2000). VCP can be taken up by mast cells and possibly persist in tissue for lengthy periods of time, thereby potentially prolonging its activity (Kotwal, GJ, et al., *In* GP. Talwat, et al. (eds), 10th International Congress of Immunology., Monduzzi Editore, Bologna, Italy, 1998). In addition, VCP can reduce chemotactic migration of leukocytes by blocking chemokine binding (Reynolds, D, et al., in S. Jameel and L. Villareal (ed., *Advances in animal virology*. Oxford and IBN Publishing, New Delhi, India, 1999).

[00184] Variola virus major and minor encode proteins that are highly homologous to VCP and are referred to as smallpox inhibitor of complement enzymes (SPICE) (Rosengard, AM, et al., *Proc. Natl. Acad. Sci.*, 99(13), 8803-8813. U.S. Pat. No. 6,551,595). SPICE from various variola strains sequenced to date differs from VCP by about 5% (e.g., about 11 amino acid differences). Similarly to VCP, SPICE binds to C3b and C4b and causes their degradation, acting as a cofactor for factor I. However, SPICE degrades C3b approximately 100 times as fast as VCP and degrades C4b approximately 6 times as fast as VCP. The amino acid sequence of SPICE is presented in Figure 6 and can be described as follows. Referring to Figure 6, a signal sequence extends from amino acid 1 to about amino acid 19. Four SCRs extend from about amino acid 20 to amino acid 263. Each SCR is characterized by four cysteine residues. The four cysteine residues form two disulfide bonds in the expressed protein. The boundaries of each SCR are best defined by the first and fourth cysteine residues in the sequence that forms the disulfide bonds of the SCR. An invariant tryptophan residue is present between cysteine 3 and cysteine 4 of each SCR. SCR1 extends from amino acid 20 or 21 to amino acid 81. Both residues are cysteines that may be involved in disulfide bonding. SCR2 extends from amino acid 86 to amino acid 143. SCR3 extends from amino acid 148 to amino acid 201. SCR4 extends from amino acid 206 to amino acid 261. The SCRs include the complement binding locations of SPICE. SPICE or any of the portions thereof that inhibit complement activation, e.g., SPICE and

SPICE-related polypeptides containing four SCRs, such as those described in U.S. Pat. No. 6,551,595, are of use in the present invention.

[00185] Complement control proteins from cowpox virus (referred to as inflammation modulatory protein, IMP) and monkeypox virus (referred to herein as monkeypox virus complement control protein, MCP) have also been identified and sequenced (Miller, CG, et al., *Virology*, 229, 126-133, 1997 and Uvarova, EA and Shchelkunov, SN, *Virus Res.*, 81(1-2), 39-45, 2001). MCP differs from the other PVCCPs described herein in that it contains a truncation of the C-terminal portion of the fourth SCR.

[00186] It will be appreciated that the exact sequence of complement control proteins identified in different virus isolates may differ slightly. Such proteins fall within the scope of the present invention. Complement control proteins from any such isolate may be used, provided that the protein has not undergone a mutation that substantially abolishes its activity. Thus the sequence of a VCCP such as SPICE or VCP may differ from the exact sequences presented herein or under the accession numbers listed in Table 1. It will also be appreciated that a number of amino acid alterations, e.g., additions, deletions, or substitutions such as conservative amino acid substitutions, may be made in a typical polypeptide such as a VCCP without significantly affecting its activity, such that the resulting protein is considered equivalent to the original polypeptide. For example, up to about 10% of the amino acids, or up to about 20% of the amino acids may frequently be changed without significantly altering the activity. Also, of course, domains known to have similar functions can be substituted for one another. Such domains may be found within a single polypeptide (e.g., repeated domains) or within different, homologous polypeptides. The effect of any particular amino acid alteration(s) or domain substitutions can readily be determined.

[00187] Figure 4 shows a sequence alignment of a variety of poxvirus complement control proteins from isolates of variola major and minor, vaccinia, cowpox virus, and monkeypox virus. Figure 5 shows a comparison of the SCR domain structure of a number of complement control proteins and fragments thereof, the number of K+R residues, %K+R residues, pI, number of putative heparin binding sites, and ability to inhibit hemolysis (indicative of complement inhibiting activity) and/or bind to heparin.

[00188] Without limitation, any of the viral polypeptides identified by accession number in Table 2 below is of use in various embodiments of the invention.

[00189] Table 2: Representative Viral Complement Control Proteins

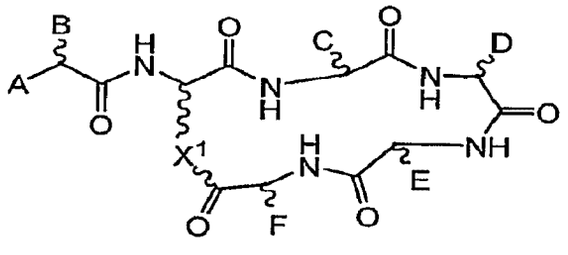
Virus	Protein	Accession	Virus Type
Variola	D12L	NP_042056	Orthopoxvirus
	D15L (SPICE)	AAA69423	Orthopoxvirus
Vaccinia	VCP	AAO89304	Orthopoxvirus
Cowpox	CPXV034	AAM13481	Orthopoxvirus
	C17L	CAA64102	Orthopoxvirus
Monkeypox	D14L	AAV84857	Orthopoxvirus
Ectromelia virus	Complement control protein	CAE00484	Orthopoxvirus
Rabbitpox	RPXV017	AAS49730	Orthopoxvirus
Macaca fuscata rhadinovirus	JM4	AAS99981	Rhadinavirus (Herpesvirus)
Cercopithecine herpesvirus 17	Complement binding protein (ORF4)	NP_570746	Herpesvirus
Human herpes virus 8	Complement binding protein (ORF4)	AAB62602	Herpesvirus

[00190] ***Compounds that Inhibit C5 Activation or Activity***

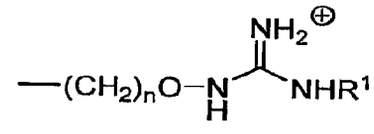
[00191] In certain embodiments the complement inhibitor inhibits activation of C5. For example, the complement inhibitor may bind to C5. Exemplary agents include antibodies, antibody fragments, polypeptides, small molecules, and aptamers. Exemplary antibodies are described in U.S. Pat. No. 6,534,058. Exemplary compounds that bind to and inhibit C5 are described in U.S. Pat. Pub. Nos. 20050090448 and 20060115476. In certain embodiments the complement inhibitor is an antibody, small molecule, aptamer, or polypeptide that binds to substantially the same binding site on C5 as an antibody described in U.S. Pat. No. 6,534,058 or a peptide described in USSN 10/937,912. U.S. Pat. Pub. No. 20060105980 discloses aptamers that bind to and inhibit C5. Also of use are RNAi agents that inhibit expression of C5 or C5R.

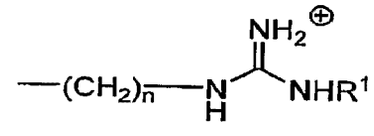
[00192] In other embodiments the agent is an antagonist of a C5a receptor (C5aR). Exemplary C5a receptor antagonists include a variety of small cyclic peptides such as those described in U.S. Pat. No. 6,821,950; USSN 11/375,587; and/or PCT/US06/08960 (WO2006/099330).

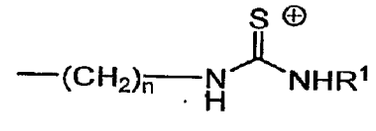
For example, the therapeutic agent may be a compound of general formula I below:

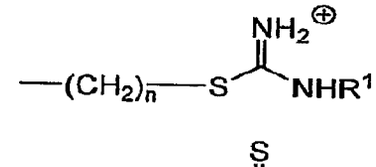


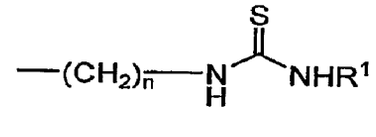
[00193] where A is H, alkyl, aryl, NH_2, NHalkyl, $N(alkyl)_2$, NHaryl or NHacyl; B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid selected from the group consisting of phenylalanine, homophenylalanine, tryptophan, homotryptophan, tyrosine, and homotyrosine; C is the side chain of a D-, L- or homo-amino acid selected from the group consisting of proline, alanine, leucine, valine, isoleucine, arginine, histidine, aspartate, glutamate, glutamine, asparagine, lysine, tyrosine, phenylalanine, cyclohexylalanine, norleucine, tryptophan, cysteine and methionine; D is the side chain of a D- or L-amino acid selected from the group consisting of cyclohexylalanine, homocyclohexylalanine, leucine, norleucine, homoleucine, homonorleucine and tryptophan; E is the side chain of a D- or L-amino acid selected from the group consisting of tryptophan and homotryptophan; F is the side chain of a D- or L-amino acid selected from the group consisting of arginine, homoarginine, lysine and homolysine or is one of the following side-chains

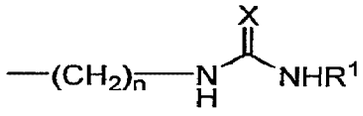


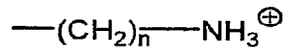






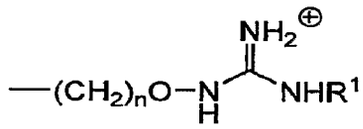


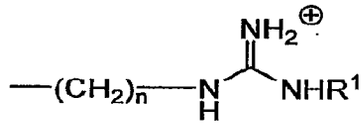


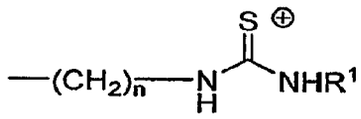


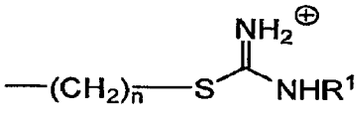
[00194] or another mimetic of an arginine side chain, where X is NCN, NNO_2, $CHNO_2$ or NSO_2NH_2; n is an integer from 1 to 4, and R^1 is H or an alkyl, aryl, CN, NH_2, OH, --CO--CH_2CH_3, --CO--CH_3, --CO--$CH_2CH_2CH_3$, --CO--CH_2 Ph, or --CO-Ph; and X^1 is --$(CH_2)_nNH$-- or $(CH_2)_n$ --S--, --$(CH_2)_2$ O--, --$(CH_2)_3$ O--, --$(CH_2)_3$ --, --$(CH_2)_4$ --, or --CH_2 COCHRNH--, where R is the side chain of any common or uncommon amino acid, and where n is an integer of from 1 to 4, e.g., 1, 2, 3, or 4.

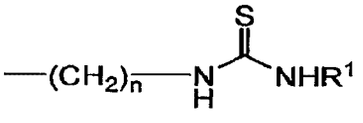
[00195] In certain embodiments of the invention F is one of the following side-chains:

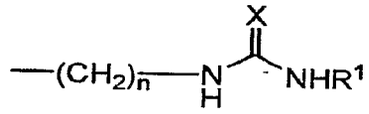


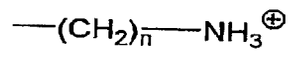












or another mimetic of an arginine side chain; where X is NCN, NNO_2, $CHNO_2$ or NSO_2NH_2; n is an integer from 1 to 4, and R^1 is H or an alkyl, aryl, CN, NH_2, OH, --CO—CH_2CH_3, --CO--CH_3, --CO--$CH_2CH_2CH_3$, --CO--CH_2 Ph, or --CO-Ph; B is an indole, indole methyl, benzyl, phenyl, naphthyl, naphthyl methyl, cinnamyl group, or any other derivative of the aromatic group; and C is D- or L-cyclohexylalanine (Cha), leucine, valine, isoleucine, phenylalanine, tryptophan or methionine. In certain embodiments of the invention A is L-arginine. In certain embodiments of the invention F is an L-amino acid. In certain embodiments F is L-arginine. In certain embodiments n = 1, 2, 3, or 4.

[00196] In certain embodiments of the invention the compound is selected from the group consisting of SEQ ID NOs: 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 and 28, as described in U.S. Pat. No. 6,821,950. Other embodiments disclosed therein may also be used. For example, A, B, C, D, E, F, and R^1 may be any of the groups mentioned in U.S. Pat. No. 6,821,950. It is noted that the letters A, B, C, D, E, and F in the formulas presented herein are to be given the meanings described herein and in U.S. Pat. No. 6,821,950 and do not stand for chemical elements or isotopes such as boron, carbon, deuterium, or fluorine. The compounds described above will be referred to collectively herein as GPCRA.

[00197] In one embodiment, the complement inhibitor is a C5a receptor inhibitor, e.g., a C5a antagonist. For example, the complement inhibitor may be a peptide having the following sequence: HC-[ORN-PRO-dCHA-TRP-ARG] (SEQ ID NO: 45) where HC = hydrocinnamate, dCHA = d-cyclohexylalaine, ORN = l-ornithine, and [ ] denotates cyclization through an amide bound. In another embodiment the complement inhibitor is a peptide having sequence Ac-PHE-[ORN-PRO-dCHA-TRP-ARG] (SEQ ID NO: 46), using the same abbreviations. In one embodiment, the therapeutic agent is the compound depicted in Figure 8. In certain embodiments of the invention the complement inhibitor is a C3a receptor inhibitor, e.g., a C3a antagonist.

[00198] Methods for making the GPCRA, confirming their structure, and testing their activity as modulators of a GPCR are disclosed in U.S. Pat. No. 6,821,950. Certain of these compounds

are available from Promics (Brisbane, Australia). In one embodiment the complement inhibitor is PMX205.

[00199] ***C. Long-acting Therapeutic Agents***

[00200] In certain embodiments of the invention at least one of the therapeutic agents is a long-acting agent. For example, certain complement inhibitors may intrinsically have a long duration of activity even if not provided as a component of a sustained release formulation. The long-acting therapeutic agent may, for example, have an activity period of at least 3 months, at least 6 months, at least 9 months, or at least 12 months when administered in solution in a liquid medium in medically acceptable quantities. The long-acting therapeutic agent may be administered in solution in a liquid medium or may be a component of a solid or semi-solid formulation which optionally contains one or more additional therapeutically active or inactive components.

[00201] In other embodiments a therapeutic agent that is not a long-acting agent is modified such that it becomes long-acting. The modification may, for example, stabilize the agent against the activity of various endogenous molecules such as proteases. Suitable modifications are known in the art and include, for example, pegylation.

[00202] In certain embodiments of the invention the long-acting therapeutic agent is administered as a component of a sustained release formulation, e.g., an ocular implant or any sustained release formulation described herein.

[00203] ***III. Liquid Compositions Comprising a Therapeutic Agent***

[00204] In certain embodiments of the invention at least one of the therapeutic agents, e.g., any of the therapeutic agents discussed above, is administered in solution in a liquid medium. Suitable preparations, e.g., substantially pure preparations of one or more therapeutic agents may be combined with pharmaceutically acceptable carriers, diluents, solvents, etc., to produce an appropriate pharmaceutical composition, i.e., one that is pharmaceutically acceptable for administration to the eye. The preparation may contain a pharmaceutically acceptable carrier, diluent, etc. Suitable carriers are known in the art and include, for example, sterile water for injection, saline, etc. Additional components may include, but are not limited to, buffers, preservatives, salts, etc.

[00205]

The therapeutic agents themselves may be provided as pharmaceutically acceptable salts, which include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate,

cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Salts derived from appropriate bases include alkali metal (e.g., sodium and potassium), alkaline earth metal (e.g., magnesium), ammonium and N+(C1-4 alkyl)4 salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

[00206] Solutions or suspensions can include components such as a sterile diluent such as water for injection, saline solution, or other solvent acceptable for administration to the eye, buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The preparation can be enclosed in ampoules, disposable syringes or single or multiple dose vials made of glass or plastic and provided for commercial sale and/or use in any such manner. The term "suspension" includes a composition comprising particles in a liquid medium. In some embodiments, the particles consist essentially of a therapeutic agent. In other embodiments the particles comprise a drug-releasing component such as a polymer and, optionally, one or more additional components such as an excipient.

[00207] In some embodiments of the invention the liquid composition comprises an agent that enhances uptake of the therapeutic agent by cells, enhances bioavailability of the agent at its site of action, or otherwise enhances activity of the therapeutic agent. For example, a variety of delivery vehicles that enhance uptake and/or activity of RNAi agents such as siRNAs are known in the art and may be included in the liquid composition.

[00208] Preferred pharmaceutical formulations are stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms such as bacteria and fungi.

[00209] ***IV. Sustained Release Formulations***

[00210] A sustained release formulation of use in the present invention provides a therapeutic concentration of a drug within the eye or a portion or region thereof for a prolonged period of time. The period of time during which a therapeutic level of the drug is present can be, e.g., at least 1, 2, 4, or 6 weeks, at least 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 24 months, or longer. Release

may begin immediately or shortly (e.g., within 24 hours) after administration of the sustained delivery formulation. Alternately, release may be delayed, e.g., it may commence at a time point at least 24 hours following administration. Without limitation, release may occur steadily or may occur intermittently (e.g., in bursts during which a substantial amount of the agent is released), or periods of steady release may alternate with bursts. In certain embodiments the therapeutic agent is released at controlled or predetermined rates when the sustained release formulation is placed in the eye. Such rates may range, for example, from about 0.003 micrograms/day to about 5000 micrograms/day, or between about .01 micrograms/day to about 5 micrograms/day, or between about .05 micrograms to about 1 microgram/day. In some embodiments the rate of release is between 1 μg and 5 μg/day.

[00211] A sustained release formulation of use in the present invention typically comprises a therapeutic agent and an additional component, element, or structure that contributes to the sustained release properties of the formulation. The additional component, element, or structure that is effective to provide sustained release is referred to herein as a "drug delivery regulating component". Optionally the drug delivery regulating element is designed to provide control over the kinetics of release. It will be appreciated that the physical nature of the formulation, e.g., the shape and total surface area of any solid or semi-solid constituents, may contribute to its sustained release properties. As another example, tight compression of particles containing an active agent may result in release that takes place over a longer time period than if the particles were not compressed. In some embodiments the structure is provided at least in part by the therapeutic agent itself and, optionally, one or more substances present at the site of administration such as an ion, protein, etc. In some embodiments no additional drug delivery regulating component need be present in the administered composition. For example, a composition comprising a therapeutic agent in a liquid medium may form a structure having properties of a gel following its administration. The therapeutic agent may be released over time, optionally as the structure degrades. The drug delivery regulating component may comprise or consist of a polymer matrix that is physically associated with the therapeutic agent. For example, the therapeutic agent may be entrapped, embedded, or encapsulated by the polymer matrix. A sustained release formulation can be in the form of an individual ocular implant, a plurality of nanoparticles, microparticles, or liposomes, a semi-solid or viscous material such as a gel, etc. The therapeutic agent may preferably be from about 1% to 90% by weight of the sustained release formulation. More preferably, the therapeutic agent is from about 20% to about 80% by weight of the of the sustained release formulation. In certain embodiments, the

therapeutic agent comprises about 40% by weight of the sustained release formulation (e.g., 30%-50%).

[00212] A number of polymeric delivery vehicles for providing sustained release have been used in an ocular context and can be used to administer the compositions of the invention. Various polymers, e.g., biocompatible polymers, which may be biodegradable, can be used. The polymers may be homopolymers, copolymers (including block copolymers), straight, branched-chain, or cross-linked. Useful polymers include, but are not limited to, poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactide-co-glycolide (PLGA), poly(phosphazine), poly (phosphate ester), polycaprolactones, polyanhydrides, ethylene vinyl acetate, polyorthoesters, polyethers, and poly (beta amino esters). Peptides, proteins such as collagen or albumin, polysaccharides such as chitosan, alginate, hyaluronic acid (or derivatives of any of these) and dendrimers (e.g., PAMAM dendrimers) are also of use. Methods for preparation of such formulations will be apparent to those skilled in the art. Certain of the materials can also be obtained commercially, e.g., from Alza Corporation Any of these polymers, or combinations thereof, can be used in various embodiments of the invention.

[00213] Additional exemplary polymers include cellulose derivatives such as carboxymethylcellulose, polycarbamates or polyureas, cross-linked poly(vinyl acetate) and the like, ethylene-vinyl ester copolymers having an ester content of 4 to 80% such as ethylene-vinyl acetate (EVA) copolymer, ethylene-vinyl hexanoate copolymer, ethylene-vinyl propionate copolymer, ethylene-vinyl butyrate copolymer, ethylene-vinyl pentantoate copolymer, ethylene-vinyl trimethyl acetate copolymer, ethylene-vinyl diethyl acetate copolymer, ethylene-vinyl 3-methyl butanoate copolymer, ethylene-vinyl 3-3-dimethyl butanoate copolymer, and ethylene-vinyl benzoate copolymer, or mixtures thereof.

[00214] Poly(ortho esters) have been introduced into the eye and demonstrated favorable properties for sustained release ocular drug delivery (Einmahl, S., *Invest. Ophthalmol. Vis. Sci.*, 43(5), 2002). Polylactide particles have been used to target an agent to the retina and RPE following intravitreous injection of a suspension of such particles (Bourges, J-L, et al, *Invest. Ophthalmol. Vis. Sci.*, 44(8), 2003).

[00215] Sustained release formulations including various ocular implants and other ocular drug delivery systems that are of use in various embodiments of the invention are described, for example, in U.S. Patent Nos. 6,692,759; 6,331,313; 5,869,079; 5,824,072; and U.S.S.N. 10/918,597 (Pub. No. 20050048099); 10/837,357 (Pub. No. 20050244469); 11/092,122 (Pub. No. 20050244472) and 11/116,698 (Pub. No. 20050281861) as well as a number of other

patents and publications referenced in the foregoing, all of which are incorporated herein by reference.

[00216] A method of making a sustained release formulation involves combining or mixing the therapeutic agent with a polymeric component to form a mixture. The mixture may then be extruded, compressed, molded, etc., to form a single composition. Optionally, heat and/or pressure can be used. The single composition may then be processed to form individual implants or particles suitable for placement in an eye of a patient. Additional methods for incorporating therapeutically active agents into polymeric matrices are known in the art. The polymeric matrix can be formed into various shapes such as rods, disks, wafers, etc., which may have a range of different dimensions (e.g., length, width, etc.) and volumes. Exemplary shapes include spherical, cylindrical, helical, coil-shaped or helical, screw-shaped, cubical, conical, ellipsoidical, biconvex, hemispherical or near-hemispherical etc.

[00217] In certain embodiments of the invention an ocular implant is so dimensioned and shaped that it fits within the hollow shaft of an injection needle, e.g., a 22, 25, 27, 30, 33, or 35 gauge needle (or needle of any gauge ranging between 22 and 35). Exemplary and nonlimiting dimensions for a cylindrical implant may be about 0.5 to 8 millimeters in length and about 0.1 to 2 millimeters in diameter, e.g., about 0.75 mm to about 1.5 mm in diameter. Implants having other shapes, e.g., other rodlike structures with cross-sections that are rectangular or square in cross-section may have a cross-section in which the two points most distant from each other are separated by at most 0.1 mm to 1 mm. In particular embodiments the intraocular implant may have a length or other longest dimension of between about 5 microns and about 2 mm, or between about 10 microns and about 1 mm for administration with a needle. Alternately, the length or other longest dimension is greater than 1 mm, or greater than 2 mm, such as 3 mm or up to 10 mm. The vitreous chamber in humans is able to accommodate relatively large implants of varying geometries, having lengths of, for example, 1 to 10 mm.

[00218] In certain embodiments of the invention the implants may also be at least somewhat flexible, which may facilitate both insertion of the implant in the eye, e.g., in the vitreous, and/or may facilitate accommodation of the implant. The total weight of the implant may be about 250-5000 micrograms, e.g., about 500-1000 micrograms. For example, an implant may be about 500 micrograms or about 1000 micrograms. Larger implants may also be formed and further processed before administration to an eye. In addition, larger implants may be desirable where relatively greater amounts of a therapeutic agent are provided in the implant, as used.

[00219] In one embodiment the sustained release formulation is a biocompatible ocular implant comprising a substantially impermeable polymeric outer layer covering a core which

comprises the drug to be delivered, wherein said outer layer has one or more orifices, by which is meant one or more openings in the outer layer through which, when the device is in use, body fluids can enter the device and the drug contained in the device (e.g., dissolved, encapsulated, or entrapped within the device) can migrate out of the device. In certain embodiments the orifices in total have a surface area of less than 10 percent of the total surface area of the device. In certain embodiments of the invention the ocular implant comprises an outer coating layer that is permeable to the therapeutic agent, allowing its slow diffusion out of the implant. The composition, structure, and/ or thickness of the coating layer may be selected to provide a particular permeability and diffusion rate.

[00220] A drug can be contained in an ocular implant as a dry powder, particles, granules, or as a compressed solid. The drug may also be present as a solution or be dispersed in a polymer matrix. Ocular implants, may be have the active agent or agents homogenously distributed through the polymeric matrix, e.g., they may be monolithic. In other embodiments the active agent(s) are heterogeneously distributed in the polymeric matrix. For example, discrete regions of the implant may contain solid particles of an active agent, or a reservoir of active agent may be encapsulated by the polymeric matrix. The therapeutic agent(s) may be distributed in a non-homogenous pattern in the matrix. For example, an implant may include a portion that has a greater concentration of the therapeutic agent relative to a second portion of the implant. Multilayered structures, with the layers having different compositions and may have different physical characteristics such as density or porosity are another possibility. For example, the layers may contain different therapeutic agents or combinations thereof. In another embodiment, layers that are relatively resistant to degradation are interspersed with layers that degrade more rapidly.

[00221] The biodegradable polymeric materials which are included to form the matrix may be subject to enzymatic or hydrolytic instability. Water soluble polymers may be cross-linked with hydrolytic or biodegradable unstable cross-links to provide useful water insoluble polymers. The degree of stability can vary widely, depending, for example, upon the choice of monomer, whether a homopolymer or copolymer or mixture, is employed, and whether the polymer includes terminal acid groups. The biodegradation of the polymer and hence the extended release profile of the sustained release formulation may also influenced by the relative average molecular weight of the polymeric materials employed. Different molecular weights of the same or different polymeric materials may be included in the formulations to modulate the release profile. For example, the average molecular weight of the polymer may range from about 5 to about 500 kD, e.g., from about 10 to 100 kD, or from about 15 to 50 kD.

[00222] Nanoparticles or microparticles can be made using any method known in the art including, but not limited to, spray drying, phase separation, single and double emulsion, solvent evaporation, solvent extraction, and simple and complex coacervation. Particulate polymeric compositions can also be made using granulation, extrusion, and/or spheronization. A composition can contain nanoparticles or microparticles having different compositions and/or properties.

[00223] The conditions used in preparing the particles may be altered to yield particles of a desired size or property (e.g., hydrophobicity, hydrophilicity, external morphology, "stickiness", shape, etc.). The method of preparing the particle and the conditions (e.g., solvent, temperature, concentration, air flow rate, etc.) used may also depend on the therapeutic agent and/or the composition of the polymer matrix.

[00224] Microparticles and nanoparticles of use in the invention can have a range of dimensions. Generally, a microparticle will have a diameter of 500 microns or less, e.g., between 1 and 500 microns, between 50 and 500 microns, between 100 and 250 microns, between 20 and 50 microns, between 1 and 20 microns, between 1 and 10 microns, etc., and a nanoparticle will have a diameter of less than 1 micron, e.g., between 10 nm and 100 nm, between 100 nm and 250 nm, between 100 nm and 500 nm, between 250 nm and 500 nm, between 250 nm and 750 nm, between 500 nm and 750 micron. If the particles prepared by any of the above methods have a size range outside of the desired range, the particles can be sized, for example, using a sieve. Particles can be substantially uniform in size (e.g., diameter) or shape or may be heterogeneous in size and/or shape. They may be substantially spherical or may have other shapes, in which case the relevant dimension will be the longest straight dimension rather than the diameter.

[00225] In certain embodiments of the invention a sustained release formulation comprises a therapeutic agent and a gel-forming material. In accordance with certain embodiments of the invention, a solution containing the soluble gel-forming material and a therapeutic agent is prepared by combining the soluble gel-forming material and therapeutic agent in solution using any suitable method, e.g., by adding the therapeutic agent to a solution containing the gel-forming material. The composition is delivered locally to an appropriate location in the eye of a subject. The solution rapidly forms a gel at or close to of the site of administration. The therapeutic agent is entrapped within the gel. The therapeutic agent diffuses out of the gel or is released as the gel degrades over time, thereby providing a continuous supply of the agent to tissues and structures that are either in direct physical contact with the gel or located nearby. In certain embodiments the solution is administered behind the sclera of the eye. Delivery can be

accomplished by injection (e.g., using a 25, 27, or 30 gauge needle or the like), by catheter, etc. In other embodiments the solution is administered intravitreally. In certain embodiments a "gel" is a structure that exhibits properties (e.g., fluidity) intermediate between solid and liquid phases. The structure may be a solid or semisolid colloid comprising a solid continuous phase and a liquid phase. The structure may have an appearance typical of a gel, which appearance is readily recognized by those of skill in the art.

[00226] In one embodiment, soluble collagen is used as the gel-forming material. The collagen is initially soluble, e.g., in an aqueous medium, and forms a solution that has a low viscosity but is capable of rapid formation of a gel under appropriate conditions, e.g., conditions encountered upon administration to a mammalian subject. A variety of different collagen preparations can be used in the present invention provided that the collagen is initially soluble and is capable of rapidly forming a gel under appropriate conditions. Suitable collagen preparations, and methods for their manufacture, are described, e.g., in U.S. Pat. Nos. 5,492,135; 5,861,486; 6,197,934; 6,204,365; and WO 00/47130, but the invention is not limited to such preparations or methods. These collagens are prepared in soluble form and rapidly form a gel upon exposure to physiological fluids or other fluids having suitable concentration of ions. In accordance with the present invention, injecting or otherwise introducing the collagen solution to the eye or near the eye results in gel formation, presumably induced by contact with physiological fluids. However it is noted that the invention is in no way limited by the mechanism by which gel formation occurs. In addition, as noted above, the gel can be formed *in vitro* and then implanted at an appropriate location.

[00227] Other gel-forming materials of use in the invention include, but are not limited to, hyaluronic acid and modified forms thereof, polysaccharides such as alginate and modified forms thereof, self-assembling peptides, etc. See, e.g., U.S. Pat. Nos. 6,129,761 for further description of alginate and modified forms thereof, hyaluronic acid and modified forms thereof, and additional examples of soluble gel-forming materials that are of use in various embodiments of the present invention. Other polymeric hydrogel precursors include polyethylene oxide-polypropylene glycol block copolymers such as Pluronics ™ or Tetronics™ which are crosslinked by hydrogen bonding and/or by a temperature change, as described in Steinleitner et al., *Obstetrics & Gynecology*, 77:48-52 (1991); and Steinleitner et al., *Fertility and Sterility*, 57:305-308 (1992). Other materials which may be utilized include proteins such as fibrin or gelatin. Polymer mixtures also may be utilized. For example, a mixture of polyethylene oxide and polyacrylic acid which gels by hydrogen bonding upon mixing may be utilized.

[00228] Typically a gel-forming material of use in the invention is capable of being at least partly dissolved, or in certain embodiments of the invention substantially or fully dissolved, e.g., in an aqueous medium. For example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more, by weight, of the gel-forming material present in a gel-forming composition may be dissolved. In certain embodiments essentially 100% of the material is dissolved. The aqueous medium can contain one or more liquids in addition to water, e.g., various alcohols. In general, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% of the liquid present in the medium is water.

[00229] Covalently crosslinkable hydrogel precursors also are useful. For example, a water soluble polyamine, such as chitosan, can be cross-linked with a water soluble diisothiocyanate, such as polyethylene glycol diisothiocyanate. The isothiocyanates will react with the amines to form a chemically crosslinked gel. Aldehyde reactions with amines, e.g., with polyethylene glycol dialdehyde also may be utilized. A hydroxylated water soluble polymer also may be utilized.

[00230] In certain embodiments of the invention a therapeutic agent is covalently or noncovalently attached to a drug delivery regulating component such as a polymer via a linking moiety. The linking moiety is cleaved to release the therapeutic agent from the drug delivery regulating component to provide sustained release. For example, the linking moiety may be a peptide containing a site that is cleaved by an endogenous enzyme such as a protease or may contain a labile or hydrolyzable bond, e.g., a disulfide bond, ester moiety, etc.

[00231] Cells that express a therapeutic agent that is a biological macromolecule such as a protein or RNAi agent can be implanted into the eye and are of use in certain embodiments of the invention to provide sustained release. U.S. Patent No. 6,436,427 provides a method for delivering biologically active molecules to the eye by implanting biocompatible capsules containing a cellular source of the biologically active molecule.

[00232] In certain embodiments of the invention the sustained release formulation comprises liposomes. For example, a liposomal suspension can be administered. Liposomes can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 and other references listed herein. Liposomes, including targeted liposomes (e.g., antibody targeted liposomes) and pegylated liposomes have been described (Hansen CB, et al., *Biochim Biophys Acta.* 1239(2):133-44,1995; Torchilin VP, et al., *Biochim Biophys Acta*, 1511(2):397-411, 2001; Ishida T, et al., *FEBS Lett.* 460(1):129-33, 1999).

[00233] One of ordinary skill in the art will appreciate that the materials and methods selected for preparation of a sustained release formulation, implant, etc., should be such as to

retain activity of the compound. For example, it may be desirable to avoid excessive heating of certain agents such as polypeptides, which could lead to denaturation and loss of activity. Furthermore, it will be appreciated that a sustained release formulation may contain a variety of additional components that lack therapeutic activity and that may or may not contribute to the sustained release features of the formulation. Examples include plasticizing agents, solubilizing agents, solubility decreasing agents, and dispersing agents (see U.S. Pat. No. 6,331,313), provided that such components are compatible with administration to the eye under the conditions used. For example, a sustained release formulation may include a β-cyclodextrin, which is effective in enhancing the solubility of the therapeutic agent. The β-cyclodextrin may be provided in an amount from about 0.5% (w/w) to about 25% (w/w) of the implant. In certain implants, the β-cyclodextrin is provided in an amount from about 5% (w/w) to about 15% (w/w) of the formulation. Other formulations include a gamma-cyclodextrin, and/or cyclodextrin derivatives.

[00234] A sustained release formulation herein may include an excipient component, such as effective amounts of buffering agents, preservatives and the like. Suitable water soluble buffering agents include, without limitation, alkali and alkaline earth carbonates, phosphates, bicarbonates, citrates, borates, acetates, succinates and the like, such as sodium phosphate, citrate, borate, acetate, bicarbonate, carbonate and the like. These agents are advantageously present in amounts sufficient to maintain a pH of the system of between about 2 to about 9 and more preferably about 4 to about 8. As such the buffering agent may be as much as about 5% by weight of the total system. Suitable water soluble preservatives include sodium bisulfite, sodium bisulfate, sodium thiosulfate, ascorbate, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, parabens, methylparaben, polyvinyl alcohol, benzyl alcohol, phenylethanol and the like and mixtures thereof. These agents may be present in amounts of from 0.001 to about 5% by weight and preferably 0.01 to about 2% by weight. These agents may also be used in certain of the liquid compositions described herein.

[00235] In some embodiments of the invention the sustained release formulation comprises an agent that enhances uptake of the therapeutic agent by cells, enhances bioavailability of the agent at its site of action, or otherwise enhances activity of the therapeutic agent. For example, a variety of delivery vehicles that enhance uptake and/or activity of RNAi agents such as siRNAs are known in the art and may be included in the sustained release formulation.

[00236] If desired, the proportions of therapeutic agent, polymer, and any other modifiers may be empirically determined by formulating several implants, for example, with varying proportions of such ingredients. A USP approved method for dissolution or release test can be used to measure the rate of release (USP 23; NF 18 (1995) pp. 1790-1798). The implants can also be tested *in vivo*.

[00237] Included within the scope of the term "sustained release formulation" of a therapeutic agent are devices or "chips" that include one or more reservoirs containing the agent and that release the agent or a portion thereof from the one or more reservoirs into the surrounding environment (see, e.g., U.S. Pat. Nos. 5,797,898 and 6,976,982). Release may occur through a variety of means. For example, the reservoirs may have a biodegradable cap that is impermeable to the agent and degrades over time, so that the therapeutic agent is released once the cap is degraded. Caps of differing thickness will cause release to occur at different times. Mechanical, electrical, or other means may be used to release the agent from a reservoir, optionally using external control means to regulate such release. Release can occur at predetermined times and/or in predetermined amounts. The device may be programmable.

[00238] ***V. Methods of Administration***

[00239] A variety of different methods, techniques, and procedures may be used to administer the first and second therapeutic agents. In certain embodiments of the invention administration is performed by intravitreal injection. While it will be appreciated that a certain amount of inter-physician variability exists.A nonlimiting example of an intravitreal procedure may be performed as follows: The tension in the eye is typically measured with a tensiometer and inflammation is graded clinically. The eyes are anesthetized with sodium channel blockers (such as novocaine) and treated with mydriatic drops (either sympathomimetic, anti-parasympathetic or myoplegic in nature), followed by further treatment with anesthetic and antibiotic drops. A speculum is then inserted under the eyelids and the patient is asked to look sideways. A caliper is used to measure a distance of 2 mm from the limbus and determine the injection site (this is done in order to avoid hitting the lens with the needle). An injection syringe (e.g., a 1 ml or 0.5 ml syringe) with a needle (e.g., a 22, 25, 27, or 30 gauge needle) is then loaded with 50-100 microliters of an agent (e.g., either 1mg Avastin or 0.3mg Macugen), or a syringe that is preloaded with the agent is used. The needle is then inserted at the injection site until the middle of the vitreous cavity is reached with the tip of the needle and the drug is slowly injected. The syringe is then slowly retracted and pressure is administered for a few seconds at the site of injection with wet gauze. More antibiotic drops are administered and the speculum is

removed. The patient is then typically observed for 10 to 60 min, during which time the intraocular pressure is measured at regular intervals.

[00240] Other methods of administration include, e.g., choroidal injection, transscleral injection or placing a scleral patch, selective arterial catheterization, intraocular administration including transretinal, subconjunctival bulbar, intravitreal injection, suprachoroidal injection, subtenon injection, scleral pocket and scleral cutdown injection, by osmotic pump, etc. In choroidal injection and scleral patching, the clinician uses a local approach to the eye after initiation of appropriate anesthesia, including painkillers and ophthalmoplegics. A needle containing the therapeutic compound is directed into the subject's choroid or sclera and inserted under sterile conditions. When the needle is properly positioned the compound is injected into either or both of the choroid or sclera.

[00241] Intraocular administration of drugs intended for treatment of macular degeneration and/or other intraocular conditions by a variety of methods is well known in the art, and any suitable method can be used in the present invention. See, e.g., U.S. Patent Nos. 5,632,984 and 5,770,589. U.S. Patent No. 6,378,526 provides methods for intrascleral injection of a therapeutic or diagnostic material at a location overlying the retina, which provide a minimally invasive technique for delivering the agent to the posterior segment of the eye.

[00242] Only minor modifications of the foregoing procedures, or no modifications at all, may be needed to administer first and second therapeutic agents in accordance with the present invention. Standard injection times and pressures can be used or appropriately modified. For example, the total injection time may be longer than in the case of injecting a single agent. It will be appreciated that the nature of the modifications, if any, will be dictated at least in part by the particular procedure as well as the nature of the therapeutic agents and their formulation in addition to the manner in which they are provided for use by the clinician. For example, in one embodiment a sustained release formulation containing the second therapeutic agent, e.g., an ocular implant, is loaded into the needle. The needle may be supplied to the clinician having already been preloaded with the sustained release formulation or the clinician may load the needle with the sustained release formulation. The clinician attaches the needle to the syringe. An appropriate volume (containing an appropriate amount) of a solution containing the first therapeutic agent is then drawn up into the syringe and the the intravitreal injection procedure is performed as described above. Depression of the plunger of the syringe will eject both the first therapeutic agent and the sustained release formulation into the vitreous (or elsewhere in the eye if a technique other than intravitreal injection is used). In another embodiment, a syringe containing an appropriate volume of a solution containing the first therapeutic agent is provided

to the clinician, optionally together with a needle that is preloaded with the sustained release formulation containing the second therapeutic agent. Thus the clinician needs to undertake no measurement, dilution, or other manipulation of the therapeutic agents themselves.

[00243] In another embodiment, both therapeutic agents are contained in individual syringes. The injection is performed with a needle and syringe assembly, wherein the syringe contains the first therapeutic agent or, more generally, the syringe contains a composition comprising the first therapeutic agent. After administration of the therapeutic agent contained in the syringe, the syringe is removed and a second syringe, containing the second therapeutic agent, or more generally, a composition comprising the second therapeutic agent, is attached to the needle. The second therapeutic agent (or composition) is then administered. The therapeutic agents can be administered in either order. Any number of therapeutic agents can be administered consecutively, without removing the tip of the needle from the subject's eye.

[00244] Figure 9 shows an exemplary embodiment of a needle and syringe assembly that may be used to practice the methods of the invention. The assembly includes a syringe having a barrel and a plunger with a stopper at its end. The syringe is attached to a needle, typically by means of a threaded tip with an opening (e.g., a Luer lock tip), which is not shown. The portion of the syringe between the stopper and the end of the barrel contains a therapeutic agent, e.g., an angiogenesis inhibitor. The needle contains an additional therapeutic agent, e.g., a sustained release formulation such as an ocular implant containing a therapeutic agent (e.g., a complement inhibitor). For purposes of clarity the implant is depicted outside the needle in Figure 9 though of course it would be located within the shaft of the needle for administration. Exerting pressure on the plunger following introduction of the needle into a subject's eye will eject both the therapeutic agent in the syringe and the ocular implant in the needle into the eye. In other embodiments, the syringe may be provided with additional or alternative means of ejecting the implant.

[00245] In certain embodiments of the invention the first and second therapeutic agents are delivered to different structures, regions, compartments, or tissues of the eye in a single procedure. For example, a needle or other instrument may pass through different structures, regions, compartments, or tissues of the eye during the process of inserting and withdrawing the needle. The first and second therapeutic agents may be ejected into different structures, regions, compartments, or tissues of the eye in the course of a single injection procedure. For example, in one embodiment one of the therapeutic agents is introduced into the vitreous and one of the therapeutic agents in introduced into a different structure, region, compartment, or tissue of the eye in a single procedure. Either the agent that provides rapid rapid improvement in the

condition of the subject's eye or the sustained release formulation of the second therapeutic agent may be introduced into the vitreous.

[00246] The volume to be administered will depend on the location within the eye to which the composition is administered. For example, for intravitreal injection volumes of 200 μl, preferably 100 μl or less are generally preferred. In certain embodiments the total volume of liquid injected is 200 μl or less, 100 μl or less, 50 μl or less. In certain embodiments of the invention the total volume of material introduced into the subject's eye (including liquid and any solid or semi-solid components) is 500 μl or less, 400 μl or less, 300 μl or less 200 μl or less, 100 μl or less, 50 μl or less, or 25 μl or less.

[00247] ***VI. Gene Therapy***

[00248] The invention also encompasses gene therapy, in which one or more of the therapeutic agents is a nucleic acid that encodes a therapeutic agent such as an siRNA or protein such as a VCCP in operable association with regulatory elements sufficient to direct expression of the nucleic acid is administered to the eye. A composition comprising a nucleic acid therapeutic can consist essentially of the nucleic acid or a gene therapy vector in an acceptable diluent, or can comprise a drug release regulating component such as a polymer matrix with which the nucleic acid or gene therapy vector is physically associated, e.g, with which it is mixed or within which it is encapsulated or embedded. The gene therapy vector can be a plasmid, virus, or other vector. Alternatively, the pharmaceutical composition can comprise one or more cells which produce a therapeutic nucleic acid or polypeptide. Preferably such cells secrete the therapeutic agent into the extracellular space.

[00249] Viral vectors that have been used for gene therapy protocols include, but are not limited to, retroviruses, lentiviruses, other RNA viruses such as poliovirus or Sindbis virus, adenovirus, adeno-associated virus, herpes viruses, SV 40, vaccinia and other DNA viruses. Replication-defective murine retroviral or lentiviral vectors are widely utilized gene transfer vectors. Chemical methods of gene therapy involve carrier-mediated gene transfer through the use of fusogenic lipid vesicles such as liposomes or other vesicles for membrane fusion. A carrier harboring a nucleic acid of interest can be conveniently introduced into the eye or into body fluids or the bloodstream. The carrier can be site specifically directed to the target organ or tissue in the body. Cell or tissue specific DNA-carrying liposomes, for example, can be used and the foreign nucleic acid carried by the liposome absorbed by those specific cells. Gene transfer may also involve the use of lipid-based compounds which are not liposomes. For example, lipofectins and cytofectins are lipid-based compounds containing positive ions that

bind to negatively charged nucleic acids and form a complex that can ferry the nucleic acid across a cell membrane.

[00250] Certain cationic polymers spontaneously bind to and condense nucleic acids such as DNA into nanoparticles. For example, naturally occurring proteins, peptides, or derivatives thereof have been used. Synthetic cationic polymers such as polyethylenimine (PEI), polylysine (PLL) etc. condense DNA and are useful delivery vehicles. Dendrimers can also be used. Many useful polymers contain both chargeable amino groups, to allow for ionic interaction with the negatively charged DNA phosphate, and a degradable region, such as a hydrolyzable ester linkage. Examples include poly(alpha-(4-aminobutyl)-L-glycolic acid), network poly(amino ester), and poly (beta-amino esters). These complexation agents can protect nucleic acids against degradation, e.g., by nucleases, serum components, etc., and create a less negative surface charge, which may facilitate passage through hydrophobic membranes (e.g., cytoplasmic, lysosomal, endosomal, nuclear) of the cell. Certain complexation agents facilitate intracellular trafficking events such as endosomal escape, cytoplasmic transport, and nuclear entry, and can dissociate from the nucleic acid.

[00251] ***VII. Articles of Manufacture***

[00252] In another aspect of the invention, an article of manufacture, which may be referred to as a pharmaceutical pack or kit, containing compositions, devices or instruments, and optionally additional materials or items useful for treating the disorders described above is provided. The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is, either alone or together with another composition effective for treating the condition. Optionally the container may have a sterile access port (for example the container may be a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert may indicate that the composition is used for treating one or more conditions of choice, e.g., an eye disorder such as macular degeneration. The article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a first therapeutic agent; and (b) a second container with a composition contained therein, wherein the composition comprises a sustained release formulation of a second therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the first and second compositions can be used to treat a particular eye disorder, e.g., exudative ARMD. Alternatively, or additionally, the article of manufacture may further comprise a second (or

third) container comprising a pharmaceutically acceptable liquid, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution.

[00253] In certain embodiments of the invention the article of manufacture may further comprise one or more devices or instruments for administering a therapeutic agent to the eye. For example, the article of manufacture may include one or more needles (e.g., a 22, 25, 27, or 30 gauge needle) and/or one or more syringes (e.g., 0.3, 0.5, or 1.0 ml syringes). Either a needle or a syringe, or both, may contain one or more compositions comprising a unit dosage form of a therapeutic agent. For example, the article of manufacture may include a needle or syringe that contains a predetermined volume and/or amount of a composition comprising a therapeutic agent. The article of manufacture may contain a needle or syringe that contains a sustained release formulation of a therapeutic agent, e.g., an ocular implant. The needle and syringe may, but need not be, attached to one another. The needle and/or syringe may be provided with a removable cap. Providing one or more of the compositions already loaded into the device that will be used to administer the agent(s) may provide increased reliability, safety, and convenience. The article of manufacture may include a plurality of syringes, each of which optionally contains a unit doseage form of a different therapeutic agent. For example, a first syringe containing a first therapeutic agent and a second syringe containing a third therapeutic agent may be included. In one embodiment a first syringe containing an angiogenesis inhibitor and a second syringe containing a complement inhibitor are provided. Either or both of the agents may be in a liquid composition. Either or both of the agents may be a component of a sustained release formulation. Each syringe may contain a composition containing a single therapeutic agent and optionally other components such as a pharmaceutically acceptable carrier. Alternately, one or more of the syringes may contain a composition containing a plurality of different therapeutic agents and optionally other components such as a pharmaceutically acceptable carrier.

[00254] The individual components described above may be packaged together in a larger container, e.g., a box, foil, styrofoam, or plastic wrapper, or other container, which may optionally contain additional packaging material. Care should be taken to use materials that will, if necessary or desirable, protect the therapeutic agent(s) from light and/or other environmental conditions and will not adversely affect them. The article of manufacture may include instructions, e.g., in the form of a package insert, that instruct the clinician as to methods by which the compositions should be administered including, if appropriate, instructions for assembling, diluting, or otherwise manipulating any individual components. In one embodiment each article of manufacture contains appropriate amounts of first and second compositions

comprising first and second therapeutic agents for performing a single procedure (i.e., a single administration of first and second therapeutic agents to the eye). Optionally included are devices or instruments such as a needle and syringe for performing the procedure. The needle, syringe, or both, may be preloaded with a composition comprising a therapeutic agent. Articles of manufacture that contain one or more of any of the therapeutic agents, sustained release formulations thereof, and/or devices or instruments for administering a therapeutic agent to the eye, and any combinations thereof, are within the scope of the invention.

[00255] Preferably any composition to be administered to the eye is sterile. The composition can be made from sterile components, or sterilization can be performed after manufacture. Methods of sterilization include irradiation, heat, etc. Preferably, the sterilization method used does not substantially reduce the activity or biological or therapeutic activity of the therapeutic agents. Devices and instruments to be used for administration to the eye are also preferably sterile, at least to the extent they will enter the eye.

[00256] ***VIII. Testing in Animal Models***

[00257] Animal models that replicate one or more features of macular degeneration, diabetic retinopathy, CNV, inflammation, or other ocular conditions are known in the art. A compound of the invention can be administered in various doses to mice, rats, dogs, primates, etc. that have spontaneous macular degeneration and/or CNV or in which macular degeneration and/or CNV have been induced by a treatment. The ability of the compound to prevent or treat one or more signs or symptoms of macular degeneration (e.g. CNV, accumulation of lipofuscin in and/or drusen beneath the RPE, photoreceptor atrophy or hypertrophy, altered RPE pigmentation, photoreceptor loss, altered electroretinogram, etc.) is assessed. Visual examination, photography, histopathology, immunohistology, etc., can be used.

[00258] Useful models include animals (e.g., mice,Yucatan pigs, monkeys, etc.) in which CNV is induced by laser treatment (see, e.g., Bora, P.S., et al., *Proc. Natl. Acad. Sci.* 100(5): 2679-2684, 2003; Zacks, DN, et al., *Invest Ophthalmol Vis Sci.* 243(7):2384-91, 2002). Other models include animals that have been treated with a variety of agents such as lipid hydroperoxide (Tamai, K., et al., *Exp Eye Res.* 74(2):301-8, 2002), pellets comprising growth factors, etc. Animals genetically engineered to overexpress or underexpress one or more genes are also useful. For example, transgenic mice (mcd/mcd mice) that express a mutated form of cathepsin D that is enzymatically inactive display features associated with geographic atrophy (Rakoczy, PE, et al, *Am. J. Path.*, 161(4), 1515-1524, 2002). Adeno-associated virus (AAV) mediated expression of vascular endothelial growth factor induces CNV in rats (Wang, F., et al., *Invest Ophthalmol Vis Sci.* 44(2):781-90, 2003). One animal model is a transgenic mouse

deficient in either monocyte chemoattractant protein (Ccl-2) or its cognate chemokine receptor (Ccr-2) (Ambati, J., et al., *Nat Med.* 9(11):1390-7, 2003; U.S.S.N. 10/685,705 – U.S. Pat. Pub. No. 20040177387). Aged mice with a deficiency in either of these proteins exhibit a number of features of ARMD including accumulation of lipofuscin in and drusen beneath the RPE, photoreceptor atrophy, and CNV. Methods for testing the efficacy of a candidate agent using this mouse model are disclosed in U.S. Pat. Pub. No. 20040177387. In general, a candidate agent is administered to the mouse either before or after development of features of ARMD, and at least one eye is monitored for development or regression of drusen and/or lipofuscin accumulation therein, for effect of the candidate agent on Bruch's membrane, effect on retinal degeneration, and/or for effect on CNV.

[00259] The therapeutic agents are administered as described herein. The eye can be analyzed by ophthalmoscopy (e.g., indirect ophthalmoscopy, slit lamp assessment), angiography (e.g., fluorescein angiography), histopathology, optical coherence tomography (OCT), fundus photography, or a combination thereof. Any of these methods can be used to assess efficacy in any animal model or in humans. Compounds that show promising results in animal studies are tested in humans, e.g., using standard protocols and endpoints for clinical trials for therapies for ARMD or diabetic retinopathy but it will be appreciated that agents may be administered to humans without evidence of efficacy in animal models.

[00260] ***IX. Identifying Subjects and Assessing Response***

[00261] The methods of the invention may include providing a subject to which a composition of the invention is to be administered. The subject is typically suffering from an eye disorder characterized by macular degeneration, CNV, or RNV. The compositions are administered to the subject according to the inventive methods with the intent of treating or preventing such condition. Thus the subject will typically have been identified as being at risk of or suffering from such a condition. Methods for diagnosis of macular degeneration, CNV, and RNV etc., and for assessing response to therapy are known in the art.

[00262] Any suitable tests and criteria can be used to identify a subject at risk of or suffering from a macular degeneration related condition, diabetic retinopathy, or CNV and/or to evaluate the condition of a subject's eye either prior to or following therapy (e.g., to determine whether the subject is in need of therapy or has responded to therapy). Visual acuity can be measured using, for example, a Snellen chart, a Bailey-Lovie chart, a decimal progression chart, a Freiburg visual acuity test, a measurement of minimum angle of resolution (MAR) etc. Metamorphopsia (visual distortion) may be measured using an Amsler chart. Contrast sensitivity may be measured using a Pelli-Robson chart. Diagnostic studies include, but are not limited to, standard

ophthalmologic examination of the fundus, stereo biomicroscopic examination of the macula, intravenous fundus fluorescein angiography, fundus photography, indocyanine green video-angiography, and optical coherence tomography (OCT). OCT may be of particular use for measuring macular thickness, an indicator of macular edema. A subject displaying an abnormality on one or more of these diagnostic studies (e.g., a subject that falls outside a range that is considered normal for a healthy eye) may be treated in accordance with the present invention.

[00263] Subjects may be classified as having early, intermediate, or advanced ARMD in accordance with the classification scheme used in the Age-Related Eye Diseases Study (AREDS), which is set forth in guidelines developed American Academy of Ophthalmology (American Academy of Ophthalmology, Age Related Macular Degeneration Preferred Practice Pattern™, 2003; available for download at URL www.aao.org/aao/education/library/ppp/amd_new.cfm). A subject falling into any of these categories may be treated in accordance with the present invention. If the subject has already developed CNV, the subject may have classic CNV, occult CNV, or a mixture of the two. Of course alternate classification schemes, of which a variety is described in the literature, could be used.

[00264] ARMD is known to have a genetic component, based on studies showing an increased incidence of ARMD in individuals with relatives suffering from ARMD (e.g., twin studies). Therefore, a subject may be considered at risk of developing ARMD if he or she has one or more close relatives (e.g., parent, grandparent, sibling, cousin, uncle, aunt), who has received a diagnosis of ARMD. Individuals who have certain polymorphic variants of genes encoding certain complement components, such as the factor H gene, are particularly prone to develop ARMD (see, e.g., Klein RJ, et al., Zeiss C, *Science*, 308(5720):385-9, 2005; Edwards AO, et al., *Science*, 308(5720):421-4, 2005; Haines JL, et al., *Science*, 308(5720):419-21, 2005). In one embodiment the method comprises providing or ascertaining a genotype of a subject, wherein the genotype includes information as to the presence of one or more polymorphisms predisposing to ARMD. Optionally the gene encodes a complement component, e.g., factor H or factor B.

[00265] Individuals who smoke and/or consume a high fat diet are also at increased risk. The incidence of ARMD increases with age. Therefore, an individual over approximately 50 years of age, generally at least 60 or at least 70 years of age may be considered at increased risk. An individual having drusen and one or more additional risk factors may be at particular risk for developing ARMD. An individual with multiple drusen, particularly if large and with indistinct

borders, may be at particular risk. An individual with RPE hyperpigmentation or hypopigmentation or geographic atrophy may be at particular risk. Specific genetic mutations are associated with various less common macular degeneration related conditions. A subject who has received a diagnosis of diabetes is at risk of developing diabetic retinopathy. In addition, a subject who has developed ARMD in one eye is at increased risk of developing the disorder in the other eye.

[00266] Response to therapy can be assessed by any of the methods mentioned above. Numerous studies have been conducted to assess the efficacy of a variety of different therapies in restoring vision, preventing visual loss, and/or resulting in improvement or slowing progression of ARMD or choroidal neovascularization as judged by diagnostic tests such as those described above. One of ordinary skill in the art will be able to select appropriate criteria by which to judge the efficacy of therapy.

[00267] Rapid improvement in the condition of the subject's eye may be, for example, any clinically significant improvement in a sign or symptom associated with the eye disorder. For example, the improvement may be an improvement in visual acuity (e.g., best corrected visual acuity) such as gaining 1, 2, 3, or more lines on an eye chart. The improvement may be a decrease in macular thickness, e.g., a decrease by at least 50 μm, at least 100 μm, at least 150 μm, etc. The improvement can be a decrease in the area of exudate evident in or on the retina.

[00268] The improvement can be an increase of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, or more, in any quantitative measure of the condition of the subject's eye, where an increase in the quantitative measure indicates improvement in the condition of the subject's eye. The improvement can be a decrease of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more, in any quantitative measure of the condition of the subject's eye, where a decrease in the quantitative measure indicates improvement in the condition of the subject's eye. If a scoring system is used as an indication of the condition of the subject's eye, (e.g., a scoring system including scores ranging from 1-3, 1-5, 1-10, etc.), the improvement may be, e.g., an increase of at least 1, 2, 3, or more units, where an increase in the score indicates improvement in the condition of the subject's eye. Alternately, if a scoring system in which a decrease in the score indicates an improvement in the condition of the subject's eye is used, the improvement may be a decrease of at least 1, 2, 3, or more units. One of ordinary skill in the art will appreciate that a variety of scoring systems may be used. For example, scores may be expressed in terms of units such as "+" or "-" rather than with integers. Of course it will be understood that individual responses will vary, and not all sujbects will

respond. In animal studies and in clinical trials, changes in the condition of a subject's eye may be expressed in terms of an average or mean change in the population studied and/or using appropriate statistical tests.

[00269] In one example, subjects with exudative ARMD are divided into two groups. A variety of parameters, for example visual acuity (e.g., best corrected visual acuity), contrast sensitivity, visual distortion, retinal hemorrhage number or area, macular thickness, etc., are evaluated to provide baseline indication of the condition of the subject's eye. One group receives a single intravitreal injection of a first therapeutic agent, e.g., an angiogenesis inhibitor such as an anti-VEGF agent, e.g., Lucentis, Avastin, or Macugen. The other group receives the same dose of the first therapeutic agent and also receives a sustained release formulation of a second therapeutic agent (e.g., a complement inhibitor such as compstatin or a derivative thereof), with both agents being administered together by a single intravitreal injection. The sustained release formulation may be, e.g., a cylindrical or screw-shaped ocular implant comprising the second therapeutic agent, a plurality of particles comprising the agent, a composition that forms a discrete solid or semi-solid structure such as a gel following administration, etc. The groups are monitored over time. Parameters such as visual acuity (e.g., best corrected visual acuity), contrast sensitivity, visual distortion, retinal hemorrhage number or area, macular thickness, etc., are evaluated, preferably using the same methods and metrics as were used in the initial evaluation to determine baseline values. For example, resolution of macular edema may be monitored by OCT. Evaluations to determine the number of subjects who experience rapid improvement in the condition of a treated eye can take place, e.g., 1 week, 10 days, or 2 weeks following treatment. The number of subjects that experience rapid improvement in the condition of a treated eye (e.g., rapid decrease in macular edema and its associated visual disturbances) is compared between the two groups. Also monitored is the average time to destabilization, e.g., the average time before an acute deterioration in one or more of the foregoing parameters occurs. Also monitored (e.g., using fluorescein angiography and/or ophthalmologic examination) is the extent of neovascularization and/or vessel leakage at various time points following treatment, e.g., at 30, 60, 90, 120, 150, and 180 day time points and at 30 day intervals thereafter (or an appropriate subset of these time points). The change from baseline in a retinal thickness score may be evaluated and compared between the two groups. A greater mean decrease in retinal thickness at one or more of the foregoing time points in the group that received the combined therapy of the present invention is indicative that the combined therapy of the present invention provides a therapeutic advantage for treating the eye disorder. The change from baseline in fluorescein leakage score (where the fluorescein leakage

score provides an indication of neovascularization and/or vessel leakage and a higher score indicates a greater amount of neovascularization and/or vessel leakage) may be evaluated. A greater mean decrease in fluorescein leakage score from baseline in the in the group that received the combined therapy of the present invention is indicative that the combined therapy of the present invention provides a therapeutic advantage for treating the eye disorder.

[00270] Each of the above examples is repeated except that the first and second therapeutic agents are both complement inhibitors or combinations thereof. For example, the first therapeutic agent is a VCCP and the second therapeutic agent is a GPCRA. Alternately, the first therapeutic agent is compstatin or a derivative thereof and the second therapeutic agent is a VCCP.

[00271] Each of the above examples is repeated except that one of the groups receives, by a single intravitreal injection, a composition comprising multiple therapeutic agents in a liquid medium and a sustained release formulation containing at least one therapeutic agent. The multiple therapeutic agents can be, for example, different angiogenesis inhibitors. Alternately, the multiple therapeutic agents can include a complement inhibitor and an angiogenesis inhibitor. The sustained release formulation may contain, for example, two or more different complement inhibitors or a complement inhibitor and an angiogenesis inhibitor. For example, in one embodiment the sustained release formulation contains a compstatin analog and Lucentis.

[00272] Each of the above examples is repeated except that at least one therapeutic agent is an RNAi agent. For example, the first or second therapeutic agent is an siRNA that inhibits expression of one or more endogenous pro-angiogenic molecules such as one or more VEGF isoforms, one or more VEGF receptors, one or more complement components, etc. In one embodiment the sustained release formulation contains at least two RNAi agents, each of which inhibits expression of a different pro-angiogenic molecule. For example, the sustained release formulation may contain Cand5 and Sirna-027.

[00273] Each of the above examples is repeated in subjects suffering from diabetic retinopathy.

[00274] In another example the ability of the inventive compositions and methods to inhibit progression of early ARMD (AREDS 2) to intermediate ARMD (AREDS 3) is assessed. Subjects with early ARMD are divided into two groups, one of which receives an inventive combination of agents as described in either of the two examples above while the other receives either no therapy or an alternative therapy such as therapy with a single agent, e.g., Lucentis, Avastin, or Macugen as described in either of the two examples above. The groups are monitored for a period of time (e.g., as described above). In addition the percentage of subjects

that progress from early to intermediate ARMD is determined. A lower proportion of subjects that progress to intermediate ARMD in the group that receives the combined therapy of the present invention is indicative that the combined therapy of the present invention provides a therapeutic advantage for treating the eye disorder.

[00275] In another example the ability of an inventive method to inhibit progression of intermediate ARMD (AREDS 3) to advanced ARMD (AREDS 4) is assessed. Subjects with intermediate ARMD are divided into two groups, one of which receives an inventive combination of agents as described in either of the two examples above while the other receives either no therapy or an alternative therapy such as Lucentis, Avastin, Macugen as described in either of the two examples above. The groups are monitored for a period of time (e.g., as described above). The percentage of subjects that progress from intermediate to advanced ARMD is determined. A lower proportion of subjects that progress to advanced ARMD in the group that receives the combined therapy of the present invention is indicative that the combined therapy of the present invention provides a therapeutic advantage for treating the eye disorder.

[00276] In addition to monitoring progression of ARMD, the incidence of side effects and complications may also be monitored. Consideration of side effects is an important aspect when evaluating the overall outcome and risk/benefit ratio of a therapy. For example, if two therapies are equally efficacious in terms of inhibiting progression of or treating ARMD, the therapy with a lower incidence of side effects (e.g., severe complications such as those mentioned above) is typically preferred for most subjects. In certain embodiments of the invention therapy of a disorder such as ARMD, or a disorder featuring CNV or RNV from any cause, using the methods and compositions of the invention is associated with fewer total side effects, e.g., severe complications, over time (e.g., over a 1-2 year period) than therapy in which multiple agents are administered individually or therapy in which only a single therapeutic agent is used.

Equivalents and Scope

[00277] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims. In the claims and elsewhere in the specification, articles such as "a,", "an" and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. For example, the indefinite articles "a" and "an", as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one". Claims or descriptions

that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims (or from the portion of the specification relevant to such claim or claim element) is introduced into another claim. For example, and without limitation, any claim that is dependent on another claim can be modified to include one or more elements or limitations found in any other claim (or from the portion of the specification relevant to such claim or claim element) that is dependent on the same base claim. Furthermore, where the claims or description recite a composition, it is to be understood that methods of administering the composition according to any of the methods disclosed herein, and methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. The invention encompasses all variations, combinations, and permutations in which one or more elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[00278] Where elements are presented as lists, e.g., in Markush group format or the like, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should it be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not been specifically set forth *in haec verba* herein in all cases.

[00279] The inclusion of a "providing a subject..." step in certain methods of the invention is intended to indicate that the composition is administered to treat an eye disorder. Thus the subject will have or be at risk of an eye disorder and the composition is administered to treat the

disorder, typically upon the sound recommendation of a medical or surgical practitioner, e.g., an ophthalmologist, who may or may not be the same individual who administers the composition. The invention includes embodiments in which a step of providing is not explicitly included and embodiments in which a step of providing is included. The invention also includes embodiments in which a step of identifying the subject as being at risk of or suffering from a eye disorder characterized by macular degeneration, CNV, or RNV, is included.

[00280] Where ranges are given, endpoints are included and the invention includes embodiments in which either or both endpoints are excluded. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00281] Any particular embodiment of the compositions or methods of the invention (e.g,. any therapeutic agent, any sustained release formulation or any method of preparing a sustained release formulation), any method of administration, any eye disorder or condition or characteristic(s) thereof, or any subject characteristic(s) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

We claim:

1. A method of treating an eye disorder characterized by macular degeneration, CNV, or RNV comprising the step of: administering effective amounts of first and second therapeutic agents to the subject's eye in a single procedure, wherein the first therapeutic agent provides rapid improvement in the condition of the subject's eye and the second therapeutic agent is administered as a sustained release formulation of the second therapeutic agent.

2. The method of claim 1, wherein the rapid improvement includes improvement in the visual acuity of the eye that occurs within 2 weeks following administration of the agents.

3. The method of claim 1, wherein the procedure is an injection procedure.

4. The method of claim 1, wherein the procedure is an injection procedure in which the first and second therapeutic agents are injected into the vitreous of the subject's eye.

5. The method of claim 1, wherein the procedure is an injection procedure in which, prior to administration, the first therapeutic agent is contained in a syringe and the sustained release formulation comprising the second therapeutic agent is contained in a needle attached to the syringe.

6. The method of claim 5, wherein the first therapeutic agent is dissolved in a liquid medium located in the syringe and the sustained formulation of the second therapeutic agent comprises an ocular implant located in the needle.

7. The method of claim 1, wherein the first therapeutic agent is an angiogenesis inhibitor.

8. The method of claim 1, wherein the first therapeutic agent is an anti-VEGF agent.

9. The method of claim 1, wherein the first therapeutic agent is an anti-VEGF agent selected from the group consisting of: antibodies that bind to VEGF and nucleic acids that bind to VEGF.

10. The method of claim 1, wherein the first therapeutic agent is selected from the group consisting of bevacizumb, ranibizumab, and pegaptanib.

11. The method of claim 1, wherein the second therapeutic agent is a complement inhibitor.

12. The method of claim 1, wherein the second therapeutic agent is a complement inhibitor selected from the group consisting of: viral complement control proteins and peptides or small molecules that bind to a complement component.

13. The method of claim 1, wherein the second therapeutic agent is compstatin or a derivative thereof.

14. The method of claim 1, wherein the second therapeutic agent is a GPCRA.

15. The method of claim 1, wherein the first therapeutic agent is an angiogenesis inhibitor and the second therapeutic agent is a complement inhibitor.

16. The method of claim 1, wherein compstatin or an analog thereof and a C5a inhibitor are adminstered.

17. The method of claim 16, wherein the C5a inhibitor is a C5a receptor antagonist.

18. The method of claim 1, wherein the first therapeutic agent is dissolved or suspended in a liquid medium prior to admininstration.

19. The method of claim 1, wherein the sustained release formulation comprises an ocular implant comprising the second therapeutic agent.

20. The method of claim 1, wherein the sustained release formulation comprises a polymeric material.

21. The method of claim 20, wherein the polymeric material is biodegradable.

22. The method of claim 20, wherein the polymeric material is selected from the group consisting of: poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactide-co-glycolide (PLGA), poly(phosphazine), poly (phosphate ester), polycaprolactones, polyanhydrides, ethylene vinyl acetate, polyorthoesters, polyethers, poly (beta amino esters), copolymers containing monomeric subunits found in any of the foregoing polymers, collagen, albumin, chitosan, alginate, hyaluronic acid, and mixtures of any of the foregoing polymers.

23. The method of claim 1, wherein the sustained release formulationcomprises nanoparticles, microparticles, dendrimers, or liposomes comprising the second therapeutic agent.

24. The method of claim 1, wherein the sustained release formulation comprises a solid or semi-solid material that entraps or encapsulates the second therapeutic agent.

25. The method of claim 1, wherein the sustained release formulation comprises an inactive material to which the second therapeutic agent is covalently attached.

26. The method of claim 1, wherein the first therapeutic agent is administered in soluble or particulate form in a liquid medium and the second therapeutic agent is administered in or attached to a solid or semi-solid matrix.

27. The method of claim 1, wherein the second therapeutic agent, when administered as a component of the sustained release formulation, has an activity period greater than that of the first therapeutic agent.

28. The method of claim 1, wherein administering the second therapeutic agent prolongs the time interval during which the subject experiences improvement in the condition of the subject's eye relative to the time interval during which the subject would have experienced improvement if the first therapeutic agent had been administered as sole therapy.

29. The method of claim 1, wherein the eye disorder is exudative ARMD.

30. The method of claim 1, wherein the subject has experienced a perceptible deterioration in the condition of the subject's eye within the two weeks preceding administration of the first and second therapeutic agents.

31. The method of claim 1, further comprising performing the method one or more additional times at time intervals greater than the activity period of the first therapeutic agent.

32. A method of treating an eye disorder characterized by macular degeneration, CNV, or RNV comprising the step of: administering first and second compositions to the subject's eye in a single procedure, wherein the first composition comprises an

angiogenesis inhibitor or complement inhibitor that provides rapid improvement in the condition of the subject's eye and the second composition comprises a sustained release formulation comprising an angiogenesis inhibitor or a complement inhibitor.

33. The method of claim 32, wherein the single procedure is an intravitreal injection.

34. The method of claim 32, wherein the angiogenesis inhibitor is an anti-VEGF agent.

35. The method of claim 32, wherein the angiogenesis inhibitor is an anti-VEGF agent selected from the group consisting of: antibodies or antibody fragments that bind to VEGF and nucleic acids that bind to VEGF.

36. The method of claim 32, wherein the angiogenesis inhibitor is selected from the group consisting of bevacizumb, ranibizumab, and pegaptanib.

37. The method of claim 32, wherein the complement inhibitor is selected from the group consisting of: viral complement control proteins and peptides that bind to a complement component.

38. The method of claim 32, wherein the complement inhibitor is compstatin or a derivative thereof.

39. The method of claim 32, wherein the angiogenesis inhibitor is selected from the group consisting of bevacizumb, ranibizumab, and pegaptanib and the complement inhibitor is compstatin or a derivative thereof.

40. The method of claim 32, wherein the first therapeutic agent is provided at least in part dissolved or suspended in a liquid medium.

41. The method of claim 32, wherein the second therapeutic agent is released from the ocular implant so as to maintain a therapeutic level in the subject's eye over a period of at least 3 months.

42. A method of administering first and second therapeutic agents to the eye of a subject comprising: injecting (i) a solution or suspension containing the first therapeutic agent and (ii) a solid ocular implant, plurality of particles, or gel-forming composition containing the second therapeutic agent into the subject's eye in a single injection procedure.

43. The method of claim 42, wherein the first and second therapeutic agents are injected into the vitreous of the subject's eye.

44. The method of claim 42, wherein (i) the solution or suspension; and (ii) the solid ocular implant, plurality of particles, or gel-forming composition, are injected using a single needle and syringe assembly.

45. The method of claim 42, wherein the first therapeutic agent provides a rapid improvement in the condition of the subject's eye.

46. The method of claim 42, wherein the activity period of the second composition is greater than the activity period of the first composition.

47. The method of claim 42, wherein the second therapeutic agent does not provide rapid improvement in the condition of the subject's eye.

48. The method of claim 42, wherein the second therapeutic agent has an activity period greater than that of the first therapeutic agent.

49. The method of claim 42, further comprising the step of: repeating the administering step once or more at time intervals greater than the activity period of the first therapeutic agent.

50. An article of manufacture comprising (i) a first therapeutic agent effective for treating an eye disorder; and (ii) a needle containing a second therapeutic agent.

51. The article of manufacture of claim 50, further comprising a syringe.

52. The article of manufacture of claim 50, further comprising a syringe, wherein the syringe contains the first therapeutic agent.

53. The article of manufacture of claim 50, wherein the article of manufacture contains a unit dosage form of the first therapeutic agent.

54. The article of manufacture ofclaim 50, wherein the article of manufacture contains a unit dosage form of the first therapeutic agent and a unit dosage form of the second therapeutic agent.

55. The article of manufactureof claim 50, further comprising a syringe, wherein the article of manufacture contains at least one compartment and the syringe and needle are housed in a single compartment of the article of manufacture.

56. The article of manufacture of claim 50, further comprising a syringe, wherein the syringe and needle are attached to one another.

57. The article of manufacture of claim 50, further comprising a syringe, wherein the article of manufacture contains at least two compartments, and wherein the syringe and needle are housed in individual compartments.

58. An article of manufacture comprising (i) a first therapeutic agent effective for treating an eye disorder; (ii) a second therapeutic agent effective for treating an eye disorder, wherein each therapeutic agent is contained in an individual syringe.

59. The article of manufacture of claim 58, further comprising a needle.

60. A method of supplying a combination therapy for an ocular disorder comprising providing the article of manufacture of any of claims 50 - 59.

61. The method of claim 60, wherein the step of providing comprises: shipping the article of manufacture to a pharmacy or to a site of health care delivery.

62. A needle and syringe assembly, wherein the needle contains a sustained release formulation comprising a first therapeutic agent for an eye disorder and the syringe contains a second therapeutic agent for the eye disorder, wherein the second therapeutic agent is dissolved or suspended in a liquid medium.

63. The needle and syringe assembly of claim 62, wherein the sustained release formulation comprises an ocular implant, plurality of particles, or gel-forming material.

64. The needle and syringe assembly of claim 62, wherein the first therapeutic agent is a complement inhibitor or an angiogenesis inhibitor and the second therapeutic agent is a complement inhibitor or an angiogenesis inhibitor.

65. The needle and syringe assembly of claim 62, wherein the first therapeutic agent is a complement inhibitor and the second therapeutic agent is an angiogenesis inhibitor.

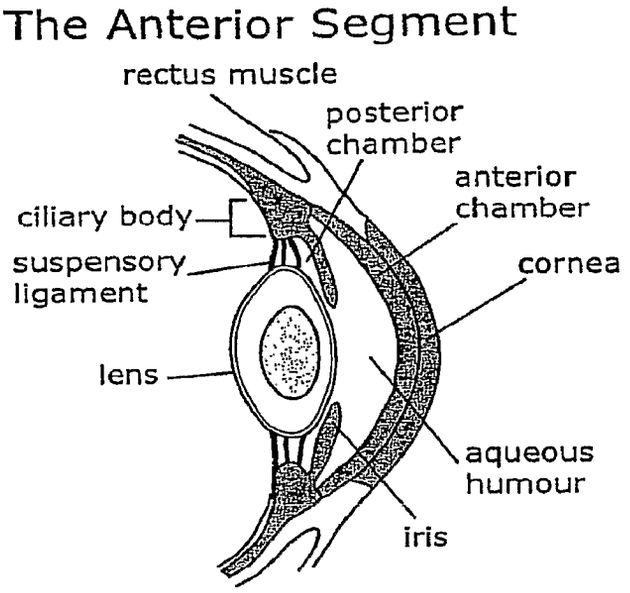


FIG. 1A

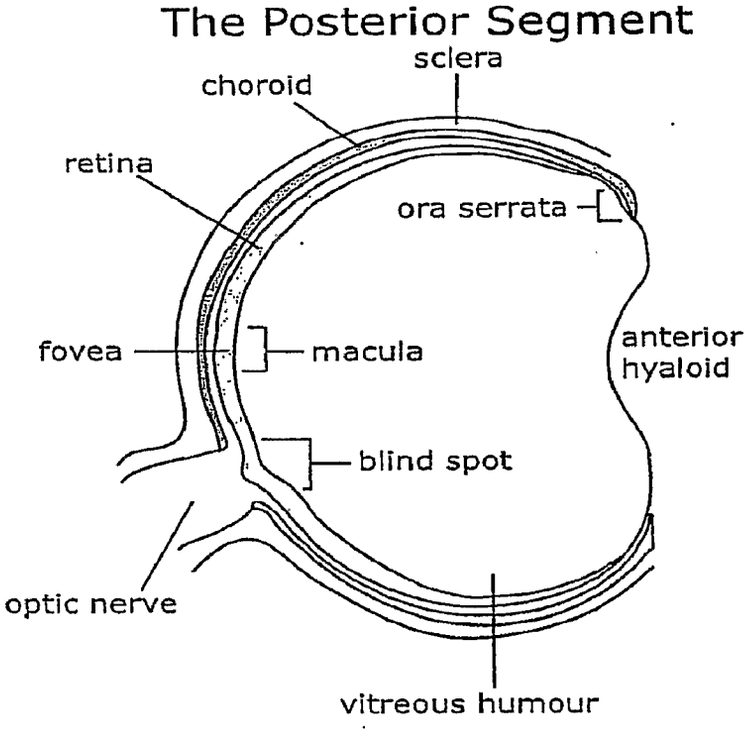


FIG. 1B

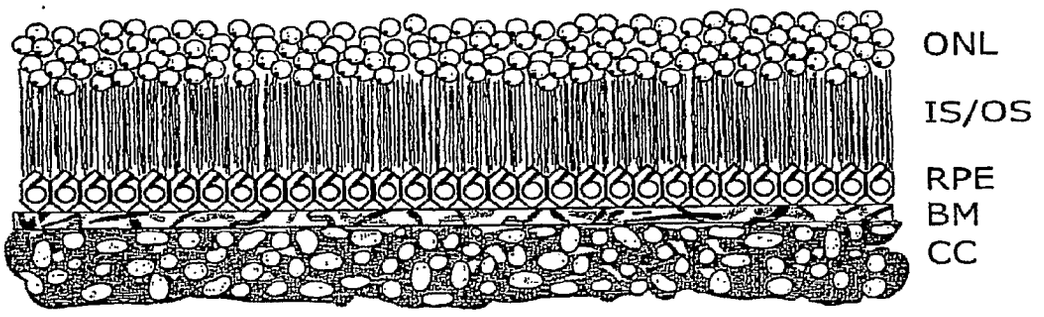


FIG. 1C

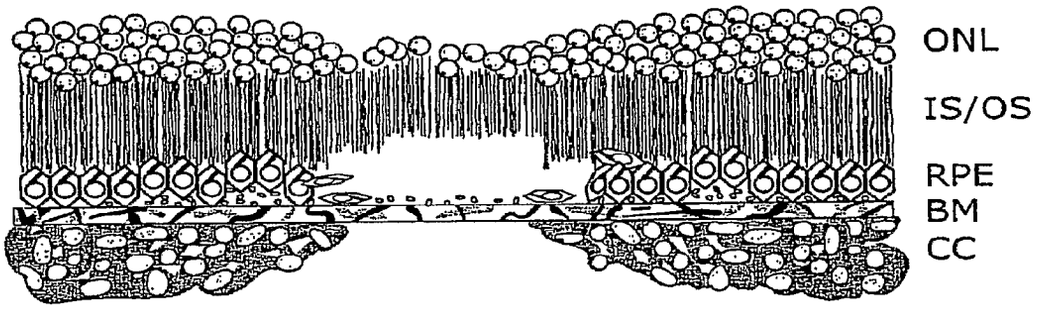


FIG. 1D

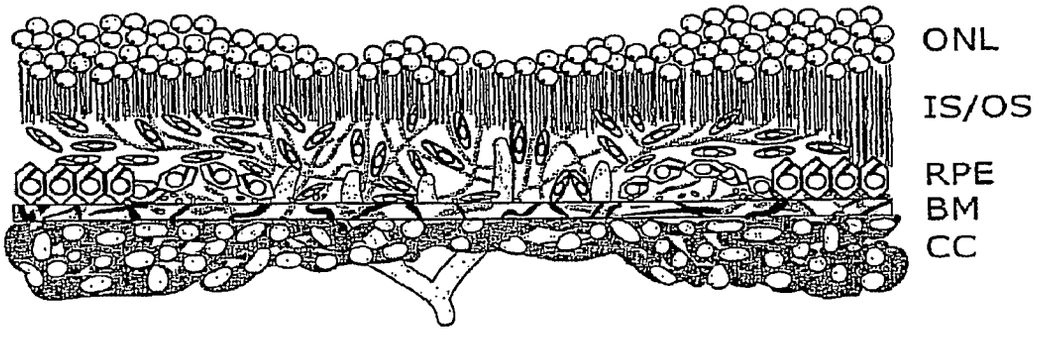


FIG. 1E

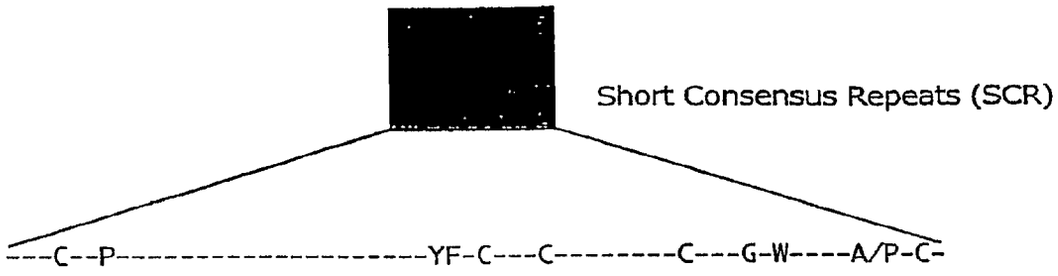


FIG. 2

Vaccinia virus complement control protein precursor: Accession number P10998

mkvesvtflt llgigcvlsc ctipsrpinm kfknsvetda nanynigdti eylclpgyrk
qkmgpiyakc tgtgwtlfnq cikrrcpspr didngqldig gvdfgssity scnsgyhlig
esksycelgs tgsmvwnpea picesvkcqs ppsisngrhn gyedfytdgs vvtyscnsgy
slignsgvlc sggewsdppt cqivkcphpt isngylssgf krsysyndnv dfkckygykl
sgsssstcsp gntwkpelpk cvr

FIG. 3A

Vaccinia virus complement control protein: Accession number 1RID_B

cctipsrpin mkfknsvetd ananynigdt ieylclpgyr kqkmgpiyak ctgtgwtlfn
qcikrrcpsp rdidngqldi ggvdfgssit yscnsgyhli gesksycelg stgsmvwnpe
apicesvkcq sppsisngrh ngyedfytdg svvtyscnsg yslignsgvl csggewsdpp
tcqivkcphp tisngylssg fkrsysyndn vdfkckygyk lsgsssstcs pgntwkpelp
kcvr

FIG. 3B

```
VAC-COP C3L   CCTIPSRPINMKFKNSVETDANANYNLGDTIEYLCLPGYRKQKMGPIYAKCTGTGWTLFNQCI
VAC-WR  C21L  ..............................................................
CPV-GRI C17L  ..P.....T....GT.--.--SH.......................................
CPV-BRI IMP   ...........................G..................................
VAR-BSH D15L  ..............................................................
VAR-IND D12L  ..........T...................................................
VAR-GAR B18L  ..............................................................
MPV-ZAI D15L  Y.....................--.....................................

VAC-COP C3L   KRRCPSPRDIDNGQLDIGGVDFGSSITYSCNSGYHLIGESKSYCELGSTGSMVWNPEAPICE
VAC-WR  C21L  ..............................................................
CPV-GRI C17L  ..............................................................
CPV-BRI IMP   ..K.........I.....E.....................Q.....................Y.....
VAR-BSH D15L  ..........H...................................Y....K.....K.....
VAR-IND D12L  ..........H...................................Y....K.....K.....
VAR-GAR B18L  ..........H...................................Y....K.....K.....
MPV-ZAI D15L  ..............................................................

VAC-COP C3L   SVKCQSPPSISNGRHNGYEDFYTDGSVVTYSCNSGYSLIGNSGVLCSGGEWSDPPTCQ
VAC-WR  C21L  ..............................................................
CPV-GRI C17L  ..............................................................
CPV-BRI IMP   ....P....VT..................................IV................
VAR-BSH D15L  .....L.....N..................................N.....
VAR-IND D12L  .....L.....N..................................N.....
VAR-GAR B18L  .....L.....N..................................N.....
MPV-ZAI D15L  ..............I..I.....................M.....N.....

VAC-COP C3L   IVKCPHPTISNGYLSSGFKRSYSYNDNVDFKCKYGYKLSGSSSSTCSPGNTWKPELPKCVR
VAC-WR  C21L  ..............................................................
CPV-GRI C17L  ..............................................................
CPV-BRI IMP   .....S.T.....................H.....RH.....................Q.....
VAR-BSH D15L  ..........L...................T..........................Q.....
VAR-IND D12L  ..........L...................T..........................Q.....
VAR-GAR B18L  .....Y...L...................T..........................Q.....
MPV-ZAI D15L  .........-....K.LAA
```

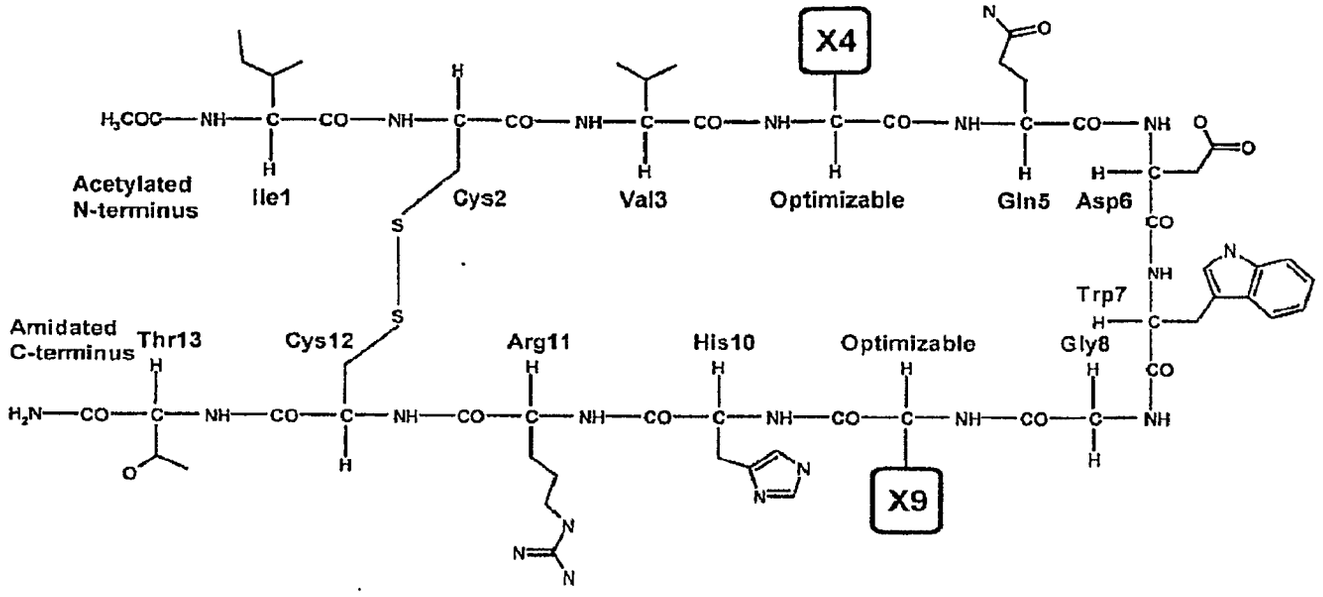
FIG. 4

	Inhibition of Hemolysis	Heparin Binding Activity	K+R	%K+R	pI	# of Putative Sites (K/R X K/R)
VCP/IMP/SPICE	+	+	23	9.43	8.80	4
MPV Homolog of VCP	+	N/D	16	8.00	7.22	3
rVCP	+	+	23	9.43	8.80	4
rVCP SCR (2,3,4)	-	+	16	8.79	7.22	2
rVCP SCR (1,2)	-	+	12	9.60	7.00	3
rVCP SCR (2,3)	-	-	7	5.83	4.41	1
rVCP SCR (3,4)	-	+	11	9.24	9.08	1

FIG. 5

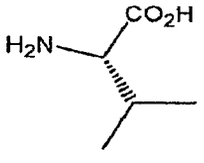
MKVERVTFLTLLGIGCVLSCCTIPSRPINMKFKNSVETDANANYNIGDTIEYLCL
PGYRKQKMGPIYAKCTGTGWTLFNQCIKRRCPSPRDIDNGHLDIGGVDFGSSIT
YSCNSGYYLIGEYKSYCKLGSTGSMVWNPKAPICESVKCQLPPSISNGRHNGY
NDFYTDGSVVTYSCNSGYSLIGNSGVLCSGGEWSNPPTCQIVKCPHPTILNGYL
SSGFKRSYSYNDNVDFTCKYGYKLSGSSSSTCSPGNTWQPELPKCVR

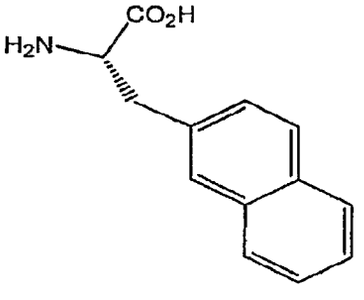
FIG. 6



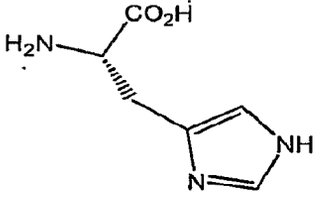
Ac-Compstatin-NH2 Ac-V4(2Nal)/H9A-NH2

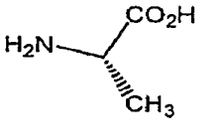
X4





X9





IC_{50} 53.6 μM 0.5 μM

Figure 7

Figure 8

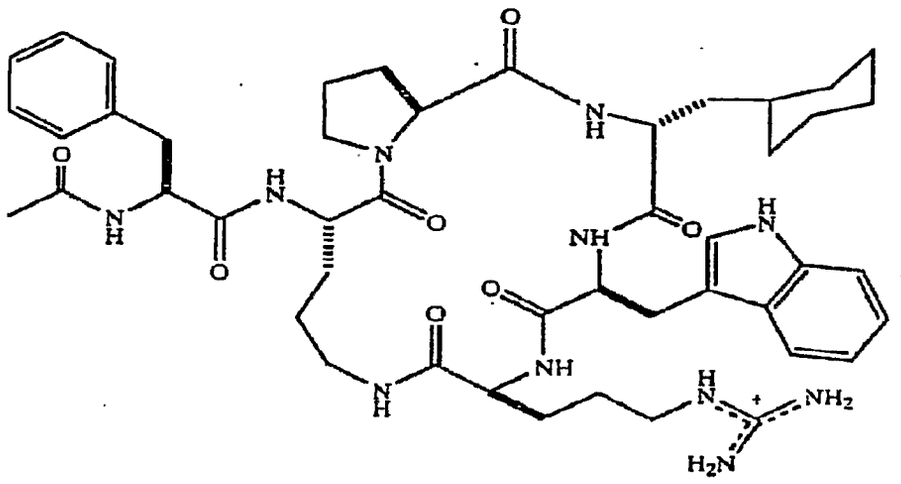
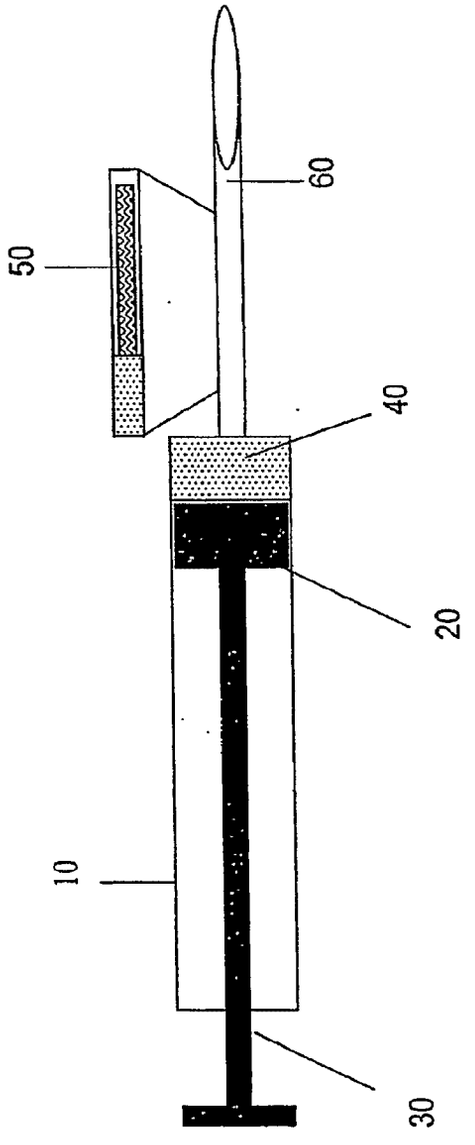


Figure 9



10 Syringe (barrel)
20 Stopper
30 Plunger
40 First therapeutic agent (e.g., anti-angiogenic drug)
50 Ocular implant containing second therapeutic agent (e.g., complement inhibitor)
60 Injection needle

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 December 2006 (07.12.2006)

PCT

(10) International Publication Number
WO 2006/128564 A1

(51) International Patent Classification:
A61M 5/315 (2006.01)

(21) International Application Number:
PCT/EP2006/004494

(22) International Filing Date: 12 May 2006 (12.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/685,830 31 May 2005 (31.05.2005) US

(71) Applicants *(for all designated States except US)*: **BAXTER INTERNATIONAL INC.** [US/US]; One Baxter Parkway, Deefield, IL 60015 (US). **BAXTER HEALTHCARE S.A.** [CH/CH]; Heristrasse 2, CH-8304 Wallisellen (CH).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **DELMOTTE, Yves, A.** [BE/BE]; 47, Rue Colonel Balaince, B-7332 Neufmaison (BE). **BLOM, Agneta** [SE/BE]; 4, Avenue de la Marbrerie, B-1440 Wauthier-Braine (BE).

(74) Agent: **PERREY, Ralf**; Müller-Boré & Partner, Grafinger Strasse 2, 81671 München (DE).

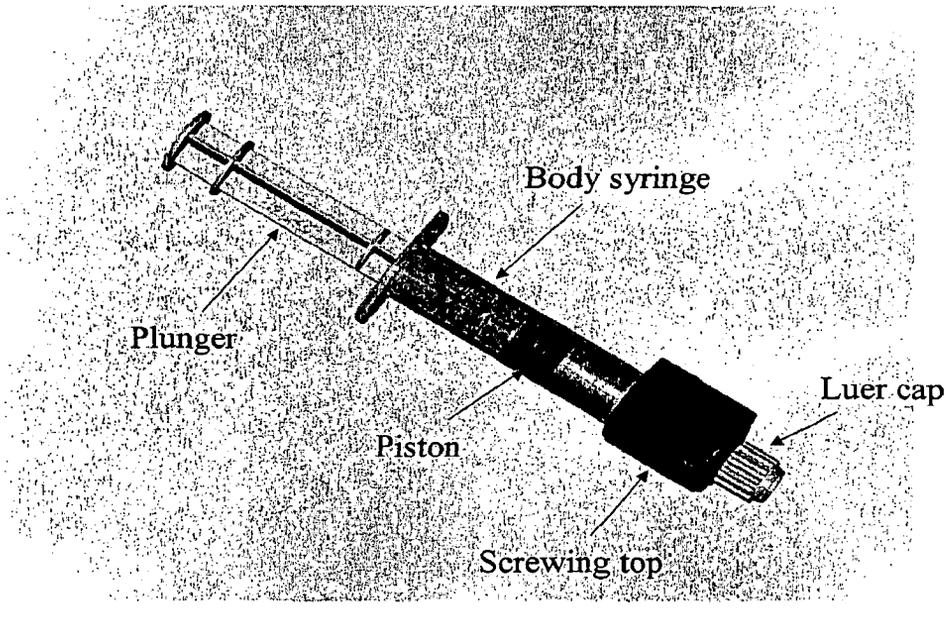
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC,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

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

Published:
— *with international search report*

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

(54) **Title:** POLYPROPYLENE HOLLOW BARREL WITH SLIDING COATED RUBBER PISTON



(57) **Abstract:** The present invention relates to a device comprising a piston sliding in a body like, for example, a syringe.

WO 2006/128564 A1

POLYPROPYLENE HOLLOW BARREL WITH SLIDING COATED RUBBER PISTON

[001] The present invention relates to a device comprising a piston sliding in a body like, for example, a syringe.

[002] In most of commercial syringes, the piston sliding in the hollow barrel of a syringe body is made of a resilient material, such as rubber or thermoplastic elastomer, to absorb the irregularity in the shape of the syringe body. In order to allow the sliding and ensure that the syringe does not become leaky when pressure is applied, the sliding piston is coated with a silicone lubricant. One disadvantage of the use of silicone coated pistons is that that the silicone oils contaminate the content of the syringe body, e.g. a liquid medicament to be applied with the syringe.

[003] In order to avoid such effect, laminated pistons were developed and disclosed in the prior art. Those pistons are of a silicone-free type in which it is not necessary to coat the sliding portion with a silicone oil layer as a lubricant.

[004] In US patent 6,090,081, pistons (rubber stoppers) are described which are capable of satisfying both the sealing property and slidable property without using silicone oils and having high sanitary and safety property. Such pistons are coated with a tetrafluoroethylene-ethylene copolymer resin as disclosed in Japanese Patent Laid-Open Publication No. 139668/1987, or with a polytetrafluoroethylene resin film, as disclosed in Japanese Patent Laid-Open Publication No. 97173/1988. The content of the said US patent and the Japanese Patent Publications are incorporated herein by reference.

[005] Efforts were made to develop new materials for the body of the syringes which can be combined with the laminated pistons.

[006] It is the merit of the present invention that it was surprisingly found that it is possible to combine certain pistons with certain coating with conventional hollow barrel bodies made of polypropylene in order to obtain a device fulfilling all functional, sanitary and regulatory

requirements for use for medical purposes, like air and water tightness and required sliding forces.

[007] In one embodiment of the invention, the surface of the resilient piston is coated with a laminated layer of polytetrafluoroethylene resin film, in another embodiment the surface of the resilient piston is coated with a laminated layer tetrafluoroethylene-ethylene resin film. The coating can be performed as described in US patent 6,090081 and the Japanese Patent Laid-Open Publication No. 139668/1987, or Japanese Patent Laid-Open Publication No. 97173/1988. In a further embodiment of the present invention, a tetrafluoroethylene polymer coated piston Flurotec commercially available from West Pharmaceutical-Daikyo as specified in Example 1 is used.

[008] A hollow barrel polypropylene body according to the present invention may be any body made of polypropylene which is a hollow barrel intended for use in combination with a sliding piston, e.g. conventional syringe bodies or the like. It is within the ordinary skill of a worker in the field to be capable to combine a piston with a certain design with the appropriate hollow barrel in order to achieve the functional requirements such as air and water tightness and requiring a sliding force that fulfills the acceptance criteria of regulatory authorities.

[009] Therefore, the invention concerns a device comprising a combination of a polypropylene body with a laminated piston defined above as possible embodiment of the invention.

[010] The device exemplified in detail in the following example shall be another embodiment of the invention. However, the examples shall illustrate the invention and not be used to limit the scope of the teaching given herein.

EXAMPLES

EXAMPLE 1: "TRICOS-Fluoro-DEVICE"

[011] In a TRICOS-device (see FIGURE 1) constituted of plunger, a body syringe, a screwing top and a luer cap as described in PCT patent publication number WO2004032808, the conventional rubber piston is replaced by 5ml piston made of Butyl rubber coated with

tetrafluoroethylene polymer resin as per BP 3P01020 obtained from West Pharmaceutical-Daikyo. Such amended TRICOS-device is named TRICOS-Fluoro-Device.

[012] The different components of one embodiment of the TRICOS-Fluoro-Device are described in detail in the following table:

Name	Description	Reference number	Lot number
Screwing top	Molded part made of 96 % natural polypropylene (Grade: HD810MO) and 4% of Blue concentrate Polybatch P45056 as per BP Ind.01.10.001-D - Bouchon	RE REF #195	10030F0020
Luer cap	Molded part made of PL 1747 as per BP PF0470	20007803	10352601
Body syringe	Molded part made of natural polypropylene (Grade: HD810MO) as per BP Ind.01.10.001-D- Corps de seringue	RE REF #194	10030F0022
Plunger	Molded part made of natural polypropylene (Grade: HD810MO) as per BP Ind.01.10.001- C- Piston de seringue	RE REF #196	10030F0021
Piston	5ml piston made of Butyl rubber coated with tetrafluoroethylene polymer resin as per BP 3P01020 obtained from West Pharmaceutical-Daikyo .	5 ml Piston FR2-2RS – formulation: D21-6-1	030110

[013] The use of a tetrafluoroethylene polymer coated piston from Daikyo with the polypropylene syringe body, presents a lot of advantages for the development of the final product, as it does not require the use of silicone oil to facilitate the sliding of the piston inside of the syringe body. This is a tremendous advantage from a regulatory but also manufacturing point of view: easy to store, does not stick, inexpensive process and equipment, no transfer of the silicone oil to the granules of calcium phosphate.

Further embodiments of TRICOS-Fluoro-Device:

[014] "5ml, 10ml and 20ml" tetrafluoroethylene polymer coated pistons(i.e pistons foreseen for conventional 5ml, 10ml, and 20ml syringes, respectively) from Daikyo were successfully used with 3.5 ml, 7.0 ml and 17ml TRICOS syringes (design History file : 001-DHF-NIV). Of course, it is understood that also pistons of other size could be used with the appropriate hollow barrel (TRICOS syringe) to produce a functional TRICOS-Fluoro-Device.

EXAMPLE 2: TESTS

[015] Tests were performed to evaluate if the tetrafluoroethylene polymer resin coated piston can fulfill the acceptance criteria of the standards applying for syringe like container made of polypropylene HD810MO.

[016] These standards are applied for commercial syringe made of polypropylene with a piston that is siliconized.

1. Air leakage between the piston and the inner wall of the syringe body during aspiration, and for separation of piston and plunger as per ISO 7886-1 (annex B)

[017] This test challenges the ability of the syringe like container to resist to leakage and piston detachment from the plunger under negative pressure. This test is an attribute test based on the ISO 7886-1, Annex B of the norm.
A pass or fail determination was made based on a visual observation for replacing bubbles and piston detachment.
Protocol of test is described in EXAMPLE 3.

Test criteria:
No leak at piston is accepted and no piston detachment is accepted.
The pressure may not increase during the 60's test under vacuum

Test results:
75 non-sterile units and 75 sterile units were tested.
All units passed successfully the piston detachment test and no increase in pressure during the 60 seconds of vacuum was observed for any of the units tested. No piston leak was detected.

Conclusions:

[018] All tested units passed successfully test "Air leakage past piston during aspiration, and for separation of piston and plunger as per ISO 7886-1 (annex B of the norm)" and by that it can be stated with 95% confidence that there is less than 3.916% defective units.

2. Piston pull-out test

[019] This test challenges the ability of the piston to remain engaged with the plunger when exposed to a potential pull out force. A pass or fail determination was made.
Protocol of test is described in EXAMPLE 4.

Test criteria:
No piston detachment from the plunger is accepted

Test results:
75 non sterile units and 75 sterile units were tested.
All units passed successfully the test.

Conclusions:

[020] All tested units passed successfully "Piston pull-out test" and by that it can be stated with 95% confidence that there are less than 3.916% defective units as per test.

3. Piston removal force

[021] This test challenges the ability of the piston/plunger to remain inserted into the body syringe when exposed to a potential pull out force. The force needed to remove the piston/plunger from the body syringe was measured thanks to a tensile machine and the maximum pull out force has to be higher than 29 N (precision movement sustained male – DEF

STAN 00-25 – part 3) and it is preferable that the maximum pull force is higher than 59 N (precision movement momentary male - DEF STAN 00-25 – part 3).
Protocol of test is described in EXAMPLE 5.

Test results:

[022] 50 non-sterile units and 50 sterile units were tested. For both the sterile and the non-sterile units there were 3 units where the part of the plunger attached to the tensile machine broke before the plunger was removed. This means that the actual force needed to remove the plunger is above the value registered.

	Peak force (N)	
	Non- sterile units	Sterile units
Average:	198.4	178.2
Min:	112.1	118.3
Max:	281.1	233.2
Standard Deviation:	42.96	28.19

Conclusions:

[023] All tested units successfully passed the test, and it can be stated with 95% confidence that at least 99% of the units of an equal production, when tested according to test , will result in a peak force above 75.8 N for the non-sterile samples and above 97.8 N for the sterile samples.

4. Liquid leakage at syringe piston under compression

[024] The test challenges the ability of the syringe piston to resist leakage under axial pressure.
This test is based on the ISO 7886-1.
A pass or fail determination was made.
Protocol of test is described in EXAMPLE 6.

Test criteria:

No leak is accepted

Test results:

75 non sterile units and 75 sterile units were tested.

No leak was detected for any of the units tested.

Conclusions:

[025] All test units passed successfully test "Liquid leakage at syringe piston under compression" and by that it can be stated with 95% confidence that there is less than 3.916% defective units.

5. Forces required to operate the plunger

[026] The test purpose is to measure the force, which is required to initiate the movement of the plunger inside of the syringe body. This test is based on the ISO 7886-1:1993 annex G

Test criteria:

[027] In ISO 7886-1:1993 annex G there is no strict requirement on the force required to initiate the movement of the plunger, but a proposed value of < 25 N is given.

It is known in the art that a piston cannot slide into the syringe body without coating with silicone oil.

Test results:

Protocol of test is described in EXAMPLE 7.

50 sterile units were tested.

	Sterile units
	Initial force (N)
Average:	18.43
Min:	12.12
Max:	22.95
Standard Deviation:	2.61

Conclusions:

[028] The force needed to initiate the movement of the plunger is below the proposed limit of 25 N for all units tested and it can be stated with 95% confidence that at least 99% of the units of an equal production when tested according to the above test, will result in an initial force to move the plunger below 25.9 N. These results are acceptable since there is no difficulty to move the piston at the forces obtained in this study

6. Check the dimensions of the pistons as per blueprint provided by Daikyo.

[029] The tests are performed before sterilization and after beta sterilization at a dose of 50 kGy onto the overall dimensions of the TRICOS devices.
This test is important to show that the tetrafluoroethylene polymer resin coated piston from Daikyo keeps its dimensions after sterilization and therefore the its functionality when mounted in the TRICOS device as shown in tests 1 to 4

Samples and raw material:

Piston:

Traceability: Production code: 5 ml Piston FR2-2RS from Daikyo
Formulation: D21-6-1
Lot No: 030110

Description: Part made by Daikyo Seiko, LTD and supplied by West Pharmaceutical. Butyl rubber part coated with Fluoro resin.

Sample preparation:

[030] 25 pistons were tested as received by the West supplier, while 25 other pistons were packed into an HDPE overpouch and sent to Ionisos for *beta sterilization at 50 kGy* before dimensional test.

Test description : visual inspection with a calibrated caliper

Performance: All 50 pistons were inspected. The 25 pistons for sterilization were inspected both before and after sterilization.

Result: No defective units were observed.

Dimensional check

Performance: 25 sterile and 25 non-sterile pistons were measured as per the attached blueprint. A letter as indicated on the blueprint identified each dimension.

Result on non-sterile units:

Unit #	Dimension (mm)			
	A	B	C	D
1	12.6	12.05	12.47	10.82
2	12.67	12.14	12.48	10.8
3	12.66	12.09	12.47	10.94
4	12.64	12.09	12.55	11.02
5	12.65	12.1	12.47	11.07
6	12.66	12.12	12.5	11.03
7	12.67	12.09	12.47	10.93
8	12.69	12.08	12.59	10.86
9	12.67	12.1	12.48	10.95
10	12.64	12.11	12.49	10.88
11	12.67	12.1	12.55	10.86
12	12.67	12.14	12.54	10.91
13	12.68	12.09	12.5	10.9
14	12.67	12.11	12.53	10.89
15	12.67	12.13	12.53	10.98
16	12.69	12.09	12.52	10.99
17	12.67	12.1	12.57	10.88
18	12.68	12.12	12.47	10.95
19	12.69	12.14	12.51	10.99
20	12.69	12.14	12.58	10.89
21	12.69	12.14	12.46	10.85
22	12.69	12.13	12.55	10.89
23	12.69	12.11	12.56	11.02
24	12.68	12.14	12.53	10.93
25	12.67	12.14	12.54	10.95
Average	12.67	12.11	12.52	10.93
Stdev	0.0208	0.0243	0.0394	0.0686

A, B, C and D are described on the attached drawing

Sterile units:

Unit #	Dimension (mm)			
	A	B	C	D
1	12.68	12.09	12.49	10.87
2	12.67	12.12	12.49	11.01
3	12.68	12.12	12.5	10.99
4	12.69	12.12	12.56	10.99
5	12.69	12.13	12.51	11.04
6	12.66	12.12	12.5	10.98
7	12.67	12.09	12.5	11
8	12.68	12.12	12.52	11
9	12.68	12.14	12.5	10.99
10	12.69	12.11	12.51	10.99
11	12.67	12.11	12.49	10.98
12	12.67	12.14	12.49	10.91
13	12.66	12.07	12.52	11.01
14	12.67	12.14	12.52	10.96
15	12.64	12.1	12.5	10.99
16	12.66	12.13	12.46	11.01
17	12.64	12.13	12.47	10.99
18	12.66	12.09	12.52	11
19	12.68	12.11	12.54	10.96
20	12.69	12.13	12.49	10.98
21	12.69	12.14	12.57	10.99
22	12.68	12.14	12.49	10.97
23	12.67	12.12	12.55	11.02
24	12.64	12.08	12.48	11.02
25	12.66	12.12	12.5	10.99
Average	12.67	12.12	12.51	10.99
Stdev	0.0155	0.0200	0.0264	0.0345

A, B, C and D are described on the attached drawing

Conclusions:

[031] All piston measurements performed were within the limits.

No significant differences between sterile and non-sterile units were observed.

The piston keeps its characteristics after irradiation at a dose of 50 kGy.

References:

Design History File : 001-DHF-NIV

EXAMPLE 3:

Test set-up: see Figure 2.

Test procedure:

- Take a Bone Substitute device assembly without luer cap.
- Draw into the syringe a volume of at least 2ml of freshly boiled water, cooled to room temperature.
- With the screwing top female luer uppermost, withdraw the plunger axially until the fiducial line is at the nominal capacity graduation line. Clamp the plunger in this position using an appropriate fixture (RE.REF#189).
- Connect the screwing cap female luer to the 3-way stopcock. Position the 3-way stopcock such that vacuum will be drawn in all directions.
- Switch on the vacuum pump and allow the vacuum to stabilize. In the protocol 173-P-NIV it was asked to stabilize the pressure at 0.88 bar, however with the vacuum pump used the pressure was stabilized between 0.88 and 0.93 bar. During the stabilisation observe for air bubbles that break free from the piston seal. No more than 2 bubbles that break free are acceptable. If more than 2 bubbles break free, it is possible that air is being withdrawn from in-between piston seals. Record the location of leaks if any.
- Position the 3-way stopcock such that the BSD and the pressure monitor are isolated from the vacuum pump. Turn off the vacuum pump and record the pressure read by the pressure manometer (initial pressure).
- Start the stopwatch and allow the test sample to remain under vacuum for 60 (+5, -0) seconds.
- During the hold period observe the piston seals for bubbles that form and break free. No replacing bubbles are acceptable.

- At the completion of the hold period record the pressure read by the pressure manometer (final pressure). Examine the syringe to determine if the piston has become detached from the plunger. No vacuum decay or piston detachment is acceptable.
- Remove the syringe from the 3-way stopcock.

EXAMPLE 4:

Test set-up: see Figure 3.

- Take a test unit (screwing cap/body syringe/plunger/piston assembly).
- Put the piston to completely inserted position.
- Check that the piston is fully inserted into body syringe and that it is firmly threaded into the plunger.
- Place the weight on a firm flat surface.
- Fix the weight to the screwing cap thanks to a suitable fixture (RE.REF#188).
- Slide the plunger push button into the plunger fixture taking care not to move the piston inside the body syringe.
- Pick up the weight and the test sample by the weight taking care not to move the piston inside the body syringe and release the weight and allow it to drop onto the landing area.
- The body syringe/screwing cap assembly should remain attached to the weight as it is pulled off of the piston /plunger assembly.
- Observe the piston/plunger assembly. If the piston remains attached to the plunger after the body syringe has been pulled off, the piston has passed the test.
- If the piston detaches from the plunger, the piston has failed the test.

EXAMPLE 5:

Test procedure:

- Take a test unit (body syringe/screwing cap/piston/plunger)
- Put the piston to completely inserted position.
- Check that the piston is fully inserted into body syringe and that it is firmly threaded into the plunger.
- Fix the screwing cap in the upper jaw of a tensile machine thanks to a suitable fixture (RE.REF#190).

- Fix the plunger push button in the lower jaw of a tensile machine thanks to a suitable fixture (RE.REF#190).
- Zero the recorder and set the tensile machine so that it can apply a tensile force
- Start the tensile machine so that it pulls the plunger/piston assembly till it is pull off of the body syringe with a crosshead speed of 500 mm/min.
- Record the peak force when the plunger passes through the body syringe undercut.
- The peak force should be higher than 29 N to pass the test and it is preferable that the peak force is higher than 59N.

Test set-up: see Figure 4.

EXAMPLE 6:

Test set-up: see Figure 5.

Test procedure:

- Take a test unit (body syringe/piston/plunger assembly);
- Screw the specific screwing cap for t
- Draw into the syringe a volume of r exceeding the nominal capacity of the syringe;
- Expel air and adjust the volume of water in the syringe at normal capacity;
- Seal the specific screwing cap for test with the water connection;
- Fix the body syringe vertically with a specific fixture;
- Apply a sideways force to the syringe to the plunger push button at right angle to the plunger to swing the plunger radially about the piston seal(s) with a force of about 3 N. During testing the set-up shown above with a weight of 300 g was not used, but the sideways force was applied by the hand of the person performing the test;
- Orientate the plunger to permit the maximum deflection from the axial position;
- Increase the water pressure till 300 kPa;
- Record the pressure measured by the pressure monitor;
- Maintain the pressure for 30 (+5, -0) seconds;
- Turn off the water pressure and remove the test unit;
- Examine the syringe for liquid leakage beyond the piston seals to the outside. If no liquid is found, the unit is acceptable.

EXAMPLE 7:

Test procedure:

- For each device, set the piston at graduation 3.4 before sterilization (only sterile units are tested).
- Take a Bone Substitute Device and remove the screwing cap and the luer cap.
- Do not move the syringe plunger. Leave it at its initial setting.
- Mount the test unit in the tensile machine as shown in the photo above.
- Start the testing machine so that it pushes the plunger at a rate of 100 mm/min, until the piston is about 1mm out of the syringe body.

CLAIMS

1. A device comprising a combination of a polypropylene body with a resilient piston selected from a piston coated with a laminated layer of polytetrafluoroethylene resin film and a piston coated with a laminated layer tetrafluoroethylene-ethylene resin film.

2. The device according to claim 1 in which the body is a syringe body.

3. The device according to claim 1 which is a TRICOS-Fluoro-Device according to Example 1.

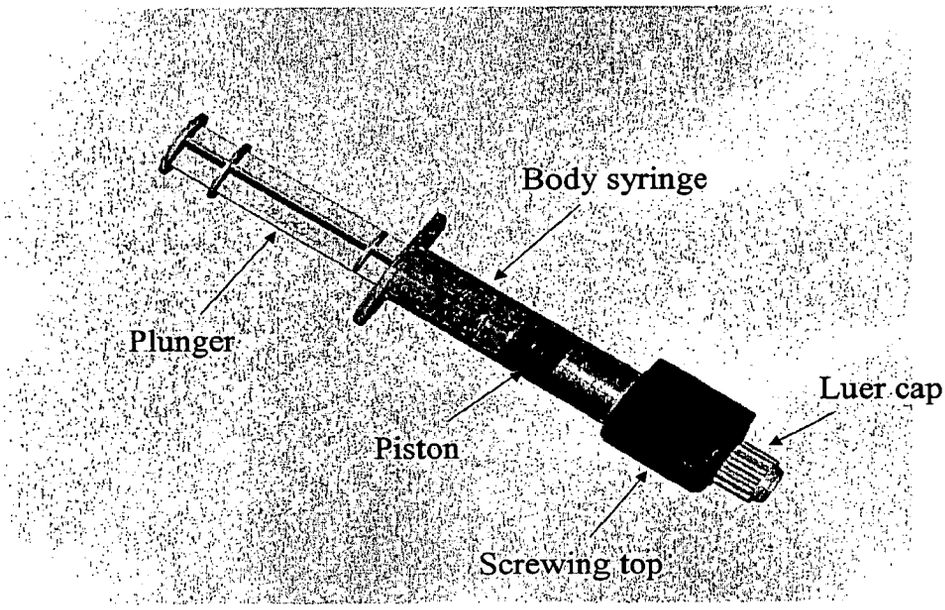


FIG. 1

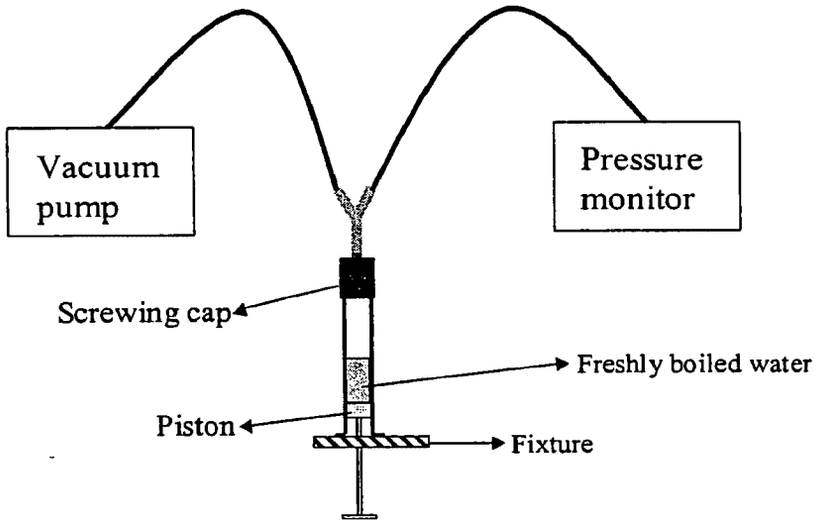


FIG. 2

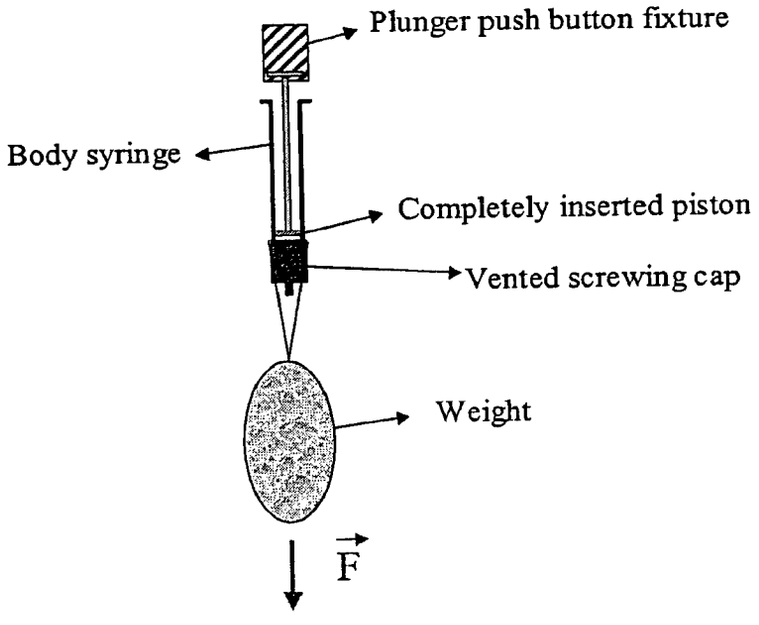


FIG. 3

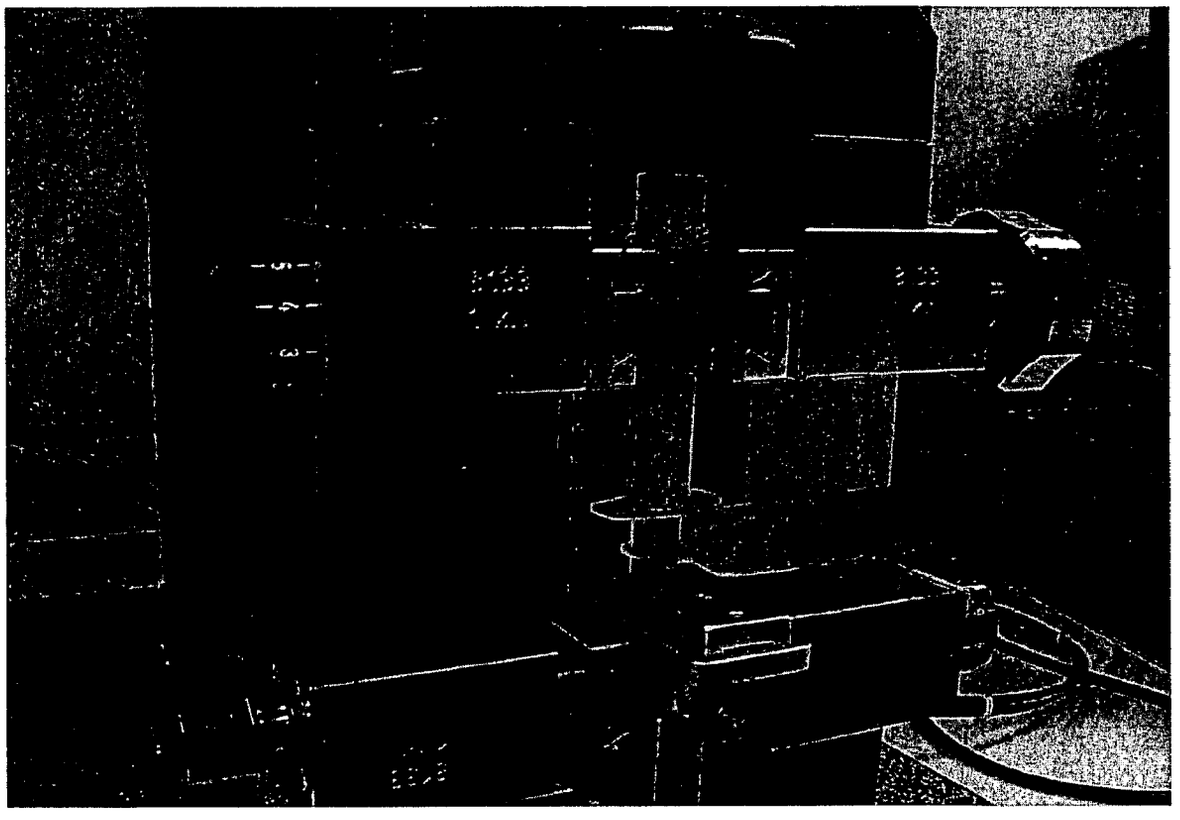


FIG. 4

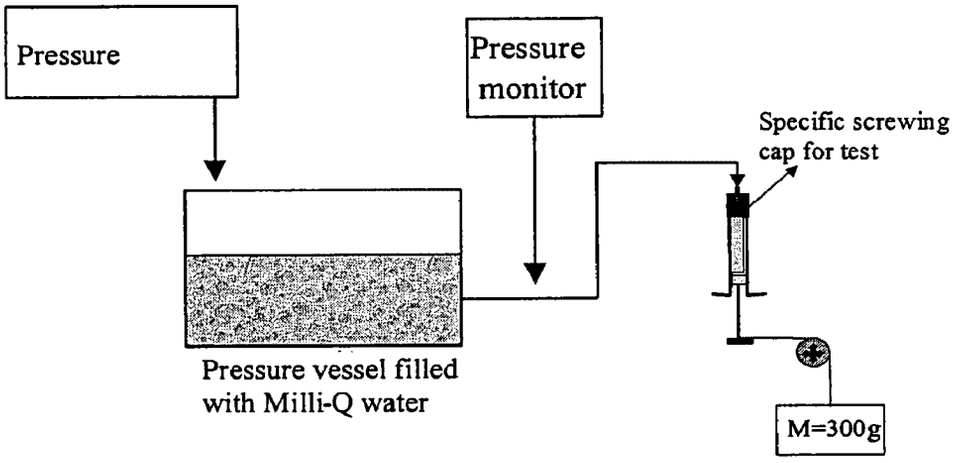


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/004494

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61M5/315

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)
A61M A61B A61F

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 6 090 081 A (SUDO ET AL) 18 July 2000 (2000-07-18) cited in the application column 3, lines 38-51; figures 1-3 column 5, lines 5-67 column 8, lines 4-24; table 3 column 9, lines 26-49 column 11, line 53 - column 12, line 19	1,2
X	WO 94/13345 A (MALLINCKRODT MEDICAL, INC) 23 June 1994 (1994-06-23) page 4, line 3 - page 5, line 27; figures 1,2	1,2

-/--

[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*
25 August 2006	08/09/2006

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	Björklund, A

2

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/004494

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 317 937 A (TERUMO KABUSHIKI KAISHA; KOKOKU INTECH CO., LTD) 11 June 2003 (2003-06-11) paragraphs [0022], [0023], [0036], [0037], [0041]; figures 1-3	1,2
X	EP 0 555 900 A (STERLING WINTHROP INC; NYCOMED IMAGING AS) 18 August 1993 (1993-08-18) column 5, lines 19-49; figures 1,2	1,2
X	WO 2004/032808 A (BAXTER INTERNATIONAL INC; BAXTER HEALTHCARE S.A) 22 April 2004 (2004-04-22) cited in the application figures 3-4C	1,2

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/004494

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

2. ☒ Claims Nos.: 3
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 3

Claim 3 makes reference to the description (Rule 6.2(a) PCT) in a manner which makes it impossible to determine the scope of the claim. It has therefore not been searched.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/004494

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 6090081 A	18-07-2000	AT	199650 T	15-03-2001
		CA	2238222 A1	22-11-1998
		DE	69800582 D1	19-04-2001
		DE	69800582 T2	28-06-2001
		DK	879611 T3	16-07-2001
		EP	0879611 A2	25-11-1998
		JP	3387775 B2	17-03-2003
		JP	10314305 A	02-12-1998
WO 9413345 A	23-06-1994	AU	5745494 A	04-07-1994
EP 1317937 A	11-06-2003	AU	8625401 A	26-03-2002
		WO	0222192 A1	21-03-2002
		US	2005212222 A1	29-09-2005
		US	2004084852 A1	06-05-2004
EP 0555900 A	18-08-1993	AT	170758 T	15-09-1998
		AU	652873 B2	08-09-1994
		AU	3022292 A	05-08-1993
		BR	9300278 A	03-08-1993
		CA	2088432 A1	01-08-1993
		CZ	9300114 A3	11-08-1993
		DE	69320845 D1	15-10-1998
		DE	69320845 T2	27-05-1999
		ES	2120473 T3	01-11-1998
		FI	930406 A	01-08-1993
		HU	63772 A2	28-10-1993
		IL	104558 A	05-12-1996
		JP	2539575 B2	02-10-1996
		JP	5253296 A	05-10-1993
		MX	9300520 A1	29-07-1994
		NO	930320 A	02-08-1993
		NZ	245796 A	27-06-1995
		RU	2093194 C1	20-10-1997
		SK	4893 A3	09-09-1993
		US	5256154 A	26-10-1993
WO 2004032808 A	22-04-2004	AU	2003275463 A1	04-05-2004
		EP	1545403 A2	29-06-2005
		US	2004068266 A1	08-04-2004

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 May 2006 (04.05.2006)

PCT

(10) International Publication Number
WO 2006/047325 A1

(51) International Patent Classification:
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2005/038006

(22) International Filing Date: 21 October 2005 (21.10.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/621,209 21 October 2004 (21.10.2004) US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 Dna Way, South San Francisco, CA 94080-4990 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **SHAMS, Naveed** [US/US]; 216 Dove Creek Lane, Danville, CA 94506 (US).

(74) Agent: **CUI, Steven X.**; GENENTECH, INC., 1 Dna Way, South San Francisco, CA 94080-4990, (US).

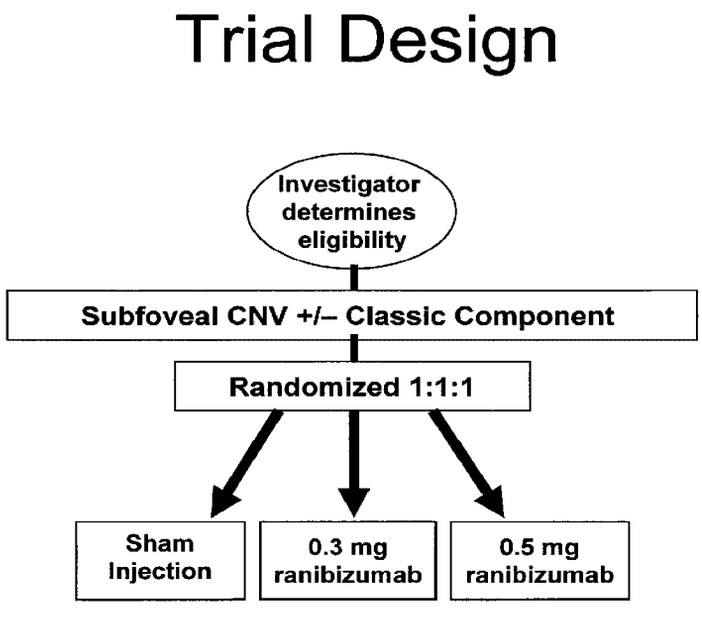
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK,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

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

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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

(54) **Title:** METHOD FOR TREATING INTRAOCULAR NEOVASCULAR DISEASES



(57) **Abstract:** A method is provided for administering to a mammal suffering from, or at risk for, an intraocular neovascular disorder with regular dosing of a therapeutically effective amount of VEGF antagonist, followed by less frequent dosing of a therapeutically effective amount of VEGF antagonist.

WO 2006/047325 A1

METHOD FOR TREATING INTRAOCULAR NEOVASCULAR DISEASES

FIELD OF THE INVENTION

This invention relates to methods for treating an intraocular neovascular disorder with a VEGF antagonist. Methods for administering to a mammal suffering from, or at risk for, an intraocular neovascular disorder include monthly dosing of a therapeutically effective amount of VEGF antagonist, followed by less frequent dosing of a therapeutically effective amount of VEGF antagonist.

BACKGROUND OF THE INVENTION

Angiogenesis is implicated in the pathogenesis of intraocular neovascular diseases, e.g., proliferative retinopathies, age-related macular degeneration (AMD), etc., as well as a variety of other disorders. These include solid tumors, rheumatoid arthritis, and psoriasis (Folkman *et al. J. Biol. Chem.* 267:10931-10934 (1992); Klagsbrun *et al. Annu. Rev. Physiol.* 53:217-239 (1991); and Garner A, *Vascular diseases. In: Pathobiology of ocular disease. A dynamic approach.* Garner A, Klintworth GK, Eds. 2nd Edition Marcel Dekker, NY, pp 1625-1710 (1994)).

The search for positive regulators of angiogenesis has yielded many candidates, including aFGF, bFGF, TGF-α, TGF-β HGF, TNF-α, angiogenin, IL-8, etc. (Folkman *et al.* and Klagsbrun *et al*). The negative regulators so far identified include thrombospondin (Good *et al. Proc. Natl. Acad. Sci. USA*. 87:6624-6628 (1990)), the 16-kilodalton N-terminal fragment of prolactin (Clapp *et al. Endocrinology*, 133:1292-1299 (1993)), angiostatin (O'Reilly *et al. Cell*, 79:315-328 (1994)) and endostatin (O'Reilly *et al. Cell*, 88:277-285 (1996)).

Work done over the last several years has established the key role of vascular endothelial growth factor (VEGF) in the regulation of normal and abnormal angiogenesis (Ferrara *et al. Endocr. Rev.* 18:4-25 (1997)). The finding that the loss of even a single VEGF allele results in embryonic lethality points to an irreplaceable role played by this factor in the development and differentiation of the vascular system (Ferrara *et al.*).

Human VEGF exists as at least six isoforms ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$) that arise from alternative splicing of mRNA of a single gene (Ferrara N, Davis Smyth T. *Endocr Rev* 18:1–22 (1997)). $VEGF_{165}$, the most abundant isoform, is a basic, heparin binding, dimeric glycoprotein with a molecular mass of ~45,000 daltons (*Id*). Two VEGF receptor tyrosine kinases, VEGFR1and VEGFR2, have been identified (Shibuya et al. *Oncogene* 5:519–24 (1990); Matthews et al., *Proc Natl Acad Sci U S A* 88:9026–30 (1991); Terman et al., *Oncogene* 6:1677–83 (1991); Terman et al. *Biochem Biophys Res Commun* 187:1579–86 (1992); de Vries et al., *Science* 255:989–91 (1992); Millauer et al. *Cell* 72:835–46 (1993); and, Quinn et al. *Proc Natl Acad Sci USA* 90:7533–7 (1993)). VEGFR1 has the highest affinity for VEGF, with a Kd of ~10–20 pM (de Vries et al., *Science* 255:989–91 (1992)), and VEGFR2 has a somewhat lower affinity for VEGF, with a Kd of ~75–125 pM (Terman et al., *Oncogene* 6:1677–83 (1991); Millauer et al. *Cell* 72:835–46 (1993); and, Quinn et al. *Proc Natl Acad Sci USA* 90:7533–7 (1993)).

VEGF has several biologic functions, including regulation of VEGF gene expression under hypoxic conditions (Ferrara N, Davis Smyth T. *Endocr Rev* 18:1–22 (1997)), mitogenic activity for micro and macrovascular endothelial cells (Ferrara N, Henzel WJ. *Biochem Biophys Res Commun* 161:851–8 (1989); Leung et al., *Science* 246:1306–9 (1989); Connolly et al. *J Clin Invest* 84:1470–8 (1989a); Keck et al. *Science* 246:1309–12 (1989); Plouet et al., *EMBO J* 8:3801–6 (1989); Conn et al. *Proc Natl Acad Sci USA* 87:2628–32 (1990); and, Pepper et al., *Exp Cell Res* 210:298–305 (1994)), and induction of expression of plasminogen activators and collagenase (Pepper et al., *Biochem Biophys Res Commun* 181:902–6 (1991)).

Furthermore, VEGF has been shown to be a key mediator of neovascularization associated with tumors and intraocular disorders (Ferrara *et al.*). The VEGF mRNA is overexpressed by the majority of human tumors examined. Berkman *et al. J Clin Invest* 91:153-159 (1993); Brown *et al. Human Pathol* 26:86-91 (1995); Brown *et al. Cancer Res* 53:4727-4735 (1993); Mattern *et al. Brit J Cancer.* 73:931-934 (1996); and Dvorak *et al. Am J Pathol* 146:1029-1039 (1995). Also, the concentration of VEGF in eye fluids are highly correlated to the presence of active proliferation of blood vessels in patients with diabetic and other ischemia-related retinopathies. Aiello *et al., N. Engl. J. Med.* 331:1480-1487 (1994). Furthermore, recent studies have demonstrated the localization of VEGF in choroidal neovascular membranes in patients affected by AMD. Lopez et al., *Invest. Ophtalmo. Vis. Sci.* 37:855-868 (1996); Kvanta et al., *Invest Ophthalmol Vis Sci* 37:1929–34 (1996).

Age related macular degeneration (AMD) is a leading cause of severe, irreversible vision loss among the elderly. Bressler, *JAMA* 291:1900-1 (2004). It is characterized by a broad spectrum of clinical and pathologic findings, such as pale yellow spots known as drusen, disruption of the retinal pigment epithelium (RPE), choroidal neovascularization (CNV), and disciform macular degeneration. The manifestations of the disease are classified into two forms: non exudative (dry) and exudative (wet or neovascular). Drusen are the characteristic lesions of the dry form, and neovascularization characterizes the wet form. Disciform AMD is the fibrotic stage of the neovascular lesion.

There is a dramatic increase in the prevalence of AMD with advancing age. *See, e.g.*, Leibowitz et al., *Surv Ophthalmol* 24(Suppl):335–610 (1980) and Klein et al., *Ophthalmology* 99:933–43 (1992). Although the wet form of AMD is much less common, it is responsible for 80%–90% of the severe visual loss associated with AMD (Ferris et al., *Arch Ophthamol* 102:1640–2 (1984)). There is an estimated 1–1.2 million prevalent cases of wet AMD. The cause of AMD is unknown; however, it is clear that the risk of developing AMD increases with advancing age. Other known risk factors include family history and cigarette smoking. Postulated risk factors also include oxidative stress, diabetes, alcohol intake, and sunlight exposure. D'Amico, *N Engl J Med* 331:95–106 (1994) and Christen et al., *JAMA* 276:1147–51 (1996).

Dry AMD is characterized by changes in the RPE and Bruch's membrane. It is thought that the RPE, compromised by age and other risk factors, deposits lipofuscin and cellular debris on Bruch's membrane. These changes may be seen ophthalmoscopically as drusen, which are scattered throughout the macula and posterior retinal pole. There are also variable degrees of atrophy and pigmentation of the RPE. Dry AMD may be asymptomatic or accompanied by variable and usually minimal visual loss and is considered to be a prelude to development of wet AMD.

Wet AMD is typically characterized by CNV of the macular region. The choroidal capillaries proliferate and penetrate Bruch's membrane to reach the RPE and may extend into the subretinal space. The increased permeability of the newly formed capillaries leads to accumulation of serous fluid or blood under the RPE and/or the neurosensory retina or within the neurosensory retina. When the fovea becomes swollen or detached, decreases in vision

occur. Fibrous metaplasia and organization may ensue, resulting in an elevated subretinal mass called a disciform scar that constitutes end-stage AMD and is associated with permanent vision loss (D'Amico DJ. *N Engl J Med* 331:95–106 (1994)).

The neovascularization in AMD can be classified into different patterns based on fluorescein angiography of subfoveal chorodial neovascular lesions. TAP and VIP Study Groups, *Arch Ophthalmol* 121:1253-68 (2003). The major angiographic patterns are termed classic and occult and are associated with different degrees of aggressiveness, vision losses, and response to different treatment options.

The diffusible nature of VEGF and its specificity of action for endothelial cells support a key role in the process of abnormal blood vessel growth and vascular leakage. Increased expression of VEGF in retinal photoreceptors or RPE of transgenic mice stimulates neovascularization within the retina, and VEGF antagonists partially inhibit retinal neovascularization in animal models (Okamoto et al. *Am J Pathol* 151:281–91 (1997); Schwesinger et al., *AM J Pathol.* Mar;158(3):1161-72 (2001)). Anti-VEGF neutralizing antibodies inhibit intraocular angiogenesis in models of ischemic retinal disorders (Adamis *et al. Arch. Ophthalmol.* 114:66-71 (1996)), and also suppress the growth of a variety of human tumor cell lines in nude mice (Kim *et al. Nature* 362:841-844 (1993); Warren *et al. J. Clin. Invest.* 95:1789-1797 (1995); Borgström *et al. Cancer Res.* 56:4032-4039 (1996); and Melnyk *et al. Cancer Res.* 56:921-924 (1996)). Therefore, anti-VEGF monoclonal antibodies or other VEGF antagonists are promising candidates for use in treatments of intraocular neovascular disorders, and new methods of administering therapeutic compounds, which increases the effectiveness of the therapeutic compound, are needed.

SUMMARY OF THE INVENTION

One object of the present invention is to provide an improved method of administering a therapeutic compound. This and other objects will become apparent from the following description.

Methods for treating intraocular neovascular disease are provided. For example, methods include administering to a mammal a number of first individual doses of a VEGF antagonist, followed by administering to the mammal a number of second individual doses of the

antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

In one embodiment of the invention, a method for treating wet form age-related macular degeneration is provided, which comprises administering to a mammal a number of first individual doses of an VEGF antagonist, followed by administering to the mammal a number of second individual doses of the antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

In one embodiment, the mammal is in need of treatment. Typically, the mammal is a human.

In one embodiment, the administration of the VEGF antagonist is ocular. In one aspect, the administration is intraocular. In another aspect, the administration is intravitreal.

A VEGF antagonist is administered in the methods of the invention. In one aspect, the VEGF antagonist is an anti-VEGF antibody, e.g., a full length anti-VEGF antibody or an antibody fragment. In one embodiment, the anti-VEGF antibody is a Fab antibody fragment. In one embodiment, the antibody fragment is Y0317.

In one embodiment of the invention, the first individual doses are administered at one month intervals (e.g., about 3 individual doses). Typically, there is more than one first individual dose. In another embodiment, the second individual doses are administered at three month intervals (e.g., about 6 individual doses). In one aspect of the invention, the second individual doses are administered beginning three months after the number of first individual doses. In one embodiment, a number of second individual doses are administered to the mammal during a period of at least 22 months following the number of first individual doses.

In one embodiment of the invention, the number of first individual doses and the number of second individual doses are administered over a time period of about 2 years. In one aspect, the first individual dose is administered at month 0, 1 and 2. In another aspect, the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23. For example, the first individual dose is administered at month 0, 1, and 2 and the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23. In one embodiment, the VEGF antagonist is administered over less than 2 years, or optionally, administered over greater than 2 years.

Other aspects of the invention will become apparent from the following description of the embodiments which are not intended to be limiting of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 schematically illustrates the study in Example 1.

Figure 2 schematically illustrates a dosing regimen for treating, e.g., age-related macular degeneration (AMD) with a VEGF antagonist.

DETAILED DESCRIPTION

Definitions

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

The term "human VEGF" as used herein refers to the 165-amino acid human vascular endothelial cell growth factor, and related 121-, 189-, and 206-, (and other isoforms) amino acid vascular endothelial cell growth factors, as described by Leung *et al.*, *Science* 246:1306 (1989), and Houck *et al.*, *Mol. Endocrin.* 5:1806 (1991) together with the naturally occurring allelic and processed forms of those growth factors.

A "VEGF antagonist" refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with VEGF activities including its binding to one or more VEGF receptors. VEGF antagonists include anti-VEGF antibodies and antigen-binding fragments thereof, receptor molecules and derivatives which bind specifically to VEGF thereby sequestering its binding to one or more receptors, anti-VEGF receptor antibodies and VEGF receptor antagonists such as small molecule inhibitors of the VEGFR tyrosine kinases, and fusions proteins, e.g., VEGF-Trap (Regeneron), $VEGF_{121}$-gelonin (Peregrine). VEGF antagonists also include antagonist variants of VEGF, antisense molecules directed to VEGF,

RNA aptamers specific to VEGF, and ribozymes against VEGF or VEGF receptors. Antagonists of VEGF act by interfering with the binding of VEGF to a cellular receptor, by incapacitating or killing cells which have been activated by VEGF, or by interfering with vascular endothelial cell activation after VEGF binding to a cellular receptor. All such points of intervention by a VEGF antagonist shall be considered equivalent for purposes of this invention. Preferred VEGF antagonists are anti-VEGF antagonistic antibodies capable of inhibiting one or more of the biological activities of VEGF, for example, its mitogenic, angiogenic or vascular permeability activity. Anti-VEGF antagonistic antibodies include, but not limited to, antibodies A4.6.1, rhuMab VEGF (bevacizumab), Y0317 (ranibizumab), G6, B20, 2C3, and others as described in, for example, WO98/45331, US2003/0190317, U.S. Patents 6,582,959 and 6,703,020; WO98/45332; WO 96/30046; WO94/10202; WO2005/044853; EP 0666868B1; and Popkov et al., *Journal of Immunological Methods* 288:149-164 (2004). More preferably, the anti-VEGF antagonistic antibody of the invention is ranibizumab, which is a humanized, affinity matured anti-human VEGF antibody Fab fragment having the light and heavy chain variable domain sequences of Y0317 as described in WO98/45331 and Chen et al *J Mol Biol* 293:865-881 (1999).

The antibody is appropriately from any source, including chicken and mammalian such as rodent, goat, primate, and human. Typically, the antibody is from the same species as the species to be treated, and more preferably the antibody is human or humanized and the host is human. While the antibody can be a polyclonal or monoclonal antibody, typically it is a monoclonal antibody, which can be prepared by conventional technology. The antibody is an IgG-1, -2, -3, or -4, IgE, IgA, IgM, IgD, or an intraclass chimera in which Fv or a CDR from one class is substituted into another class. The antibody may have an Fc domain capable of an effector function or may not be capable of binding complement or participating in ADCC.

The term "VEGF receptor" or "VEGFr" as used herein refers to a cellular receptor for VEGF, ordinarily a cell-surface receptor found on vascular endothelial cells, as well as variants thereof which retain the ability to bind hVEGF. One example of a VEGF receptor is the *fms*-like tyrosine kinase (*flt*), a transmembrane receptor in the tyrosine kinase family. DeVries *et al.*, *Science* 255:989 (1992); Shibuya *et al.*, *Oncogene* 5:519 (1990). The *flt* receptor comprises an extracellular domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity. The extracellular domain is involved in the binding of VEGF, whereas the intracellular domain is involved in signal transduction. Another example of a

VEGF receptor is the *flk-1* receptor (also referred to as KDR). Matthews *et al.*, *Proc. Nat. Acad. Sci.* 88:9026 (1991); Terman *et al.*, *Oncogene* 6:1677 (1991); Terman *et al.*, *Biochem. Biophys. Res. Commun.* 187:1579 (1992). Binding of VEGF to the *flt* receptor results in the formation of at least two high molecular weight complexes, having apparent molecular weight of 205,000 and 300,000 Daltons. The 300,000 Dalton complex is believed to be a dimer comprising two receptor molecules bound to a single molecule of VEGF.

The term "epitope A4.6.1" when used herein, unless indicated otherwise, refers to the region of human VEGF to which the A4.6.1 antibody disclosed in Kim *et al.*, *Growth Factors* 7:53 (1992) and Kim *et al. Nature* 362:841 (1993), binds.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Typically, the mammal is human.

The term "antibody" is used in the broadest sense and includes monoclonal antibodies (including full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments (see below) so long as they exhibit the desired biological activity.

Unless indicated otherwise, the expression "multivalent antibody" is used throughout this specification to denote an antibody comprising three or more antigen binding sites. The multivalent antibody is typically engineered to have the three or more antigen binding sites and is generally not a native sequence IgM or IgA antibody.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges.

Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light- chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework region (FR). The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), pages 647-669). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.*

196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteine(s) from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain

constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

"Antibody fragments" comprise only a portion of an intact antibody, generally including an antigen binding site of the intact antibody and thus retaining the ability to bind antigen. Examples of antibody fragments encompassed by the present definition include: (i) the Fab fragment, having VL, CL, VH and CH1 domains; (ii) the Fab' fragment, which is a Fab fragment having one or more cysteine residues at the C-terminus of the CH1 domain; (iii) the Fd fragment having VH and CH1 domains; (iv) the Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (v) the Fv fragment having the VL and VH domains of a single arm of an antibody; (vi) the dAb fragment (Ward et al., *Nature* 341, 544-546 (1989)) which consists of a VH domain; (vii) isolated CDR regions; (viii) F(ab')2 fragments, a bivalent fragment including two Fab' fragments linked by a disulphide bridge at the hinge region; (ix) single chain antibody molecules (e.g. single chain Fv; scFv) (Bird et al., *Science* 242:423-426 (1988); and Huston et al., *PNAS (USA)* 85:5879-5883 (1988)); (x) "diabodies" with two antigen binding sites, comprising a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (see, e.g., EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. *Protein Eng.* 8(10):1057 1062 (1995); and US Patent No. 5,641,870).

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the

monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al., Nature* 321:522-525 (1986); Reichmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al. *Nature Biotechnology* 14:309-314 (1996): Sheets et al. *PNAS (USA)* 95:6157-6162 (1998)); Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.,* 222:581 (1991)). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild et al., *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995). Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized *in vitro*). *See, e.g.,* Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147 (1):86-95 (1991); and US Pat No. 5,750,373.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at about position Cys226, or from about position Pro230, to the carboxyl-terminus of the Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain.

By "Fc region chain" herein is meant one of the two polypeptide chains of an Fc region.

The "CH2 domain" of a human IgG Fc region (also referred to as "Cg2" domain) usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec. Immunol.*22:161-206 (1985). The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain.

The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (i.e. from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced "protroberance" in one chain thereof and a corresponding introduced "cavity" in the other chain thereof; see US Patent No. 5,821,333, expressly incorporated herein by reference). Such variant CH3 domains may be used to make multispecific (e.g. bispecific) antibodies as herein described.

"Hinge region" is generally defined as stretching from about Glu216, or about Cys226, to about Pro230 of human IgG1 (Burton, *Molec. Immunol.*22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions. The hinge region herein may be a native sequence hinge region or a variant hinge region. The two polypeptide chains of a variant hinge region generally retain at least one cysteine residue per polypeptide chain, so that the two polypeptide chains of the variant hinge region can form a disulfide bond between the two chains. The preferred hinge region herein is a native sequence human hinge region, e.g. a native sequence human IgG1 hinge region.

A "functional Fc region" possesses at least one "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding

domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature.

A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will typically possess, e.g., at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% sequence identity therewith, or at least about 95% sequence or more identity therewith.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. *PNAS (USA)* 95:652-656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Typically, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with

PBMCs and NK cells being generally preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (reviewed in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel et al., *Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976); and Kim et al., *J. Immunol.* 24:249 (1994)).

"Complement dependent cytotoxicity" and "CDC" refer to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996), may be performed.

An "affinity matured" antibody is one with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et

al. *Proc Nat. Acad. Sci, USA* 91:3809-3813 (1994); Schier et al. *Gene* 169:147-155 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):3310-9 (1995); and Hawkins et al, *J. Mol. Biol.* 226:889-896 (1992).

A "flexible linker" herein refers to a peptide comprising two or more amino acid residues joined by peptide bond(s), and provides more rotational freedom for two polypeptides (such as two Fd regions) linked thereby. Such rotational freedom allows two or more antigen binding sites joined by the flexible linker to each access target antigen(s) more efficiently. Examples of suitable flexible linker peptide sequences include gly-ser, gly-ser-gly-ser, ala-ser, and gly-gly-gly-ser.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

The expression "linear antibodies" when used throughout this application refers to the antibodies described in Zapata *et al. Protein Eng.* 8(10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

A "variant" anti-VEGF antibody, refers herein to a molecule which differs in amino acid sequence from a "parent" anti-VEGF antibody amino acid sequence by virtue of addition,

deletion and/or substitution of one or more amino acid residue(s) in the parent antibody sequence. In the preferred embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable region(s) of the parent antibody. For example, the variant may comprise at least one, *e.g.* from about one to about ten, and preferably from about two to about five, substitutions in one or more hypervariable regions of the parent antibody. Ordinarily, the variant will have an amino acid sequence having at least 75% amino acid sequence identity with the parent antibody heavy or light chain variable domain sequences, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. 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 the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind human VEGF and preferably has properties which are superior to those of the parent antibody. For example, the variant may have a stronger binding affinity, enhanced ability to inhibit VEGF-induced proliferation of endothelial cells and/or increased ability to inhibit VEGF-induced angiogenesis *in vivo*. To analyze such properties, one should compare a Fab form of the variant to a Fab form of the parent antibody or a full length form of the variant to a full length form of the parent antibody, for example, since it has been found that the format of the anti-VEGF antibody impacts its activity in the biological activity assays disclosed, e.g., in WO98/45331 and US2003/0190317. In one embodiment, the variant antibody is one which displays at least about 10 fold, preferably at least about 20 fold, and most preferably at least about 50 fold, enhancement in biological activity when compared to the parent antibody.

The "parent" antibody herein is one which is encoded by an amino acid sequence used for the preparation of the variant. Preferably, the parent antibody has a human framework region and, if present, has human antibody constant region(s). For example, the parent antibody may be a humanized or human antibody.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In

preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The term "epitope tagged" when used herein refers to the anti-VEGF antibody fused to an "epitope tag." The epitope tag polypeptide has enough residues to provide an epitope against which an antibody thereagainst can be made, yet is short enough such that it does not interfere with activity of the VEGF antibody. The epitope tag preferably is sufficiently unique so that the antibody thereagainst does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues). Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al. Mol. Cell. Biol.* 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al., Mol. Cell. Biol.* 5(12):3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al., Protein Engineering* 3(6):547-553 (1990)). In certain embodiments, the epitope tag is a "salvage receptor binding epitope". As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

An "angiogenic factor or agent" is a growth factor which stimulates the development of blood vessels, e.g., promotes angiogenesis, endothelial cell growth, stability of blood vessels, and/or vasculogenesis, etc. For example, angiogenic factors, include, but are not limited to, e.g., VEGF and members of the VEGF family, PlGF, PDGF family, fibroblast growth factor family (FGFs), TIE ligands (Angiopoietins), ephrins, ANGPTL3, ANGPTL4, etc. It would also include factors that accelerate wound healing, such as growth hormone, insulin-like growth factor-I (IGF-I), VIGF, epidermal growth factor (EGF), CTGF and members of its family, and TGF-α and TGF-β. *See, e.g.,* Klagsbrun and D'Amore, *Annu. Rev. Physiol.*, 53:217-39

(1991); Streit and Detmar, *Oncogene*, 22:3172-3179 (2003); Ferrara & Alitalo, *Nature Medicine* 5(12):1359-1364 (1999); Tonini et al., *Oncogene*, 22:6549-6556 (2003) (e.g., Table 1 listing angiogenic factors); and, Sato *Int. J. Clin. Oncol.*, 8:200-206 (2003).

An "anti-angiogenesis agent" or "angiogenesis inhibitor" refers to a small molecular weight substance, a polynucleotide, a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent as defined above, e.g., antibodies to VEGF, antibodies to VEGF receptors, small molecules that block VEGF receptor signaling (e.g., PTK787/ZK2284, SU6668). Anti-angiogensis agents also include native angiogenesis inhibitors, e.g., angiostatin, endostatin, etc. *See, e.g.,* Klagsbrun and D'Amore, *Annu. Rev. Physiol.*, 53:217-39 (1991); Streit and Detmar, *Oncogene*, 22:3172-3179 (2003) (e.g., Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo, *Nature Medicine* 5(12):1359-1364 (1999); Tonini et al., *Oncogene*, 22:6549-6556 (2003) (e.g., Table 2 listing antiangiogenic factors); and, Sato *Int. J. Clin. Oncol.*, 8:200-206 (2003) (e.g., Table 1 lists Anti-angiogenic agents used in clinical trials).

The term "effective amount" or "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of age-related macular degeneration (AMD), the effective amount of the drug can reduce or prevent vision loss. For AMD therapy, efficacy in vivo can, for example, be measured by one or more of the following: assessing the mean change in the best corrected visual acuity (BCVA) from baseline to a desired time, assessing the proportion of subjects who lose fewer than 15 letters in visual acuity at a desired time compared with baseline, assessing the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired time compared with baseline, assessing the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at desired time, assessing the NEI Visual Functioning Questionnaire, assessing the size of CNV and amount of leakage of CNV at a desired time , as assessed by fluorescein angiography, etc.

A therapeutic dose is a dose which exhibits a therapeutic effect on the patient and a sub-therapeutic dose is a dose which does not exhibit a therapeutic effect on the patient treated.

An "intraocular neovascular disease" is a disease characterized by ocular neovascularization. Examples of intraocular neovascular diseases include, but are not limited to, e.g., proliferative retinopathies, choroidal neovascularization (CNV), age-related macular degeneration (AMD), diabetic and other ischemia-related retinopathies, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, retinal neovascularization, etc.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody. The label may itself be detectable by itself (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.* controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.* an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-VEGF antibodies) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

MODES OF THE INVENTION

It has been discovered that the treatment effects of a VEGF antagonist, e.g., Ranibizumab, are maintained for an extended period of time, such as more than one month. Treatment with the VEGF antagonist was also found to be well tolerated for up to 2 years. The present invention describes a treatment schedule comprising an initial interval of administration of a therapeutic compound, followed by a subsequent, less frequent interval of administration of the therapeutic compound. The methods of the present invention allow one to decrease subsequent doses of the therapeutic compound, while at the same time maintaining the therapeutic efficacy.

The therapeutic compound which is administered using the treatment schedule of the present invention is a VEGF antagonist, preferably an anti-VEGF antibody (e.g., Ranibizumab). VEGF is a secreted homodimeric protein that is a potent vascular endothelial cells mitogen (Ferrara N, Davis Smyth T. *Endocr Rev* 18:1–22 (1997). VEGF stimulates vascular endothelial cell growth, functions as a survival factor for newly formed vessels, and induces vascular permeability. VEGF expression is upregulated by hypoxia as well as by a number of other stimuli.

In methods of the invention, therapeutic effects of a VEGF antagonist are provided by administering to a mammal a number of first individual doses of an VEGF antagonist; followed by, administering to the mammal a number of second individual doses of the antagonist, where the second individual doses are administered less frequently than the first individual doses.

The term "therapeutic" in this context means that the compounds binds to the ligand, VEGF, and produce a change in the symptoms or conditions associated with the disease or condition which is being treated. It is sufficient that a therapeutic dose produce an incremental change in the symptoms or conditions associated with the disease; a cure or complete remission of symptoms is not required. One having ordinary skill in this art can easily determine whether a dose is therapeutic by establishing criteria for measuring changes in symptoms or conditions of the disease being treated and then monitoring changes in these criteria according to known methods. External physical conditions, histologic examination of affected tissues in patients or the presence or absence of specific cells or compounds, associated with a disease may provide objective criteria for evaluating therapeutic effect. In one example, methods of the invention may be used to treat AMD where therapeutic effect is assessed by changes in preventing vision loss. Other indicators of therapeutic effect will be readily apparent to one having ordinary skill in the art and may be used to establish efficacy of the dose. See also section entitled herein, "Efficacy of the Treatment."

The doses may be administered according to any time schedule which is appropriate for treatment of the disease or condition. For example, the dosages may be administered on a daily, weekly, biweekly or monthly basis in order to achieve the desired therapeutic effect and reduction in adverse effects. The dosages can be administered before, during or after the development of the disorder. The specific time schedule can be readily determined by a

physician having ordinary skill in administering the therapeutic compound by routine adjustments of the dosing schedule within the method of the present invention. The time of administration of the number of first individual and second individual doses as well as subsequent dosages is adjusted to minimize adverse effects while maintaining a maximum therapeutic effect. The occurrence of adverse effects can be monitored by routine patient interviews and adjusted to minimize the occurrence of side effects by adjusting the time of the dosing. Any dosing time is to be considered to be within the scope of the present invention so long as the number of first individual doses of the VEGF antagonist is administered followed by a number of second individual doses, which are less frequently administered. For example, doses may be administered on a monthly schedule followed by subsequent quarterly or more dose schedule. Maintenance doses are also contemplated by the invention.

In a further embodiment, the first individual dose may be repeated one or more times before the second individual dose is administered. The first dose may be administered, for example, one, two or three times, typically three times before the less frequent administration dose(s) is (are) administered. In one embodiment of the invention, the first individual doses are administered at one month intervals (e.g., about 3 individual doses). The second dose is administered less frequently, e.g., at three month intervals (e.g., about 6 individual doses). In one aspect of the invention, the second individual doses are administered beginning three months after the number of first individual doses.

In one embodiment of the invention, the number of first individual doses and the number of second individual doses are administered over a time period of about 2 years. Shorter and longer time periods of 2 years are also included in the invention. In one aspect, the first individual dose is administered at month 0, 1 and 2. In another aspect, the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23. In one example, the first individual dose is administered at month 0, 1, and 2 and the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23.

Another aspect of the invention is the treatment of an intraocular neovascular disease, e.g., wet form AMD, by administering to a mammal, preferably a human patient, a number of first individual doses of a compound, e.g., a VEGF antagonist, followed by administering a number of second individual doses of the compound, where the number of second individual doses are administered less frequently than the number of first individual doses. This aspect of the

invention is different than previous dosing methods for the treatment of such diseases which generally treat with regularly spaced, even doses of a therapeutic compound. For example, the Ranibizumab (rhuFab V2), which is an antihuman VEGF, affinity-matured Fab has been administered in equal monthly (about 28 days) doses of 0.3 mg or 0.5 mg. In contrast, the method of the invention provides a number of first individual doses which are typically evenly spaced follow by a number of second individual doses that are less frequently administered.

The patient receives an initial dose of the VEGF antagonist. Since the VEGF antagonist treatment effects are maintained for more than a month, the patient can receive less frequent doses of the therapeutic compound in subsequent doses. However, it is possible to give more frequent doses, within the scope of the invention, to patients who do not experience effects on first administration.

The dosage amount depends on the specific disease or condition which is treated and can be readily determined using known dosage adjustment techniques by a physician having ordinary skill in treatment of the disease or condition. The dosage amount will generally lie with an established therapeutic window for the therapeutic compound which will provide a therapeutic effect while minimizing additional morbidity and mortality. Typically, therapeutic compounds are administered in a dosage ranging from 0.001 mg to about 100 mg per dose, preferably 0.1-20 mg.

Also within the scope of the present invention are additional doses, which may be administered after the number of first individual doses and after the number of second individual doses. For example, an additional, third set of doses can be administered.

Typically, the therapeutic compound used in the methods of this invention is formulated by mixing it at ambient temperature at the appropriate pH, and at the desired degree of purity, with physiologically acceptable carriers, i.e., carriers that are non-toxic to recipients at the dosages and concentrations employed. The pH of the formulation depends mainly on the particular use and the concentration of antagonist, but preferably ranges anywhere from about 3 to about 8. Where the therapeutic compound is an anti-VEGF antibody (e.g., ranibizumab), a suitable embodiment is a formulation at about pH 5.5.

The therapeutic compound, e.g. an anti-VEGF antibody, for use herein is preferably sterile. Sterility can be readily accomplished by sterile filtration through (0.2 micron) membranes. Preferably, therapeutic peptides and proteins are stored as aqueous solutions, although lyophilized formulations for reconstitution are acceptable.

The therapeutic compound may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the time scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the therapeutic compound to be administered is governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat an intraocular neovascular disease.

The therapeutic compound for treatment of an intraocular neovascular disease is typically administered by ocular, intraocular, and/or intravitreal injection. Other methods administration by also be used, which includes but is not limited to, topical, parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. As described herein, the therapeutic compound for treatment of an intraocular neovascular syndrome may be formulated, dosed, and administered in a fashion consistent with good medical practice.

The efficacy of the treatment of the invention can be measured by various endpoints commonly used in evaluating intraocular neovascular diseases. For example, vision loss can be assessed. Vision loss can be evaluated by, but not limited to, e.g., measuring by the mean change in best correction visual acuity (BCVA) from baseline to a desired time point (e.g., where the BCVA is based on Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at a desired time point, measuring the NEI Visual Functioning Questionnaire, measuring the size of CNV and amount

of leakage of CNV at a desired time point, e.g., by fluorescein angiography, etc. Ocular assessments can be done, e.g., which include, but are not limited to, e.g., performing eye exam, measuring intraocular pressure, assessing visual acuity, measuring slitlamp pressure, assessing intraocular inflammation, etc.

Any compound which binds to VEGF or a VEGF receptor and reduces the severity of symptoms or conditions associated with an intraocular neovascular disease may be used in this embodiment of the invention. Preferred compounds are peptide or protein compounds, more preferably are compounds which are or which contain an antibody or fragment thereof or which are fusions to an antibody fragment such as an immunoadhesin. Particularly preferred compounds are anti-VEGF antibodies or compounds containing fragments thereof.

VEGF is expressed in a variety of cells in the normal human retina. Co-localization of VEGF mRNA and protein is observed in the ganglion cell, inner nuclear and outer plexiform layers, the walls of the blood vessels, and photoreceptors (Gerhardinger et al., *Am J Pathol* 152:1453–62 (1998)). Retinal pigment epithelium, Muller cells, pericytes, vascular endothelium, and ganglion cells all produce VEGF (Miller et al., *Diabetes Metab Rev* 13:37–50 (1997); and, Kim et al. *Invest Ophthalmol Vis Sci* 40:2115–21 (1999)).

Studies have documented the immunohistochemical localization of VEGF in surgically resected CNV membranes from AMD patients. Kvanta et al. (1996) demonstrated the presence of VEGF mRNA and protein in RPE cells and fibroblast like cells. *See* Kvanta et al., *Invest Ophthalmol Vis Sci* 37:1929–34 (1996). Lopez et al. (1996) noted that the RPE cells that were strongly immunoreactive for VEGF were present primarily in the highly vascularized regions of CNV membranes, whereas the RPE cells found in fibrotic regions of CNV membranes showed little VEGF reactivity. *See* Lopez et al., *Invest Ophthalmol Vis Sci* 37:855–68 (1996). Kliffen et al. (1997) also demonstrated increased VEGF expression in RPE cells and choroidal blood vessels in maculae from patients with wet AMD compared with controls. *See* Kliffen et al., *Br J Ophthalmol* 81:154–62 (1997).

An increase in VEGF expression has been noted in experimental models of CNV in rats and in non human primates (Husain et al., *Ophthalmology* 104:1242̄50 (1997); and, Yi et al. Vascular endothelial growth factor expression in choroidal neovascularization in rats. *Graefes Arch Clin Exp Ophthalmol* 235:313–9 (1997)). In addition, transgenic mice with increased VEGF

expression in photoreceptors (Okamoto et al. 1997, *supra*) or retinal pigment epithelium (Schwesinger et al., *AM J Pathol.* 158(3):1161-72 (2001)) developed neovacularization reminiscent of CNV seen in humans with neovascular AMD. This further supports the involvement of VEGF in ocular neovascularization.

Of particular relevance to wet AMD are the angiogenic properties of VEGF, which have been demonstrated in a variety of in vivo models, including the chick chorioallantoic membrane (Leung et al., *Science* 246:1306–9 (1989); and, Plouet J, Schilling J, Gospodarowicz D. *EMBO J* 8:3801–6 (1989)), rabbit cornea (Phillips et al., *In Vivo* 8:961–5 (1994)), and rabbit bone (Connolly et al. *J Clin Invest* 84:1470–8 (1989a)). VEGF also functions as a survival factor for newly formed endothelial cells (Dvorak HF. *N Engl J Med* 315:1650–9 (1986); and, Connolly et al. *J Biol Chem* 264:20017–24 (1989b)). Consistent with pro survival activity, VEGF induces expression of the anti apoptotic proteins Bcl 2 and A1 in human endothelial cells (Connolly et al. *J Biol Chem* 264:20017–24 (1989b)).

VEGF has been shown to induce vascular leakage in guinea pig skin (Connolly et al. *J Biol Chem* 264:20017–24 (1989b)). Dvorak (1986) and colleagues (1987) proposed that an increase in microvascular permeability is a crucial step in angiogenesis associated with tumors and wound healing. Dvorak HF. *N Engl J Med* 315:1650–9 (1986); and, Dvorak et al., *Lab Invest* 57:673–86 (1987). A major function of VEGF in the angiogenic process can be the induction of plasma protein leakage. This effect would result in the formation of an extravascular fibrin gel, which serves as a substrate for endothelial cells. This activity can have relevance for AMD, as it is well established that permeability of the CNV membranes results in transudation of serum components beneath and into the retina, leading to serous macular detachment, macular edema and vision loss.

Thus, VEGF antagonists are good therapeutic compounds for treating intraocular neovascular diseases.

Many therapeutic compounds are well known to exert a therapeutic effect by binding to a selective cell surface marker or receptor or ligand. These known therapeutic compounds, e.g., anti-angiogenesis agents, are apparent to one having ordinary skill in the art and may be used in the method of the present invention. Suitable therapeutic compounds include non-peptidic organic compounds, preferably having a molecular weight less than about 1,000 g/mol, more

preferably less than about 600 g/mol; peptide therapeutic compounds, generally containing 8 to about 200, preferably about 15 to about 150, more preferably about 20 to about 100 amino acid residues; and protein therapeutic compounds, generally having secondary, tertiary and possibly quaternary structure. Suitable peptides compounds can be prepared by known solid-phase synthesis or recombinant DNA technology which are well known in the art.

A particularly preferred method of selecting a peptide compound is through the use of phage display technology. Using known phage display methods, libraries of peptides or proteins are prepared in which one or more copies of individual peptides or proteins are displayed on the surface of a bacteriophage particle. DNA encoding the particular peptide or protein is within the phage particle. The surface-displayed peptides or proteins are available for interaction and binding to target molecules which are generally immobilized on a solid support such as a 96-well plate or chromatography column support material. Binding and/or interaction of the display peptide or protein with a target molecule under selected screening conditions allows one to select members of the library which bind or react with the target molecule under the selected conditions. For example, peptides which bind under particular pH or ionic conditions may be selected. Alternatively, a target cell population can be immobilized on a solid surface using known techniques and the peptide or protein phage library can be panned against the immobilized cells to select peptides or proteins which bind to cell surface receptors on the target cell population. Phage display techniques are disclosed, for example, in U.S. Pat. Nos. 5,750,373; 5,821,047; 5,780,279; 5,403,484; 5,223,407; 5,571,698; and others.

One category of polypeptide compounds, are compounds containing an antibody or a fragment thereof which immunologically recognize and bind to cell surface receptors or ligands. Methods of preparing antibodies are well known in the art and have been practiced for many years. Suitable antibodies may be prepared using conventional hybridoma technology or by recombinant DNA methods. Preferred antibodies are humanized forms of non-human antibodies. Alternatively, antibodies may be prepared from antibody phage libraries using methods described, for example, in U.S. Pat. Nos. 5,565,332; 5,837,242; 5,858,657; 5,871,907; 5,872,215; 5,733,743, and others. Suitable compounds include full-length antibodies as well as antibody fragments such as Fv, Fab, Fab' and F $(ab')_2$ fragments which can be prepared by reformatting the full length antibodies using known methods.

Additional preferred polypeptide therapeutic compounds are immunoadhesin molecules also known as hybrid immunoglobulins. These polypeptides are useful as cell adhesion molecules and ligands and also useful in therapeutic or diagnostic compositions and methods. An immunoadhesin typically contains an amino acid sequence of a ligand binding partner protein fused at its C-terminus to the N-terminus of an immunoglobulin constant region sequence. Immunoadhesins and methods of preparing the same are described in U.S. Pat. Nos. 5,428,130; 5,714,147; 4,428,130; 5,225,538; 5,116,964; 5,098,833; 5,336,603; 5,565,335; etc.

Pharmaceutical Compositions

Therapeutic compounds of the invention used in accordance with the present invention are prepared for storage by mixing a polypeptide(s) having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in

macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be 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 VEGF antagonist. 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 (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ 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. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

EXAMPLES

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Dosing Regiment

This study assesses the efficacy and safety of intravitreal injections of VEGF antagonist (e.g., ranibizumab) administered monthly for 3 doses followed by doses every 3 months compared with sham injections administered at the same schedule in subjects with primary or recurrent subfoveal choroidal neovascularization (CNV) with or without a classic CNV component secondary to AMD.

In this study, two treatment groups receive multiple intravitreal doses of VEGF antagonist from 0.3 mg to 0.5 mg for 24 months. See **Figure 1**. Each dose of VEGF antagonist is administered every month for 3 doses (Day 0, Month 1 and Month 2) followed by doses every 3 months (Months 5, 8, 11, 14, 17, 20, and 23) until study termination. See **Figure 2**. Subjects randomized to sham injections follow the same schedule as subjects receiving ranibizumab. During the 24 month study period, a total of 10 ranibizumab or 10 sham injections can be administered. Typically, the dosing does not occur earlier than 14 days after the previous treatment. If a dose is withheld or is missed, it may be optionally administered within 14 days following the previous treatment during the monthly injection period or within 45 days after the previous treatment during the 3-month dosing period. A maximum of 10 doses of study drug is administered during this study. Ranibizumab is administered in one eye only (study eye) during this study.

An example of a VEGF antagonist is ranibizumab (LUCENTIS™). Ranibizumab (rhuFab V2) is a humanized, affinity-matured anti-human VEGF Fab fragment. Ranibizumab is produced by standard recombinant technology methods in Escherichia coli expression vector and bacterial fermentation. Ranibizumab is not glycosylated and has a molecular mass of ~48,000 daltons. See WO98/45331 and US20030190317.

Ranibizumab Injection: For intravitreal administration, the study drug, ranibizumab, is supplied in a liquid-filled vial of ranibizumab. Each vial contains 0.7 mL of either 6 mg/mL (0.3 mg dose level) or 10 mg/mL (0.5-mg dose level) of ranibizumab aqueous solution (pH 5.5) with 10 mM of histidine, 100 mg/mL of trehalose, and 0.01% polysorbate 20. All study drug is stored at 2°C–8°C (36°F–46°F), and should not be frozen. Drug should be protected vials from direct sunlight.

Procedures are implemented to minimize the risk of potential adverse events associated with serial intraocular injections (e.g., endophthalmitis). Aseptic technique is observed for the injection tray assembly, anesthetic preparation and administration, and study drug preparation and administration.

Intravitreal injections are performed by the injecting physician(s) following the slitlamp examination. After thorough cleansings of the lid, lashes, and periorbital area with an antiseptic, local anesthesia and antimicrobials can administered prior to study drug injection.

A 30 gauge, ½-inch needle attached to a low volume (e.g., tuberculin) syringe containing 50 μL of study drug solution is inserted through the pre anesthetized conjunctiva and sclera, approximately 3.5–4.0 mm posterior to the limbus, avoiding the horizontal meridian and aiming toward the center of the globe. The injection volume should be delivered slowly. The needle is then be removed slowly to ensure that all drug solution is in the eye. Immediately following the intraocular injection, antimicrobial drops can be administered and the subject is instructed to self-administer antimicrobial drops four times daily for 3 days following each intraocular injection of ranibizumab. The scleral site for subsequent intravitreal injections should be rotated.

Sham Injection: The injecting physician(s) performs the same pre-injection cleansing and anesthetizing procedures (including subconjunctival injection of anesthesia) outlined above for subjects receiving ranibizumab. An empty syringe without a needle is used in the sham injection. The injecting physician(s) mimics an intraocular injection by making contact with the conjunctiva and applying pressure without the needle. Immediately following the sham injection, the injecting physician(s) performs the same post-injection procedures as those performed on subjects receiving ranibizumab.

Pre-Injection Procedures for All Subjects (Raninizumab or Sham Injection): The following procedures can be implemented to minimize the risk of potential adverse events associated with serial intravitreal injections (e.g., endophthalmitis). Aseptic technique is observed for injection tray assembly, anesthetic preparation, and study drug preparation and administration. The following procedures (except where noted) can be conducted by the physician performing the intravitreal injection of ranibizumab or sham injection. Subjects receive antimicrobials

(e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution) for self-administration four times daily for 3 days prior to treatment.

- The supplies are assembled and and a sterile field is prepared. Supplies can include 10% povidone iodine swabs, sterile surgical gloves, 4X4 sterile pads, pack of sterile cotton tipped applicators, eyelid speculum, sterile ophthalmic drape, 0.5% proparacaine hydrochloride, 5% povidone iodine ophthalmic solution, 1% lidocaine for injection, ophthalmic antimicrobial solution (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial), and injection supplies.
- 2 drops of 0.5% proparacaine hydrochloride are instilled into the study eye, followed by 2 drops of a broad spectrum antimicrobial solution (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial).
- The periocular skin and eyelid of the study eye are disinfected in preparation for injection. The eyelid, lashes, and periorbital skin are scrubbed with 10% povidone iodine swabs, starting with the eyelid and lashes and continuing with the surrounding periocular skin. The eyelid margins and lashes are swabbed, e.g., in a systematic fashion, from medial to temporal aspects.
- A sterile ophthalmic drape can be placed to isolate the field, and the speculum can be placed underneath the eyelid of the study eye.
- 2 drops of 5% povidone iodine ophthalmic solution are instilled in the study eye, making sure the drops cover the planned injection site on the conjunctiva.
- Wait 90 seconds.
- A sterile cotton-tipped applicator is saturated with 0.5% proparacaine hydrochloride drops and the swab is held against the planned intravitreal injection site for 10 seconds in preparation for the subconjunctival injection of 1% lidocaine hydrochloride ophthalmic solution for injection (without epinephrine).
- 1% lidocaine (without epinephrine) is injected subconjunctivally.
- A sterile 4X4 pad in a single wipe can be used to absorb excess liquid and to dry the periocular skin.
- The subject is instructed to direct gaze away from syringe prior to ranibizumab or sham injection.

Ranibizumab Preparation and Administration Instructions: The ranibizumab injection can be prepared as herein. Dose solutions are typically prepared immediately before dosing. Dose solutions are typically for single use only.

After preparing the study eye as outlined above, 0.2 mL ranibizumab dose solution is withdrawn through a 5-μm filter needle. The filter needle is removed and replaced with a 30-gauge, ½ inch Precision Glide® needle, and excess ranibizumab is expelled so that the syringe contains 0.05 mL ranibizumab solution. The syringe is inserted through an area 3.5–4.0 mm posterior to the limbus, avoiding the horizontal meridian and aiming toward the center of the globe. The injection volume should be delivered slowly. The needle is then removed slowly to ensure about all drug solution is in the eye. The scleral site for subsequent intravitreal injections should be rotated. Refer to next section for detailed post injection procedures.

The subject can be monitored with a finger count test for the study eye within, e.g., 15 minutes of the ranibizumab injection. A measurement of intraocular pressure of the study eye can be obtained, e.g., 60 minutes (±10 minutes) following the ranibizumab injection.

Post-Injection Procedures for All Subjects: Immediately following the ranibizumab or sham injection, 2 drops of antimicrobial drops (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial) are instilled in the study eye. The subject is instructed to self-administer antimicrobial drops (e.g., ofloxacin ophthalmic solution or trimethoprim polymyxin B ophthalmic solution, single-use vial) four times daily for 3 days following each injection (ranibizumab or sham).

Preparation and Administration of the Sham Injection: See above for detailed instructions for pre-injection procedures.

Subjects receiving sham injections do not receive an actual injection of study drug. The physician follows the procedures for cleansing and anesthetizing the study eye as outlined above. The subject should be instructed to direct his or her gaze away from the syringe prior to administration of the sham injection. The tuberculin syringe plunger is withdrawn to the 0.05 mL mark on the syringe, the hub of the syringe—without the needle—is then placed against the pre-anesthetized conjunctival surface. The syringe hub is pressed firmly against the globe and then the plunger is slowly depressed, mimicking the action of an intravitreal injection.

For subsequent sham injections, the procedure of rotating the location of the injection site, as is done with ranibizumab injections is followed. See above for detailed post-injection procedures.

The subject can be monitored using a finger count test within, e.g., 15 minutes of the sham injection. A measurement of intraocular pressure can be obtained, e.g., 60 minutes (±10 minutes) following the sham injection.

Safety is assessed by the incidence of ocular and non-ocular adverse events, including but not limited to, serious adverse events, ocular assessments, deaths, laboratory test results, vital signs, antibodies to Raninizumab, intraocular inflammation, visual acuity, intraocular pressure, slitlamp pressure, indirect ophthalmoscopy, fluorescein angiography, fundus photography, vitreous hemorrhage, sensory rhegmatogenous retinal break or detachment (including macular hole), subfoveal hemorrhage, local or systemic infection, intraocular surgery, etc. In one embodiment, if verteporfin PDT was given within the last 28 days, the ranibizumab/sham injection is withheld. Efficacy is assessed by changes in preventing vision loss, e.g., measured by the mean change in best correction visual acuity (BCVA) from baseline to 12 months or 24 months (where the BCVA is based on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), other means include but are not limited to measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at 12 months or 24 months compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at 12 months or 24 months compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at 12 months or 24 months, measuring the NEI Visual Functioning Questionnaire, measuring the size of CNV and amount of leakage of CNV at 12 months or 24 months, e.g., by fluorescein angiography.

The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

We claim:

1. A method for treating wet form age-related macular degeneration in a mammal, comprising the steps of:

a) administering to the mammal a number of first individual doses of an VEGF antagonist; and

b) administering to the mammal a number of second individual doses of the VEGF antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

2. The method of claim 1, wherein the mammal is a human.

3. The method of claim 1, wherein the administration is ocular.

4. The method of claim 3, wherein the administration is intraocular.

5. The method of claim 4, wherein the administration is intravitreal.

6. The method of claim 1, wherein the VEGF antagonist is an anti-VEGF antibody.

7. The method of claim 6, wherein the anti-VEGF antibody is a full length anti-VEGF antibody.

8. The method of claim 6, wherein the anti-VEGF antibody is an antibody fragment.

9. The method of claim 6, wherein the anti-VEGF antibody is a Fab antibody fragment.

10. The method of claim 8, wherein the antibody fragment is Y0317.

11. The method of claim 1, wherein the first individual doses are administered at one month intervals.

12. The method of claim 1, wherein the second individual doses are administered at three month intervals.

13. The method of claim 1, wherein the second individual doses are administered beginning three months after the number of first individual doses.

14. The method of claim 1, wherein the number of second individual doses are administered to the mammal during a period of at least 22 months following the number of first individual doses.

15. The method of claim 1, wherein the number of the first individual doses comprises about 3 individual doses.

16. The method of claim 1, wherein the number of the second individual doses comprises about 6 individual doses.

17. The method of claim 1, wherein the number of first individual doses and the number of second individual doses are administered over a time period of about 2 years.

18. The method of claim 1, wherein the first individual dose is administered at month 0, 1 and 2.

19. The method of claim 1, wherein the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23.

20. The method of claim 1, wherein the first individual dose is administered at month 0, 1, and 2 and the second individual dose is administered at month 5, 8, 11, 14, 17, 20 and 23.

21. A method for treating intraocular neovascular disease, comprising:
administering to a mammal a number of first individual doses of an VEGF antagonist; followed by,
administering to the mammal a number of second individual doses of the antagonist, wherein the second individual doses are administered less frequently than the first individual doses.

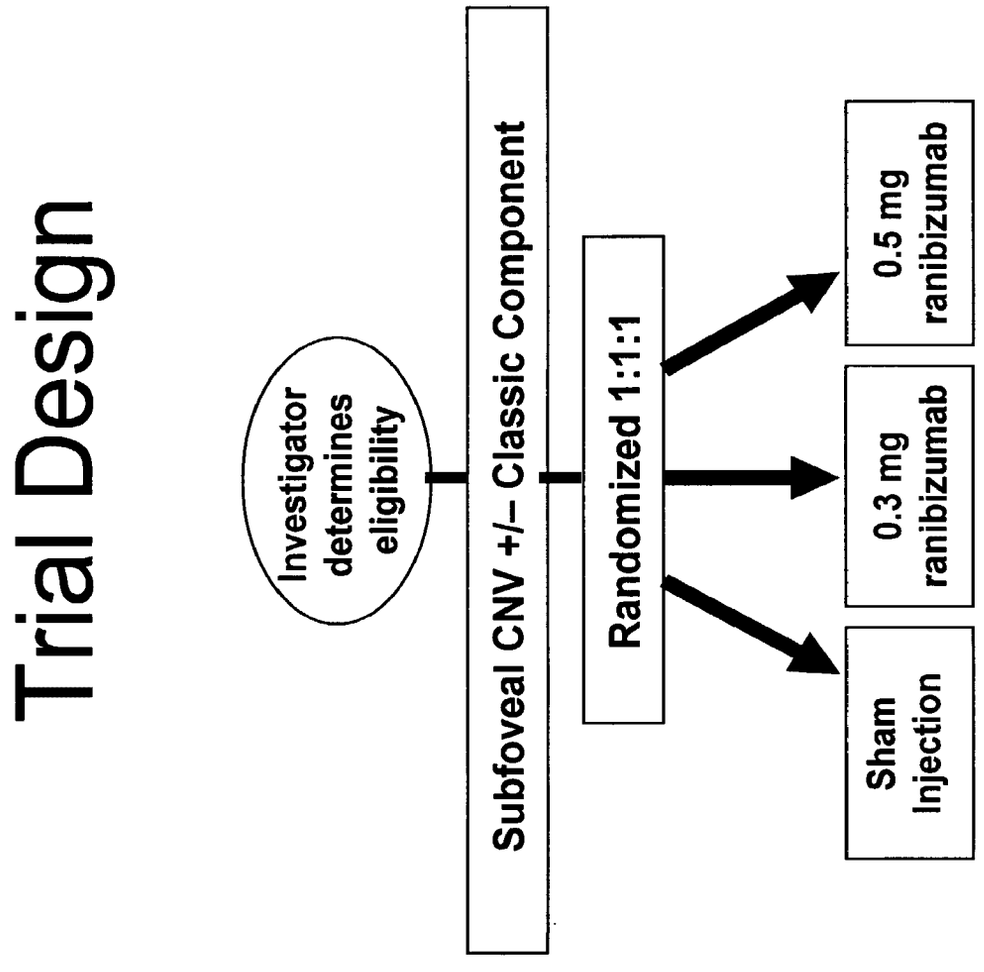


Figure 1

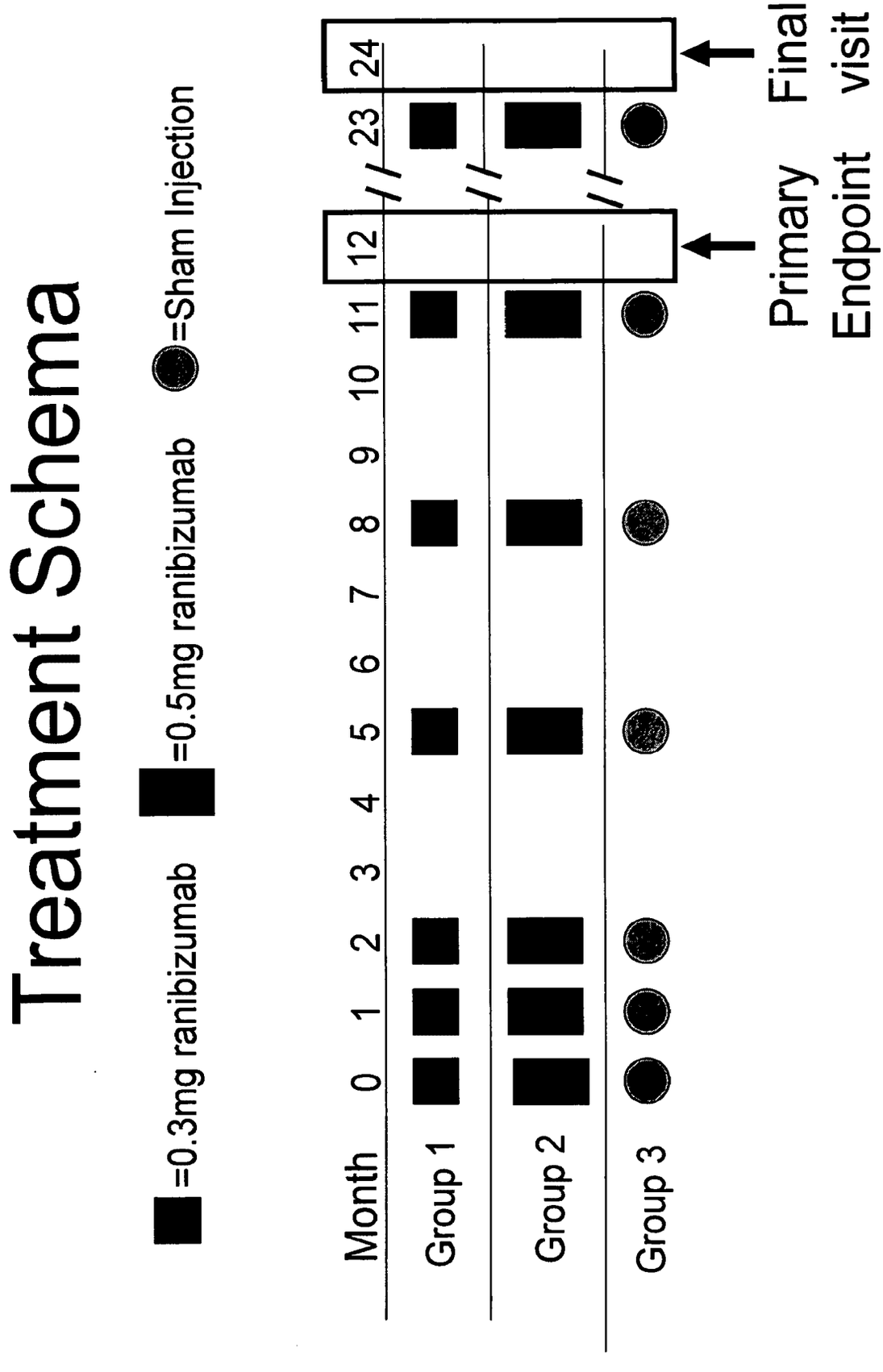

Treatment Schema
=0.3mg ranibizumab
=0.5mg ranibizumab
=Sham Injection
Month 0 1 2 3 4 5 6 7 8 9 10 11 12 23 24
Group 1
Group 2
Group 3
Primary Endpoint visit
Final visit

Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/038006

A. CLASSIFICATION OF SUBJECT MATTER
A61K39/00

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)
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, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KRZYSTOLIK MAGDALENA G ET AL: "Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment" ARCHIVES OF OPHTHALMOLOGY, vol. 120, no. 3, March 2002 (2002-03), pages 338-346, XP009061383 ISSN: 0003-9950 page 339; figures 4,5; tables 1,4 ----- -/--	1-21

[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
10 February 2006	17/03/2006

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	Trommsdorff, M

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/038006

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EARL R. NICOLS: "AAO:Ranibizumab(rhuFab) May Improve Vision in Age-Related Macular Degeneration" INTERNET ARTICLE, 'Online! 24 November 2003 (2003-11-24), XP002366839 Retrieved from the Internet: URL:http://www.pslgroup.com/dg/23f2aa.htm> 'retrieved on 2006-02-08! the whole document	1-21
Y	WO 98/45331 A (GENENTECH, INC; BACA, MANUEL; WELLS, JAMES, A; PRESTA, LEONARD, G; LOW) 15 October 1998 (1998-10-15) page 45 - page 46; table 14	1-21
Y	WO 96/30046 A (GENENTECH, INC) 3 October 1996 (1996-10-03) page 14 - page 15; claims 1-5	1-21
Y	THE EYETECH STUDY GROUP ET AL: "Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: Phase II study results." OPHTHALMOLOGY, vol. 110, no. 5, May 2003 (2003-05), pages 979-986, XP002366840 ISSN: 0161-6420 table 1A tables 1A,2,3	1,2,4, 11-15, 18,21
P,Y	ANONYMOUS: "EINE MULTIZENTRISCHE,RANDOMISIERTE,DOPPELT MASKIERTE,WIRKSTOFF-KONTROLIERTE PHASE III STUDIE ZUR WIRKSAMHEIT UND SICHERHEIT VON RHUFAB V2 (RHANIBIZUMAB) IM VERGLEICH MIT EINER PHOTODYNAMISCHEN VERTEPORFIN-THERAPIE (VISUDYNE) AN PATIENTEN MIT VORWIEGEND KLASSISCHER SUBFOVEALER NEOVASKULÄRER ..." INTERNET ARTICLE - ANCHOR-STUDIE, 'Online! XP002366841 Retrieved from the Internet: URL:http://www.medizin.uni-koeln.de/kliniken/augenklinik/angiolab/klinischestudien/anchor.shtml?print> 'retrieved on 2006-02-09! the whole document	1-21

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/038006

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAMPOCHIARO PETER ANTHONY ET AL: "Ocular neovascularization: A valuable model system." ONCOGENE, vol. 22, no. 42, 29 September 2003 (2003-09-29), pages 6537-6548, XP002366842 ISSN: 0950-9232 the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/038006

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 1-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

The present claims 1-5, 11-21 encompass compounds defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed, except for the compounds disclosed in the description which are also structurally defined, see p.6-7. Undue experimentation would be required to screen compounds randomly. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search for claims 1-5, 11-21.
The search of said claims was consequently restricted to antibodies against VEGF or structurally well defined other antagonists (such as e.g., aptamers disclosed in the art).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

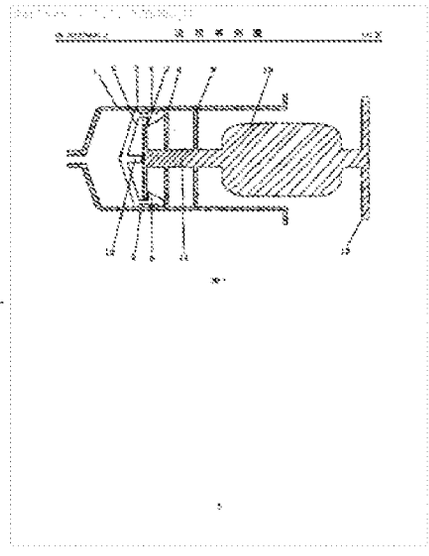
International application No
PCT/US2005/038006

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9845331 A	15-10-1998	AT 293640 T	15-05-2005
		AU 743758 B2	07-02-2002
		AU 7100798 A	30-10-1998
		BR 9809387 A	11-09-2001
		CA 2286330 A1	15-10-1998
		CN 1259962 A	12-07-2000
		DE 69829891 D1	25-05-2005
		DE 69829891 T2	06-10-2005
		DE 122005000026 I1	04-08-2005
		DE 122005000050 I1	29-12-2005
		EP 0973804 A2	26-01-2000
		ES 2236634 T3	16-07-2005
		JP 2001509817 T	24-07-2001
		NO 994869 A	06-12-1999
		NZ 500078 A	26-10-2001
		PT 1325932 T	30-06-2005
		TR 9903123 T2	22-05-2000
WO 9630046 A	03-10-1996	AT 311902 T	15-12-2005
		AT 285251 T	15-01-2005
		AU 696487 B2	10-09-1998
		AU 5378796 A	16-10-1996
		CA 2213833 A1	03-10-1996
		DE 69634079 D1	27-01-2005
		DE 69634079 T2	19-01-2006
		EP 0817648 A1	14-01-1998
		ES 2233967 T3	16-06-2005
		IL 117645 A	31-08-2005
		JP 11502853 T	09-03-1999
		NZ 305699 A	28-02-2000
		PT 817648 T	29-04-2005

PatBase - Express 29 April 2013

CN201578690U PatBase number: 4860510

Title: Syringe



Abstract:
Source: CN201578690U A syringe, which is a clinical syringe, consists of a cylinder, a piston and a push rod. The piston is made of medical soft plastic and comprises a front body, a seal ring, a trimming ring, a curved groove and a reverse hooking ring, wherein a retaining rib is arranged on the inner side of the front body, the seal ring and the trimming ring are smooth in surfaces and identical in size, and the reverse hooking ring has certain slope. The push rod consists of a front ring, a retaining ring, a fixing ring, a high ribbed bar, a low ribbed bar and a pull ring, wherein the diameter of the fixing ring is slightly smaller than that of an inner cavity of the cylinder, and the height of the high ribbed bar is smaller than the diameter of the fixing ring. The syringe without harmful substances such as sulfide is labor-saving when drawing, does not need to be coated with silicon oil, can not generate liquid medicine residues or leak liquid medicine, is labor-saving for assembly, can replace the conventional syringe and is applied to clinical operations.

Owner(s) / Assignee(s): JIANYOU WANG WANG JIANYOU

Inventor(s): JIANYOU WANG ; WANG JIANYOU

Published in: (family)

Country	Publication number	Publication date	Application number	Application date	
CHINA	CN201578690 U	Sep 15, 2010	CN2010201147380	Jan 21, 2010	PUBL. UTILITY MODEL APPLICATION

Priority: CN2010201147380 20100121

International class: A61M5/178 (Advanced/Invention)

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

(11) EP 2 371 406 A1

(12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 153(4) EPC

(43) Date of publication:
05.10.2011 Bulletin 2011/40

(21) Application number: **09830433.0**

(22) Date of filing: **03.12.2009**

(51) Int Cl.:
A61M 5/28 *(2006.01)*

(86) International application number:
PCT/JP2009/070285

(87) International publication number:
WO 2010/064667 (10.06.2010 Gazette 2010/23)

(84) Designated Contracting States:
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 SE SI SK SM TR

(30) Priority: **03.12.2008 JP 2008308978**
19.12.2008 JP 2008323575
09.01.2009 JP 2009003518

(71) Applicants:
- **Denki Kagaku Kogyo Kabushiki Kaisha**
  Tokyo 103-8338 (JP)
- **Taisei Kako Co., Ltd.**
  Kita-ku
  Osaka-shi
  Osaka 531-0072 (JP)

(72) Inventors:
- **HIOKI, Kazutoshi**
  Itoigawa-shi
  Niigata 949-0393 (JP)
- **NOHMI, Kenji**
  Itoigawa-shi
  Niigata 949-0393 (JP)
- **MIYATA, Yoshiaki**
  Tokyo 103-8338 (JP)
- **MATSUMOTO, Ippei**
  Ibaraki-shi
  Osaka 567-0054 (JP)
- **ASAHI, Norihiko**
  Ibaraki-shi
  Osaka 567-0054 (JP)

(74) Representative: **Gulde Hengelhaupt Ziebig & Schneider**
Patentanwälte - Rechtsanwälte
Wallstraße 58/59
10179 Berlin (DE)

(54) SYRINGE

(57) Provided is a syringe with which it is possible to hold with greater stability a liquid inside the barrel while maintaining the slidability and air-tightness between the barrel and the gasket without requiring fixation of silicone oil, and which is excellent in terms of accuracy of visual inspection. The syringe has a resin barrel, a gasket slidably inserted inside the barrel, a plunger attached to the gasket, and a silicone film obtained by applying silicone oil having a kinematic viscosity of 500 to 10,000 cSt over the inner peripheral surface of the barrel in an amount of 5 to 50 μg per 1 cm^2 of area.

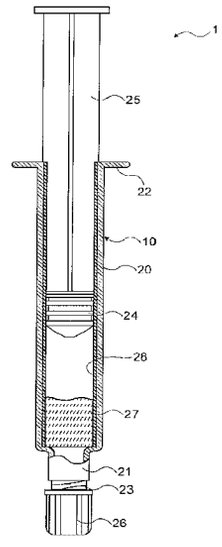


Fig. 1

Printed by Jouve, 75001 PARIS (FR)

EP 2 371 406 A1

Description

TECHNICAL FIELD

[0001] The present invention relates to a syringe, and more particularly relates to a syringe which is excellent in terms of accuracy of visual inspection of the content and a prefilled syringe filled with a high viscosity drug that are suitable for injection of high viscosity drugs.

BACKGROUND ART

[0002] In recent years, prefilled syringes prefilled with drugs have been used for reasons such as prevention of mistakes during medical treatment and prevention of bacterial contamination. A prefilled syringe has the tip opening of a barrel sealed with a cap member, is filled with a drug inside the barrel, has the rear end portion of the barrel sealed with a gasket, and is transported and stored in that state. When administering, an injection needle or an apparatus for administration is attached to the tip of the barrel, and by pushing a plunger attached to the gasket towards the tip and sliding the gasket inside the barrel, the drug flows out from the injection needle and is administered. As such, prefilled syringes have various advantages, such as allowing drugs to be administered in accurate doses without mistakes even during emergencies as there is no need to prepare the drugs at the point of treatment, being highly sanitary as there is no transferring of drugs, and being easy to operate.

[0003] Since prefilled syringes are stored and circulated in a state of being filled with a drug, it may be several years from the filling of the drug in production factories to administration. As such, while it goes without saying that long-term stability is needed, it is also necessary to be able to confirm the safety of the drug by visually inspecting for contamination by impurities. For that reason, the material constituting the barrel needs to be highly transparent, and barrels made of glass, which ensures transparency, have been frequently used in conventional prefilled syringes.

[0004] However, glass barrels crack relatively easily, need to be separated from the other parts and cannot be incinerated together therewith when discarded, and cost more, so there has been a demand for barrels made of resin. Resins with transparency comparable to that of glass barrels have appeared in recent years, and there has been a gradual transition towards resin barrels.

[0005] Regardless of the material of the barrel, to ensure sufficient slidability between the barrel and gasket, a lubricant layer composed of silicone or the like is generally provided on the inner peripheral surface of the barrel and/or the outer peripheral surface of the gasket.

[0006] In the case of conventionally used glass barrels, typically, silicone, in the form of an emulsion, is applied to the inner peripheral surface of the barrels and is fixed by baking at a high temperature (200 to 300°C). Silicone in itself is not harmful to the human body, but the silicone is fixed to the inner peripheral surface of the barrels to avoid the silicone contaminating the drugs.

[0007] In the case of resin barrels, since the glass transition point of resins is lower than the baking temperature of silicone, the same fixing treatment as for glass barrels cannot be used. In the case of resin barrels, methods in which a radiation or ultraviolet-curable organopolysiloxane is used and methods in which a photopolymerization catalyst such as benzophenone is added to silicone have been proposed as examples of methods for fixing silicone instead of baking at a high temperature (Patent Document 1).

[0008] On the other hand, as methods not involving such a fixing treatment, methods in which a silicone oil is simply applied to the inner peripheral surface of a barrel have also been widely used. In particular, in order to prevent the silicone oil from dripping from the inner peripheral surface of the barrel and contaminating the drug and to suppress increases in the sliding resistance of the gasket, the addition of a fine silica powder to a silicone oil has been proposed (Patent Document 2).

[0009] Additionally, in order to ensure sufficient slidability between the barrel and gasket, a prefilled syringe involving the use of a sealing stopper (gasket) for a syringe, which is a rubber stopper with its surface laminated with a tetrafluoroethylene resin film or an ultrahigh molecular weight polyethylene film, has also been proposed (Patent Document 3).

Patent Document 1: JP-A 2007-244606
Patent Document 2: JP-A 2006-94895
Patent Document 3: JP-A H10-314305

SUMMARY OF THE INVENTION

[0010] However, since methods for the lubrication treatment of resin barrels comprising fixation require a step of curing by radiation etc. as described in the above Patent Document 1, production efficiency is inevitably poor. Additionally, some curing agents etc. may affect the human body when contaminating a drug.

[0011] On the other hand, when the fixing treatment is not performed, naturally, there is a risk of the applied silicone oil separating from the inner peripheral surface of the barrel during filling of a drug, storage or transport and contaminating the drug, causing turbidity. This is, as described in the above Patent Document 2, not a problem that can be completely overcome even when, for example, the silicone oil contains a fine silica powder. Rather, in that case, there is a risk of not only the silicone oil, but also the fine silica powder contaminating the drug.

[0012] Such contamination by the silicone oil from the inner peripheral surface of the barrel is particularly notable when the viscosity of the drug is high. While the exact mechanism is unclear, this is thought to be due to the high shear stress exerted on the silicone oil adhering to the inner peripheral surface of the barrel when filling the syringe with a drug of high viscosity. As mentioned above, silicone oil is not necessarily harmful to the human body, but it is not possible to clearly distinguish between turbidity caused by contamination due to silicone oil and turbidity caused by substantial contamination due to impurities by visual inspection alone, so such syringes may be determined to be defective products during inspection or medical practice and be forced to be discarded without ever being used.

[0013] Further, even when the silicone oil adheres normally to the inner peripheral surface of the barrel, the refractive index of the applied silicone oil differs from the refractive index of the drug and the refractive index of the synthetic resin constituting the syringe, resulting in glare on the inner peripheral surface of the barrel, which may interfere with visual inspection or make it seem as if there has been contamination by impurities or a defect such as a scratch on the barrel.

[0014] Moreover, in the case of the sealing stopper (gasket) for a syringe described in Patent Document 3, since the surface of the rubber stopper is laminated with a resin film, the error in the inner diameter of the sealing stopper (gasket) for a syringe or the barrel could be increased due to the disparity of the actual dimensions with respect to the dimensions of the original design, and there tended to be problems in the slidability or sealing properties of the sealing stopper (gasket) for a syringe with respect to the inner surface of the barrel.

[0015] As such, there has been a need for syringes capable of reducing the risk of separation and contamination by silicone oil while not requiring fixation of the silicone oil, in which glare rarely occurs on the inner peripheral surface of the barrel, and equipped with sufficient gasket slidability and sealing properties.

[0016] The present invention was achieved in view of the above circumstances, with an object of providing a syringe excellent in inspection accuracy while ensuring slidability and sealing properties between the barrel and gasket, and in particular, a syringe that is also suitable for filling with a high viscosity drug.

[0017] As a result of diligent studies, the present inventors found that by spraying a silicone oil of a predetermined kinematic viscosity onto the inner peripheral surface of a resin barrel at a predetermined application amount per unit area, it is possible to suppress separation and contamination by the silicone oil and glare on the inner peripheral surface of the barrel in addition to providing sufficient slidability.

[0018] That is, the syringe of the present invention is characterized by having a resin barrel, a gasket slidably inserted in the barrel, a plunger attached to the gasket, and a silicone film formed by applying a silicone oil having a kinematic viscosity of 500 to 100,000 cSt to the inner peripheral surface of the above-described barrel in an amount of 5 to 50 μg per 1 cm^2 of area.

[0019] Since a silicone oil having a kinematic viscosity of at least 500 cSt is used as the silicone constituting the silicone film in this syringe, when spraying the silicone oil, the silicone oil is appropriately maintained on the inner peripheral surface of the barrel without running. For that reason, even when a small amount of silicone oil is applied, it is possible to ensure sufficient slidability with the gasket. Additionally, since a silicone oil having a kinematic viscosity of at most 100,000 cSt is used, it can be applied to the inner peripheral surface of the barrel by spraying, and the silicone oil can be evenly applied in the above predetermined application amount per unit area.

[0020] Further, by using a silicone oil having a kinematic viscosity within that range, it is possible to ensure sufficient slidability between the barrel and gasket even when the amount of the silicone oil applied is at most 50 μg per 1 cm^2 of area on the inner peripheral surface of the barrel, and the amount of the silicone oil applied can be suppressed to a low amount. As a result thereof, when filling with a drug, even if the silicone oil becomes mixed into the drug, the amount of contamination can be kept extremely low. As such, the occurrence of turbidity due to contamination by the silicone oil can be suppressed, the causes of turbidity in a drug in a prefilled syringe can be limited to cases of contamination by impurities other than silicone oil, and accuracy in visual inspection to ensure safety can be substantially improved. This is particularly applicable to cases where a high viscosity drug which is susceptible to contamination by silicone oil is loaded. Further, when the application amount is within this range, as long as observation is performed by the naked eye, there is also a low likelihood of glare being detected on the inner peripheral surface of the barrel. Moreover, when the amount of the silicone oil applied to the inner peripheral surface of the barrel is at least 5 μg per 1 cm^2 area, sufficient slidability between the barrel and the gasket can be ensured.

[0021] Since the viscosity of a silicone oil having a kinematic viscosity within the above range is high, it is generally not easy to evenly spray the oil. However, even spraying is possible by appropriately adjusting the liquid temperature, air pressure, nozzle diameter and application time etc. In particular, a fine mist can be sprayed to achieve an extremely thin film such as one within the above range by heating the silicone oil within such a range as not to cause denaturation at the time of spraying.

[0022] Moreover, by designing the maximum outer diameter of the gasket to be greater than the inner diameter of the barrel such that the difference between the maximum outer diameter of the gasket and the inner diameter of the barrel is at least 0.02 mm and at most 0.50 mm, it is possible to suppress drug leakage from the gap between the gasket and barrel while maintaining the sealing properties of the gasket and ensure sufficient slidability between the barrel and gasket.

[0023] Further, as a result of diligent studies, it was found that when, upon shining incident light with an optical axis orthogonally intersecting the central axis of the barrel and measuring the angle of refraction from the optical axis of the transmitted light scattered along the same direction as the central axis, glare on the inner peripheral surface of the barrel can be remarkably suppressed if the angle of refraction is within a predetermined range.

[0024] That is, it was found that the glare could be remarkably suppressed when, upon shining an incident beam with a wavelength of 635 nm to 690 nm and a beam width of at most 3.0 mm on a barrel filled with a drug at an optical axis orthogonally intersecting the central axis of the barrel, the angle of refraction from the optical axis of the transmitted light scattered in the same direction as the above-described central axis was within a range of 0.1 to 0.5°.

[0025] The "angle of refraction" in the present invention refers to the aperture angle from the optical axis of transmitted light scattered along the same direction as the central axis of the barrel of a prefilled syringe filled with a drug when shining an incident beam with an optical axis orthogonally intersecting the central axis of the barrel.
The barrel of a prefilled syringe will cause a transmitted beam in a direction perpendicular to the central axis to be highly refracted with the center of curvature as the central axis. Accordingly, refraction occurring in the direction perpendicular to the central axis is affected by solely the shape of the barrel, and cannot indicate small variations in the application state of the silicone oil on the inner peripheral surface of the barrel. On the other hand, as the barrel is not substantially curved in the direction of the central axis, the divergence from the optical axis occurring in the same direction as the central axis, i.e. the "angle of refraction" in the present invention, is not significantly affected by the shape of the barrel, and can directly reflect the state of application of the silicone oil.

[0026] It was found that when the angle of refraction of a prefilled syringed filled with a drug is within the range of 0.1 to 0.5°, as long as the observation is performed by the naked eye, there is an extremely low likelihood of glare being detected on the inner peripheral surface of the barrel. As such, a prefilled syringe having such an angle of refraction can remarkably improve the visual inspection accuracy of the drug.

[0027] According to the present invention, a drug can be more stably stored in the barrel and the accuracy of inspection of the content can be substantially improved while ensuring the sealing properties and the slidability between the barrel and gasket. This makes safe and accurate operation possible. As such, the syringe according the present invention has great utility as a medical apparatus and as a cosmetic apparatus.

BREIF DESCRIPTION OF THE DRAWINGS

[0028]

[Fig. 1] A schematic view of a prefilled syringe according to an embodiment of the present invention.
[Fig. 2] A schematic view showing an embodiment of a device for measuring an "angle of refraction" in the present invention.

Description of Reference Numbers

[0029]

1	Prefilled syringe
10	Syringe
20	Barrel
21	Tip opening
22	Flange
23	Screw thread portion
24	Gasket
25	Plunger
26	Cap member
27	Drug
28	Silicone film
31	Laser oscillator
32	Projection plate
33	Incident beam
34	Transmitted beam

40 Central axis
41 Optical axis
42 Projection image

MODES FOR CARRYING OUT THE INVENTION

[0030] Herebelow, preferred embodiments of the present invention shall be explained in detail with reference to the attached drawings. Fig. 1 is a schematic view of a prefilled syringe which is a preferred embodiment of the present invention.

[0031] Prefilled syringe 1 according to the present embodiment can basically adopt the constitution of a conventional prefilled syringe as is, and as shown in Fig. 1, is constituted by a syringe 10 comprising a barrel 20 with a tip opening 21 at the tip, a liquid-tight, air-tight and slidable gasket 24 in barrel 20, and a plunger 25 attached to the rear end of gasket 24; a cap member 26 for sealing tip opening 21 of barrel 20; and a drug 27 stored inside syringe 10. Moreover, a silicone film 28 formed by spraying a silicone oil is provided on the inner peripheral surface of barrel 20. In Fig. 1, for the sake of illustration, silicone 28 is shown as a film seemingly applied at a fixed thickness, but as long as the amount of silicone oil applied to the inner peripheral surface of barrel 20 is within the range of 5 to 50 μg per 1 cm^2 area, the desired effects can be sufficiently achieved, so it does not necessarily need to be even.

<Barrel>

[0032] Barrel 20, as shown in Fig. 1, is a cylindrical body provided with tip opening 21 at the tip for the attachment of an injection needle, and a pair of opposing flanges 22 at the rear end for the placement of fingers during drug injection.

[0033] Additionally, the below-described sealing member, cap member 26, is attached to tip opening 21 of barrel 20. Moreover, an injection needle (not shown) instead of cap member 26 may be directly attached. In the present embodiment, a screw thread portion 23 is provided on the outer peripheral surface of tip opening 21 for attaching cap member 26 or an injection needle.

[0034] Barrel 20 is formed with a transparent resin material in order to enable visual inspection of the filled drug 27. While there is no particular limitation to the material forming barrel 20, when considering optical transparency, strength and dimensional accuracy, various resins, for example, polystyrenes, polyamides, polycarbonates, polyvinyl chloride, polyvinylidene chloride, poly-(4-methylpentene-1), polyvinyl alcohols, acrylic resins, acrylonitrile-butadiene-styrene co-polymer, polyesters such as polyethylene terephthalate, cyclic polyolefins and cyclic olefin copolymers may be mentioned.

[0035] In the interest of visual inspection efficiency and accuracy of the content, cyclic olefin polymers (COP) and cyclic olefin copolymers (COC) which have excellent transparency are particularly preferred. As such resins, thermoplastic saturated norbornene resin compositions commercially available under Zeonex (trademark) from the (Japan) Zeon Corporation, particularly those dispersed with a compounding agent such as a gum polymer that is immiscible with the thermoplastic saturated norbornene resin, are preferred. In particular, those having the following properties are most preferred.
Optical transparency: 92%
Refractive index: 1.53

<Gasket>

[0036] While there is no particular limitation to the material of gasket 24, in order to maintain air-tightness, it is preferably formed by an elastic body such as rubber or a thermoplastic elastomer. Among them, butyl rubber, which changes little in dimensions upon autoclave sterilization, is particularly preferred as the main ingredient. As the butyl rubber, a halogenated butyl halide that has been chlorinated or brominated in order to improve crosslinkability and adhesiveness etc. may be used. As long as the material is permitted to be used as a medical apparatus or has been conventionally used as a material for forming the gasket of a syringe, there is no particular limitation. Additionally while there is no particular limitation on the surface material of the gasket, from the aspect of cost reduction, for example, materials not surface-treated with a tetrafluoroetilylene resin film or ultra high molecular weight polyethylene film are preferred. Moreover, in order to further reduce the possibility of the gasket being stuck, a silicone oil may be applied to the surface of the gasket.

[0037] Gasket 24 preferably has a plurality of ridge portions (ring-shaped convex portions) as shown in Fig. 1. By having such a plurality of ridge portions and valley portions (ring-shaped concave portions) provided in between, the sliding area between gasket 24 and barrel 20 can be reduced, and therefore the sliding resistance between gasket 24 and barrel 20 can be reduced. Additionally, by having such a plurality of ridge portions and valley potions provided in between, drug 27 can be blocked at multiple stages, suppressing leakage of drug 27 from the gap between gasket 24 and barrel 20.

[0038] Moreover, the maximum outer diameter of gasket 24 preferably corresponds to the outer diameter of the first

ridge portion closest to the tip among the plurality of ridge portions. This is because the first ridge portion closest to the tip among the plurality of ridge portions of gasket 24 is in fact directly in contact with drug 27, so by maximizing the outer diameter of this ridge portion, leakage of drug 27 from the gap between gasket 24 and barrel 20 can be effectively suppressed.

<Dimensional difference between barrel and gasket>

[0039] In syringe 10 of the present embodiment, the maximum outer diameter of gasket 24 needs to be greater than the inner diameter of barrel 20. By making the maximum outer diameter of gasket 24 greater than the inner diameter of barrel 20, leakage of drug 27 from the gap between gasket 24 and barrel 20 can be suppressed, and the sealing properties of gasket 24 can be maintained.

[0040] Additionally, in syringe 10 of the present embodiment, the difference between the maximum outer diameter of gasket 24 and the inner diameter of barrel 20 needs to be at least 0.02 mm and at most 0.50 mm. This is because by making the difference between the maximum outer diameter of gasket 24 and the inner diameter of barrel 20 at least 0.02 mm and at most 0.50 mm, leakage of drug 27 from the gap between gasket 24 and barrel 20 can be suppressed while maintaining the sealing properties of gasket 24, and sufficient slidability between barrel 20 and gasket 24 can be ensured.

[0041] Moreover, the difference between the maximum outer diameter of gasket 24 and the inner diameter of barrel 20 is preferably at least 0.10 mm, and more preferably at least 0.15 mm. This is because the greater this difference is, the easier it is to suppress drug 27 from leaking from the gap between gasket 24 and barrel 20. On the other hand, the difference between the maximum outer diameter of gasket 24 and the inner diameter of barrel 20 is preferably at most 0.40 mm and more preferably at most 0.35 mm. This is because the smaller this difference is, the better is the slidabillty between the barrel and gasket.

[0042] The tolerance (variability in dimensional accuracy of the actual product with respect to the designed dimensions) of the maximum outer diameter of gasket 24 after autoclave sterilization is preferably controlled be at most $\pm$ 0.10 mm, and is more preferably controlled to be at most $\pm$ 0.05 mm. This is because when the variability in dimensional accuracy of gasket 24 is within this range, it is stabilized by the entire syringe 10, and sufficient slidability and sealing properties of the gasket can be ensured.

[0043] On the other hand, the tolerance (variability in dimensional accuracy of the actual product with respect to the designed dimensions) of the inner diameter of barrel 20 is preferably controlled to be at most $\pm$ 0.10 mm, and is more preferably controlled to be at most $\pm$ 0.05 mm. This is because when the variability in dimension accuracy of barrel 20 is within this range, it is stabilized by almost the entire syringe 10, and sufficient slidability and sealing properties of the gasket can be ensured.

[0044] If gasket 24 is a structure in which a tetrafluoroethylene resin film or ultra high molecular weight polyethylene film is laminated on the surface of a rubber stopper, keeping the difference between the maximum outer diameter of gasket 24 and the inner diameter of barrel 20 within these ranges might be difficult. This is because when making a gasket 24 with such a complex laminated structure, the production process becomes complicated, and as a consequence thereof, there is a tendency for the disparity in the actual dimensions of gasket 24 with respect to the dimensions of the original design to be greater. For that reason, even if the inspection process of the dimensional accuracy of gasket 24 were applied strictly, the proportion of gaskets 24 outside the predetermined dimensional accuracy would be too great, stalling and lowering the production of gasket 24, the production costs would soar significantly, and too much a burden would be placed on the inspection process, so the actual construction of a production line could be difficult.

[0045] As such, in order to control such a highly accurate maximum outer diameter of gasket 24, in addition to improving the dimensional accuracy in the production process for both gasket 24 and barrel 20 or strictly applying the inspection process for dimensional accuracy, gasket 24 is preferably one that is not surface-treated with a resin film. This is because the structure of the gasket itself can be designed into a simple shape, and the production process of the gasket itself can be simplified by doing so. That is, in syringe 10 of the present embodiment, the highly accurate maximum outer diameter of gasket 24 is preferably controlled by improving the dimensional accuracy in the production process for both gasket 24 and barrel 20 or strictly applying the inspection process for dimensional accuracy in addition to using gasket 24 that is not surface-treated with a resin film.

<Plunger>

[0046] Additionally plunger 25 only needs to be equipped with a strength that can withstand the bending and pressing force required to make gasket 24 slide inside barrel 20, and may be made of, for example, a hard plastic material such as polyethylene or polypropylene, but as long as the material is permitted to be used as a medical apparatus or has been conventionally used as a material for forming the gasket of a syringe, there is no particular limitation.

<Cap member>

[0047] Cap member 26 tightly adheres to tip opening 21 of barrel 20, air-tight seals tip opening 21, and may be made using an elastic body or hard resin such as butyl rubber, high-density polyethylene, polypropylene, polystyrene, or polystyrene terephthalate, but as long as the material is permitted to be used as a medical apparatus or has been conventionally used as a material for forming the gasket of a syringe, there is no particular limitation. In the present embodiment, a female thread portion for threading thread portion 23 formed on the outer peripheral surface of tip opening 21 of barrel 20 is formed on the inner peripheral surface of cap member 26.

<Silicone film>

[0048] Silicone film 28 formed by spraying a silicone oil having a predetermined kinematic viscosity as described below is provided on the inner peripheral surface of barrel 20. Since the silicone oil applied to barrel 20 only needs to satisfy the predetermined application amount per unit area, the thickness of silicone film 28 does not necessarily need to be even across the entirety of barrel 20.

(Silicone oil)

[0049] While the silicone oil forming silicone film 28 applied to the inner peripheral surface of the barrel is basically polydimethylsiloxane, a polydimethylsiloxane with a side chain or terminal substitution within a range not impairing lubricity may be used. Specifically for example, polymethylphenylsiloxane and polymethylhydrogen siloxane may be mentioned. Various additives may be added to the silicone oil as necessary.

[0050] The above-described silicone oil preferably has a kinematic viscosity of 500 to 100,000 cSt at 25 °C, and in particular, one having a kinematic viscosity of 1,000 to 30,000 cSt is more preferably used. When the kinematic viscosity is at least 500 cSt, the silicone oil is appropriately maintained at the spraying site on the inner peripheral surface of barrel 20 without running from the inner peripheral surface of barrel 20, so the slidability between barrel 20 and gasket 24 can be sufficiently ensured with a small amount of application. Moreover, when the kinematic viscosity is at most 100,000 cSt, application to the inner peripheral surface of barrel 20 by spraying is possible.

(Thickness of silicone film)

[0051] The application amount of the silicone oil constituting silicone film 28 is preferably 5 to 50 μg, and particularly preferably 10 to 30μg, per 1 cm^2 of the inner peripheral surface of barrel 20.

If the application amount of the silicone oil is at least 5 μg per 1 cm^2 of the inner peripheral surface of the barrel, a sufficient slidability between barrel 20 and gasket 24 can be ensured. Moreover, if the application amount is at most 50 μg per 1 cm^2 of the inner peripheral surface of the barrel, even if the silicone oil is mixed into the drug when loading drug 27, the amount of contamination can be kept extremely small. Further, as long as observation is performed by the naked eye, glare will not be detected on the inner peripheral surface of barrel 20.

(Method for forming silicone film)

[0052] Silicone film 28 is formed by evenly spraying a silicone oil having the above-described kinematic viscosity on the inner peripheral surface of barrel 20 using a spray system compatible with high viscosity solutions. Since the silicone oil applied in the present invention has a high kinematic viscosity, liquid temperature, air pressure, nozzle diameter and application etc. need to be appropriately adjusted in order to be able to evenly spray the silicone oil on the inner peripheral surface of barrel 20.

Particularly, in the case of the above silicone oil of a high kinematic viscosity heating the silicone oil when spraying in particular makes the silicone oil easier to spray.

(Silicone oil applied to surface of gasket)

[0053] When spraying a silicone oil on the gasket, similar to applying a silicone oil to the inner peripheral surface of the barrel, a silicone oil having a kinematic viscosity of 500 to 100,000 cSt at 25 °C is preferably used, and in particular, one with a kinematic viscosity of 1,000 to 50,000 cSt is more preferably used. When the kinematic viscosity is at least 500 cSt, the applied silicone oil does not run and the lubricating action is maintained for a long period of time. Moreover, when the kinematic viscosity is at most 100,000 cSt, even application over the entire surface of the gasket is possible. As the method for application, a conventionally used method can be used, for example, a method in which the silicone oil is directly added to a tank containing the gasket and mixed or a method in which the gasket is mixed in water suspended

with the silicone oil may be used.

(Amount of silicone oil applied to surface of gasket per unit area)

[0054] Since the application amount should be kept at the required minimum so as to suppress intermixture of the silicone oil into the drug even when applying the silicone oil to the gasket, the application amount of the silicone oil is preferably at most 0.3 mg and is more preferably at most 0.15 mg per 1 cm^2 of the surface area of the gasket

<Drug>

[0055] While there is no particular limitation to drug 27 as long as it can be loaded into a prefilled type syringe, syringe 10 of the above constitution is particularly suitable for loading a high viscosity drug. Usually, when a high viscosity drug is loaded, a high shear force is exerted on the inner peripheral surface of the barrel, so the silicone oil applied to the inner peripheral surface of the barrel is easily mixed into the drug, and as a result thereof, turbidity occurs easily. However, if a silicone oil of the above predetermined viscosity is applied to the inner peripheral surface of the barrel at the above predetermined application amount per unit area, the amount of contamination by the silicone oil can be kept extremely small. For that reason, syringe 10 of the above constitution can be considered to be particularly suitable for high viscosity drugs in which turbidity occurs easily.

[0056] Additionally, since the maximum value of the extrusion pressure during sliding is higher when using a high viscosity drug 27 as compared to cases where a low viscosity drug 27 is used, the tolerances of barrel 20 and gasket 24 must be made higher in order to suppress the maximum value of the extrusion pressure during sliding and to improve the operability of syringe 10. For that reason, syringe 10 of the above constitution can be considered to be particularly suitable for high viscosity drugs that tend to lead to higher maximum values of the extrusion pressure during sliding. Consequently, for example, syringe 10 of the above constitution allows stable storage of a high viscosity drug, even with a viscosity of approximately 60,000 mPa·s, and as such a drug, an aqueous solution of 1% high molecular weight sodium hyaluronate with a weight average molecular weight of 600,000 to 3,700,000 may be mentioned in particular.

<Angle of refraction measuring device>

[0057] Fig. 2 is a schematic view showing an embodiment of a device for measuring an angle of refraction. This angle of refraction measuring device uses a laser oscillator 31 for shining a light beam (incident beam 33) on a prefilled syringe 1 and a projection plate 32 for projection of a light beam (transmitted beam 34) leaving prefilled syringe 1.

[0058] Laser oscillator 31 is a device for shining incident beam 33 of an optical axis 41 orthogonally intersecting the central axis 40 of barrel 20 onto prefilled syringe 1 filled with a drug. The wavelength of the oscillating laser is not particularly limited, and while a visible laser of any of red, green, blue and purple etc. may be used, the value of the angle of refraction changes with the wavelength, so the measurement needs to be carried out at a predetermined wavelength. As such, one within a wavelength range of 635 to 690 nm, which is that of common red lasers, is preferably used.

Projection plate 32 is not particularly limited as long as it is an opaque flat plate without any distortion on the surface. Projection plate 32 is arranged such that it is perpendicular to optical axis 41 of the light beam shone from laser oscillator 31.

<Method for measuring angle of refraction>

[0059] To measure the angle of refraction using the above device, the position of laser oscillator 31 is first fixed, then projection plate 32 is fixed such that it is perpendicular to the optical axis 41 of the light beam shone from laser oscillator 31. In this state, i.e. a state in which the object of measurement, prefilled syringe 1, is not positioned, the light beam shone from laser oscillator 31 is projected onto projection plate 32. When using a laser oscillator wherein the shape of a projection image 42 is more or less round, the diameter of the projection image 42 shall be considered to be the beam width "A" of incident beam 33. When using one that makes the shape of projection image 42 more or less oval, the direction of the laser oscillator is adjusted such that the direction of the short axis of the oval matches with the direction of the central axis of the barrel. In that case, the length of the short axis of the oval shall be considered to be the beam width "A" of incident beam 33. Additionally, laser oscillators making projection image 42 a shape other than round or oval are not suitable for measuring the angle of refraction in the present invention. Since it is more difficult to detect the difference in angle of refraction when the beam width "A" of incident beam 33 is large, it is preferably at most 3.0 mm and more preferably at most 2.0 mm.

[0060] Next, the object of measurement, prefilled syringe 1, is placed at a predetermined position on optical axis 41. At that time, the position of prefilled syringe 1 is adjusted such that the central axis 40 of prefilled syringe 1 orthogonally intersects optical axis 41.

In a state in which prefilled syringe 1 is arranged in the above manner, laser oscillator 31 shines a light beam (incident beam 33) on prefilled syringe 1, and transmitted beam 34 leaving prefilled syringe 1 is projected onto projection plate 32. The width "D" in the same direction as central axis 40 of projection image 42 projected on projection plate 32 and the distance "L" from central axis 40 of prefilled syringe 1 to projection plate 32 are measured.

[0061] The angle of refraction is an aperture angle "θ" from optical axis 41 of transmitted beam 34 scattered in the same direction as central axis 40 when shining incident beam 33 of optical axis 41 orthogonally intersecting central axis 40 of barrel 20 onto prefilled syringe 1 filled with a drug. Consequently, the angle of refraction can be obtained by the following formula using beam width "A" of incident beam 33 shone from laser oscillator 31, distance "L" from central axis 40 of prefilled syringe 1 to projection plate 32 and width "D" in the same direction as central axis 40 of projection image 42 of transmitted beam 34 projected on projection plate 32.

$$\text{Angle of Refraction } \theta = \tan^{-1}((D-A)/2L)$$

[0062] While embodiments of the present invention have been described with reference to the drawings above, they only serve to illustrate the present invention, and various constitutions other than the above may be adopted.
For example, in the above embodiments, the entire barrel 20 took the form of a single compartment filled with a drug, but the inside of barrel 20 may be separated into multiple compartments using at least one sealing stopper to achieve the form of a multi-compartment syringe. In that case, drug contamination and leakage can be more certainly prevented, and multiple drugs can be loaded into a single syringe.

Examples

[0063] Herebelow, the present invention shall be further explained using examples, but the present invention is not limited thereto.

<Example 1>

[0064] On the inner peripheral surface of a 5 ml volume barrel that was formed with a COP resin as the main ingredient, had a cylindrical outer diameter of 15.05 mm, a cylindrical inner diameter of 12.45 mm and a full length of 79.0 mm, a silicone oil of a kinematic viscosity of 5,000 cSt ("KF-96-5000cs" manufactured by Shin-Etsu Chemical Co., Ltd.) was sprayed under the following conditions such that the average application amount was 18 μg within a range of 12 to 25 μg per 1 cm^2. A thermoplastic saturated norbornene resin composition commercially available as Zeonex (trademark) from the (Japan) Zeon Corporation was used as the COP resin.
(Silicone oil spraying conditions)
Spraying time: 0.05 second
Air pressure: 0.5 MPa
Silicone oil heating temperature: 180 °C
Nozzle diameter: 1.0 mm

<Change in light transmittance due to formation of a silicone film>

[0065] Other than not spraying the silicone oil, a barrel similar to that of Example 1 (Comparative Example 1) was prepared, and compared with the barrel of the above Example 1 for light transmittance. The following device and method were used to measure light transmittance.

(Device)

[0066]

- Spectrophotometer (manufactured by Hitachi High-Technologies Corporation; Model No.: U-3310)
- Wavelength: 660 nm

(Method)

[0067]

- align the 0 point of the spectrophotometer in a state where nothing is in the sample chamber of the spectrophotometer.
- fix the barrel to the sample chamber of the spectrophotometer. At this time, keep the distance from the light source to the barrel constant, and adjust the light beam to shine on a position 20 mm from the tip of the barrel on the central axis of the barrel.
- read the absorptance value in a state where nothing is in the control cell holder.

The measurement results are shown in Table 1 below.

[0068]

[Table 1]

Sample	Measurement	Measured Value	Average
Example 1 (with silicone oil application)	1st	0.008	0.006
	2nd	0.006	
	3rd	0.005	
Comparative Example 1 (without silicone oil application)	1st	0.007	0.007
	2nd	0.009	
	3rd	0.006	

[0069] As shown in the above Table 1, even when a silicone film was formed under the conditions described in Example 1, the absorptance did not appear to change substantially as compared to the case where silicone oil was not applied. Accordingly, the silicone film formed under the conditions of Example 1 was confirmed to not affect the efficiency of visual inspection.

<Example 2>

[0070] A prefilled syringe was assembled by preparing a barrel on which a silicone film was formed by the same method as Example 1, and attaching a cap member to this barrel, then filling 2.9 ml of an aqueous solution of 1% high molecular weight sodium hyaluronate with a weight average molecular weight of 3,000,000 (viscosity = 25,000 mPa·s), and capping it with a gasket.

<Example 3>

[0071] Other than using a silicone oil with a kinematic viscosity of 1,000 cSt ("KF-96-1000cs" manufactured by Shin-Etsu Chemical Co., Ltd.) as the silicone oil, a barrel on which a silicone film was formed was prepared in the same manner as Example 1, and a prefilled syringe was assembled using this barrel in the same manner as Example 2.

<Example 4>

[0072] Other than using a silicone oil with a kinematic viscosity of 30,000 cSt ("KF-96H-30000cs" manufactured by Shin-Etsu Chemical Co., Ltd.) as the silicone oil, a barrel on which a silicone film was formed was prepared in the same manner as Example 1, and a prefilled syringe was assembled using this barrel in the same manner as Example 2.

<Example 5>

[0073] A silicone oil was further applied to the surface of the gasket at 0.1 mg per 1 cm^2 of the surface. Specifically, a silicone oil with a kinematic viscosity of 5,000 cSt was added to a tank filled with water in an amount that would achieve 0.13 mg per 1 cm^2 with respect to the total surface area of the entire gasket, and mixed for 10 minutes to disperse it. A gasket was put into the tank, and after mixing for 10 minutes at 100°C while blowing a vapor from the bottom, the water was drained, rinsing was performed and autoclave sterilization was carried out. The application amount of the silicone oil was confirmed by gravimetry, and verified to be 0.10 mg per 1 cm^2 of the gasket surface. Other than using this gasket, a prefilled syringe was assembled in the same manner as Example 2.

<Example 6>

[0074] A silicone oil was further applied to the surface of the gasket at 0.2 mg per 1 cm^2 of the surface. While the application method was the same as Example 5, the silicone oil was added in an amount that would achieve 0.26 mg per 1 cm^2 with respect to the total surface area of the entire gasket. The application amount of the silicone oil was confirmed in the same manner as Example 5, and was 0.20 mg per 1 cm^2 of the gasket surface. Other than using this gasket, a prefilled syringe was assembled in the same manner as Example 2.

Comparative Example 2>

[0075] Other than using a silicone oil with a kinematic viscosity of 350 cSt ("KP-96-350cs" manufactured by Shin-Etsu Chemical Co., Ltd.) as the silicone oil, a barrel on which a silicone film was formed was prepared in the same manner as Example 1, and a prefilled syringe was assembled using this barrel in the same manner as Example 2.

<Comparative Example 3>

[0076] Other than using a mixed silicone oil with a kinematic viscosity of 150,000 cSt prepared by mixing 370 g of a silicone oil with a kinematic viscosity of 300,000 cSt ("KF-96-300000cs" manufactured by Shin-Etsu Chemical Co., Ltd.) and 630 g of a silicone oil with a kinematic viscosity of 100,000 cSt ("KF-96-100000cs" manufactured by Shin-Etsu Chemical Co., Ltd.), a barrel on which a silicone film was formed was prepared in the same manner as Example 1, and a prefilled syringe was assembled using this barrel in the same manner as Example 2.

<Comparative Example 4>

[0077] Other than spraying a silicone oil to form a silicone film with an average application amount of 100 μg per 1 cm^2, a barrel on which a silicone film was formed was prepared in the same manner as Example 1, and a prefilled syringe was assembled using this barrel in the same manner as Example 2.

<Comparative Example 5>

[0078] A silicone oil was further applied to the surface of the gasket at 0.4 mg per 1 cm^2 of the surface. While the application method is the same as Example 5, the silicone oil was added in an amount that would achieve 0.52 mg per 1 cm^2 with respect to the total surface area of the entire gasket. The application amount of the silicone oil was confirmed in the same manner as Example 5, and was 0.41 mg per 1 cm^2 of the gasket surface. Other than using this gasket, a prefilled syringe was assembled in the same manner as Example 2.

[0079] The prefilled syringes prepared in the above Examples 2 to 6 and Comparative Examples 2 to 5 were evaluated by the following methods for intermixture of silicone oil into the drug, glare on the inner peripheral surface of the barrel and sliding resistance.

<Visual evaluation>

[0080] The presence of turbidity in the drugs and the presence of glare on the inner peripheral surface of the barrels were visually evaluated by a group of five panelists consisting of skilled quality inspectors. The results are shown in Table 2.
Turbidity evaluation criteria:

A (good): no turbidity confirmed.
B (poor): turbidity confirmed.

Glare evaluation criteria:

A (good): no glare observed.
B (poor): glare observed.

<Sliding resistance evaluation>

[0081] Injection needles (23G x 1 ¼; manufactured by Terumo Corporation) were affixed to the tip of the prefilled injection needles, and initial pressure and extrusion pressure when discharging the drugs at an extrusion speed of 100 mm/min. were measured using a testing machine ("EZ-TEST" manufactured by Shimadzu Corporation). Additionally,

for the initial pressure measurement, samples stored for a month at 40 °C after production were used. The results are shown in Table 2.
Initial pressure evaluation criteria:

AA (best): local pressure maximum when gasket starts moving not confirmed in data less than 5 mm from the start of compression.
A (good): local pressure maximum when gasket starts moving 30 N or lower in data less than 5 mm from the start of compression.
B (poor): local pressure maximum when gasket starts moving over 30 N in data of less than 5 mm from the start of compression.

Extrusion pressure evaluation criteria:

A (good): dispersion in extrusion pressure within 5 N and extrusion pressure maximum 30 N or lower in data 5 mm or greater from the start of compression.
B (poor): dispersion in extrusion pressure over 5 N and extrusion pressure maximum over 30 N in data 5 mm or greater from the start of compression.

[0082]

[Table 2]

	Example 2	Example 3	Example 4	Example 5	Example 6
Kinematic viscosity of silicone oil applied on barrel (cSt)	5,000	1,000	30,000	5,000	5,000
Average application amount per 1 cm^2 of silicone oil on barrel (μg)	18	18	18	18	18
Kinematic viscosity of silicone oil applied on gasket (cSt)	Not applied	Not applied	Not applied	5,000	5,000
Average application amount per 1 cm^2 of silicone oil on gasket (μg)	0	0	0	0.10	0.20
Turbidity	A	A	A	A	A
Glare	A	A	A	A	A
Initial pressure after one month storage	A	A	A	AA	AA
Extrusion pressure	A	A	A	A	A

	Comparative Example 2	Comparative Example 3	Comparative Example 4	Comparative Example 5
Kinematic viscosity of silicone oil applied on barrel (cSt)	350	150,000	5,000	5,000
Average application amount per 1 cm^2 of silicone oil on barrel (μg)	18	0-3000 In the form of unsprayable droplets	100	18
Kinematic viscosity of silicone oil applied on gasket (cSt)	0	0	0	5,000
Average application amount per 1 cm^2 of silicone oil on gasket (μg)	0	0	0	0.41
Turbidity	B	A	B	B
Glare	A	B	B	A
Initial pressure after one month storage	A	B	A	A
Extrusion pressure	B	B	A	A

[0083] As shown in the above Table 2, the prefilled syringes on which a silicone film was formed satisfying the predetermined conditions according to the invention (Examples 2 to 6) were observed to have no silicone oil contaminating the drugs and no glare on the inner peripheral surface of the barrels, and they exhibited excellent properties in terms of slidability. In particular, when the silicone oil was also applied at the predetermined amount to the surface of the gaskets, the initial pressure could be suppressed so much that a local maximum was not observed.

[0084] On the other hand, when the kinematic viscosity of the silicone oil was too low (Comparative Example 2), while it was fine in terms of glare, the sliding resistance was unstable and some turbidity in the drug, i.e. incorporation of silicone oil, was observed. Moreover, when the kinematic viscosity of the silicone oil was too high (Comparative Example 3), it could not be evenly sprayed in the form of fine particulates, and adhered unevenly as droplets with diameters ranging from several hundred μm to several mm, as a consequence of which there were portions where it was not applied and portions where it was excessively applied, and glare, and unstable sliding resistance were confirmed.

[0085] Further, even if the kinematic viscosity was within the optimal range, when the application amount of the silicone oil was too high (Comparative Example 4), glare was confirmed on the inner peripheral surface of the barrel, and intermixture of the silicone oil in the drug was observed. Additionally, when the amount of silicone oil applied to the surface of the gasket was too high (Comparative Example 5), intermixture of the silicone oil into the drug was observed.

<Example 7>

[0086] On the inner peripheral surface of a 5 ml volume barrel formed with a COP resin as the main ingredient, and produced and inspected by controlling the tolerance of the outer diameter to 15.05 ± 0.1 mm, the inner diameter to 12.45 ± 0.05 mm, the full length to 79.0 ± 0.2 mm, and the flange diameter 0 to 22.0 ± 0.2 mm, a silicone oil of a kinematic viscosity of 5,000 cSt ("KF-96" manufactured by Shin-Etsu Chemical Co., Ltd.) was sprayed under the following conditions to form a silicone film where the average application amount was 18 μg per 1 cm^2. A thermoplastic saturated norbornene resin composition commercially available as Zeonex (trademark) from the (Japan) Zeon Corporation was used as the COP resin. Additionally, during the production and inspection of the barrel, the tolerance control of the inner diameter (± 0.05 mm) was particularly strictly observed.

(Silicone oil spraying conditions)
Spraying time: 0.05 second
Air pressure: 0.5 MPa
Silicone oil heating temperature: 180 °C
Nozzle diameter: 1.0 mm

[0087] On the other hand, a gasket made of butyl rubber, a kind of rubber material whose surface is not resin treated, was produced and inspected by controlling the tolerance of the outer diameter 0 to 12.70 ± 0.10 mm (first ridge portion), Ø to 12.0 ± 0.10 mm (valley portion) and full length to 10.0 ± 0.30 mm. Additionally, since a butyl rubber was used for this gasket, the dimensional changes due to autoclave sterilization were small as compared to general gaskets, and even when sterilized, would be kept within a tolerance range of ± 0.10 mm. Additionally, the gasket was applied with the silicone oil (KF-96-5000cs manufactured by Shin-Etsu Chemical Co., Ltd.) at 0.1 mg per 1 cm^2 area.

<Comparative Example 6>

[0088] A syringe was prepared in basically the same manner as Example 7 but differed in that a gasket of butyl rubber whose surface was laminated with a tetrafluoroethylene resin film, having a tolerance of an outer diameter Ø of 12.70 ± 0.10 mm (first ridge portion), Ø of 12.0 ± 0.10 mm (valley portion) and full length of 10.0 ± 0.30 mm was used and silicone oil was not applied.

[0089] Additionally, the gaskets of Example 7 and Comparative Example 6 were preliminarily checked for how the dimensions of the gaskets before autoclaving would change after autoclaving. The results revealed that there was almost no dimensional change in the gasket of Example 7 consisting of butyl rubber whose surface was not resin treated after autoclaving as compared to before autoclaving, and dimensional accuracy was kept within the tolerance range (data not shown). On the other hand, it was revealed that there was a large dimensional change in the gasket of Comparative Example 6 made of butyl rubber whose surface was laminated with a tetrafluoroethylene resin film after autoclaving as compared to before autoclaving, and the dimensional accuracy for the outer diameter Ø became 12.70 ± 0.20 mm (first ridge portion), Ø 12.0 ± 0.20 mm (valley portion) and full length 10.0 ±0.40 mm after autoclaving.

<Comparative Example 7>

[0090] A syringe was prepared in basically the same manner as Example 7 but differed in that a 5 ml volume barrel formed with a COP resin as the main ingredient, and produced and inspected by controlling the tolerance of the outer diameter to 15.05 ± 0.1 mm, the inner diameter to 12.45 ± 0.20 mm, the full length to 79.0 ± 0.2 mm and the flange

diameter Ø to 22.0 ± 0.2 mm was used.

<Slidability and air-tightness tests>

[0091] Since the most lenient combined tolerances in Example 7 for the barrel inner diameter Ø is 12.50 mm and for the first ridge portion of the gasket Ø is 12.60 mm, the difference between them is 0.10 mm. Even in that case, when real liquid leakage tests were performed as described below, the ability to secure air-tightness was confirmed. On the other hand, since the tightest combined tolerances in Example 7 for the barrel inner diameter Ø is 12.40 mm and for the first ridge portion of the gasket Ø is 12.80 mm, the difference between them is 0.40 mm. Even in that case, a good slidability was confirmed (data not shown).

<Real liquid leakage test>

[0092] In order to confirm that the drug solution would not leak from the gaps in the gasket even when a certain degree of pressure was applied, real liquid leakage tests were performed in accordance with the following steps.

1) affix an injection needle sealed at the tip onto the syringe.
2) push the plunger rod using an extrusion tester (EZ-TEST manufactured by Shimadzu Corporation), adjust the position of the pusher such that the extrusion pressure will be within a range of 19 to 24 N, and keep for 30 seconds.
3) remove the syringe, and visually confirm whether the drug solution has leaked from the gap of the gasket.

According to the results, in the case of Example 7, real liquid leakage tests were performed n = 50 times, but there was no sample where drug solution leakage occurred, so the ability to secure air-tightness was confirmed.

<Initial pressure and maximum pressure tests>

[0093] In the following sliding resistance test, injection needles (23 G x 1 ¼; manufactured by Terumo Corporation) were affixed to the tip of the syringes and plungers were affixed thereto, the extrusion pressures when compressing the plungers at a speed of 100 mm/min. were measured using an extrusion tester (EZ-TEST manufactured by Shimadzu Corporation). Table 3 shows the results of measurements of the initial pressure (extrusion pressure at a peak appearing within 5 mm from the start of compression) in ten syringes of each sample in a state not filled with drug solutions. Table 4 shows the results of measuring the maximum pressure (maximum value of the extrusion pressure) in 10 syringes of each sample filled with drug solutions.

[0094]

[Table 3]

Sample	Average value of initial pressure at each number of days of storage (N)		
	7 days	30 days	90 days
Example 7	4.0	4.4	5.3
Comparative Example 6	11.5	12.1	13.7
Comparative Example 7	13.7	16.1	16.2

[0095]

[Table 4]

Sample	Average value of maximum pressure at each number of days of storage (N)		
	7 days	30 days	90 days
Example 7	23.4	23.8	23.1
Comparative Example 6	25.9	25.6	24.5
Comparative Example 7	31.0	31.8	32.1

[0096] As shown above, when comparing Example 7 and Comparative Example 7 in Table 3, it is clear that the control

of gasket tolerances greatly improved the initial pressure (locking). Additionally, from Table 4, it is clear that the control of gasket tolerances also improved the maximum pressure.

<Tests for comparing slidability in samples with different viscosities>

[0097] After filling the syringes of Example 7 and Comparative Example 7 with solutions of different viscosities and storing them at 40 °C for one month, slidability was measured and compared (same gaskets). The test results are shown in Table 5.

[0098]

[Table 5]

Syringe used	Loaded liquid	Viscosity of loaded liquid (mPa·s)	Maximum pressure (N)
Example 7	1% hyaluronic acid solution (weight average molecular weight 3,000,000)	25000	23.8
	1% hyaluronic acid solution (weight average molecular weight 800,000)	1800	21.4
	Water	1	5.1
Comparative Example 7	1 % hyaluronic acid solution (weight average molecular weight 3,000,000)	25000	31.8
	1% hyaluronic acid solution (weight average molecular weight 800,000)	1800	27.8
	Water	1	11.2

[0099] As shown in Table 5, the maximum pressure changes greatly with the viscosity of the drug solution. As such, it is clear that the higher the viscosity of a drug solution, the greater the need to control the tolerances in order to suppress the maximum pressure.

<Example 8 to 13 and Comparative Examples 8 to 12>

[0100] he inner peripheral surface of a 5 ml volume barrel that was formed with a COP resin as the main ingredient, had a cylindrical outer diameter of 15.05 mm, a cylindrical inner diameter of 12.45 mm and a full length of 79.0 mm, was sprayed with a silicone oil of a kinematic viscosity of 5,000 cSt ("KF-96-5000cs" manufactured by Shin-Etsu Chemical Co., Ltd.) under the following conditions to be within a range of 0 to 150 μg per 1 cm^2. A thermoplastic saturated norbornene resin composition commercially available as Zeonex (trademark) from the (Japan) Zeon Corporation was used as the COP resin.

(Silicone oil spraying conditions)
Spraying time: 0.05 second
Air pressure: 0.5 MPa
Silicone oil heating temperature: 180 °C
Nozzle diameter: 1.0 mm

A prefilled syringe was assembled by attaching a cap member to this barrel, then loading 2.9 ml of an aqueous solution of 1% high molecular weight sodium hyaluronate with a weight average molecular weight of 3,000,000 (viscosity = 25,000 mPa·s), and capping it with a gasket coated with the same silicone oil as above at 0.10 mg per 1 cm^2.

<Angle of refraction measurement>

[0101] To measure the angle of refraction of the above prefilled syringe, as shown in Fig.2, the following device, conditions and method were used.

(Device)

[0102]

- Laser oscillator: RX-4N (manufacture by Sakura Color Products Corp. Japan)
- Beam width ("A") of light beam shone from laser oscillator: 2 mm
- Wavelength: 650 nm
- Output: less than 1 mW

(Conditions)

[0103]

- Distance from laser oscillator to central axis of prefilled syringe: 50 mm
- Distance ("L") from central axis of prefilled syringe to projection plate: 200 mm
- Site of incidence: center of the region in the barrel filled with the drug on the central axis of the barrel

(Method)

[0104]

- for the portion filled with the drug in each prefilled syringe, measure the width ("D") in the direction of the central axis of the image projected on the projection plate in three installments by rotating 120° each time with the central axis as the rotation axis, and calculate the average.
- based on the obtained width of projection ("D"), the optical width of the light beam shone ("A"), and the distance from the central axis of the prefilled syringe to the projection plate ("L"), calculate angle of refraction θ.

<Glare evaluation>

[0105] The presence of glare on the inner peripheral surface of the barrel of each prefilled syringe was visually evaluated by a group of five panelists consisting of skilled quality inspectors.
Glare evaluation criteria:

A (good): no glare observed.
B (poor): glare observed.

<Sliding resistance evaluation>

[0106] The sliding resistance between the barrel and gasket of each prefilled syringe was evaluated using the following criteria.

AA (best): local pressure maximum when gasket starts moving not confirmed, and no variation in extrusion pressure after gasket starts moving.
A (good): pressure when gasket starts moving being within the permitted range, and no variation in extrusion pressure after gasket starts moving.
B (poor): pressure when gasket starts moving being within the permitted range, but variations present in extrusion pressure after gasket starts moving.

[0107] The measurement and evaluation results are shown in Table 6 below.

[0108]

[Table 6]

	Application amount of silicone oil ($\mu g/cm^2$)	Projection image width (D) (mm)	Angle of Refraction θ (°)	Glare	Sliding resistance
Example 8	5	3.0	0.14	A	A

(continued)

	Application amount of silicone oil (μg/cm^2)	Projection image width (D) (mm)	Angle of Refraction θ (°)	Glare	Sliding resistance
Example 9	10	3.0	0.14	A	A
Example 10	20	3.0	0.14	A	AA
Example 11	30	3.7	0.24	A	AA
Example 12	40	4.3	0.33	A	AA
Example 13	50	5.0	0.43	A	AA
Comparative Example 8	0	3.0	0.14	A	B
Comparative Example 9	60	6.7	0.67	B	AA
Comparative Example 10	80	7.3	0.76	B	AA
Comparative Example 11	100	9.0	1.00	B	AA
Comparative Example 12	150	10.3	1.19	B	AA

[0109] As shown in the above Table 6, no glare was observed on the inner peripheral surfaces of barrels of the prefilled syringes with angles of refraction within a range of 0.1 to 0.5° (Examples 8 to 13), and they exhibited excellent slidability. On the other hand, glare was confirmed on the inner peripheral surface of the barrel when the angle of refraction exceeded the range of 0.1 to 0.5° (Comparative Examples 9 to 12). Additionally, when a silicone oil was not applied to the inner peripheral surface of the barrel (Comparative Example 8), no glare was confirmed, but sliding resistance was confirmed to be unstable.

[0110] The present invention has been explained with reference to examples above. These examples are only exemplifications, and those skilled in the art will recognize that various modifications are possible, and that such modifications are also within the scope of the present invention.

Claims

1. A syringe having a resin barrel, a gasket slidably inserted in the barrel, a plunger attached to the gasket, and a silicone film formed by applying a silicone oil having a kinematic viscosity of 500 to 10,000 cSt to the inner peripheral surface of said barrel in an amount of 5 to 50 μg per 1 cm^2 of area.

2. The syringe according to claim 1, **characterized in that** a silicone oil having a kinematic viscosity of 500 to 10,000 cSt is applied to the surface of said gasket at 0 to 0.3 mg per 1 cm^2 of area.

3. The syringe according to claim 1 or 2, wherein the tolerance of the inner diameter of said barrel is controlled to be at most ± 0.10 mm.

4. The syringe according to any one of claims 1 to 3, **characterized in that** said barrel consists of a thermoplastic saturated norbornene resin composition.

5. The syringe according to any one of claims 1 to 4, wherein said gasket has a maximum outer diameter greater than the inner diameter of said barrel, and the difference between the maximum outer diameter of said gasket and the inner diameter of said barrel is at least 0.02 mm and at most 0.50 mm.

6. The syringe according to claim 5, wherein said gasket has a plurality of ridge portions, the outer diameter of a first ridge portion closest to the tip among said plurality of ridge portions corresponding to said maximum outer diameter.

7. The syringe according to any one of claims 1 to 6, wherein the tolerance of the maximum outer diameter of said gasket after autoclave sterilization is controlled to be ± 0.10 mm

8. The syringe according to any one of claims 1 to 7, wherein said gasket consists of a rubber or thermoplastic elastomer.

9. The syringe according to claim 8, wherein said rubber is butyl rubber.

10. The syringe according to any one of claims 1 to 9, wherein when shining an incident beam with a wavelength of 635 nm to 690 nm and a beam width of at most 3.0 mm on the barrel filled with a drug with an optical axis orthogonally intersecting the central axis of the barrel, the angle of refraction from the optical axis of a transmitted beam scattered in the same direction as said central axis is within a range of 0.1 to 0.5°.

11. The syringe according to any one of claims 1 to 10, which is a prefilled syringe having a cap member sealing the tip opening of said barrel and a drug loaded inside said barrel.

12. The syringe according to claim 11, **characterized in that** said drug has a viscosity of 1,000 to 60,000 mPa·s.

13. The syringe according to claim 11 or 12, wherein said drug is an aqueous sodium hyaluronate solution.

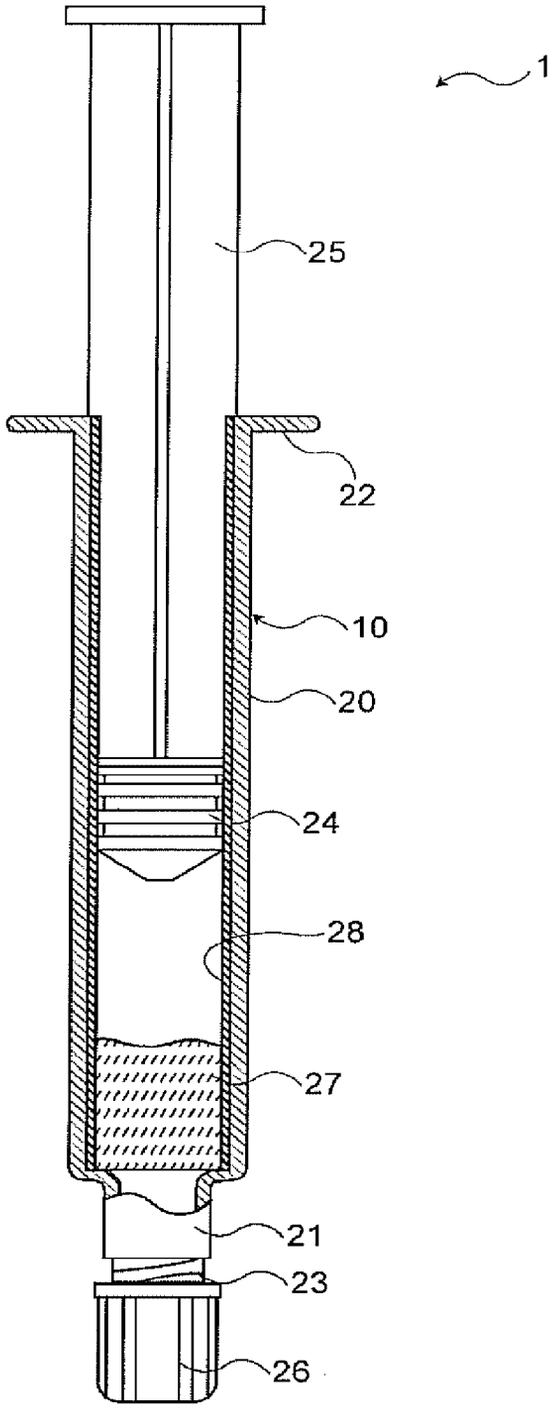


Fig. 1

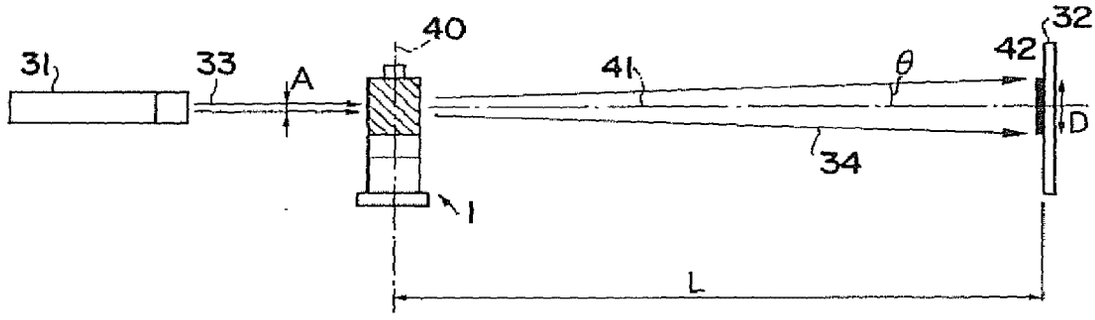


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/070285

A. CLASSIFICATION OF SUBJECT MATTER
A61M5/28(2006.01)i

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)
A61M5/28

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

Jitsuyo Shinan Koho	1922-1996	Jitsuyo Shinan Toroku Koho	1996-2010
Kokai Jitsuyo Shinan Koho	1971-2010	Toroku Jitsuyo Shinan Koho	1994-2010

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP 10-201844 A (Yugen Kaisha Koki Enjiniaringu), 04 August 1998 (04.08.1998), claim 3; paragraph [0035] (Family: none)	1,3,7,8,10 2,4-6,9, 11-13
Y	JP 2004-321614 A (Terumo Corp.), 18 November 2004 (18.11.2004), all drawings (Family: none)	2,11-13

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 March, 2010 (04.03.10)	16 March, 2010 (16.03.10)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/070285

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 6-197965 A (Nippon Zeon Co., Ltd.), 19 July 1994 (19.07.1994), paragraphs [0039], [0047] to [0049] & US 5561208 A (specification, column 10, lines 10 to 16; column 11, lines 35 to 62) & EP 0559146 A1 (specification, page 8, lines 11 to 14; column 9, lines 10 to 26)	4
Y	JP 2003-180832 A (Hori Garasu Kabushiki Kaisha), 02 July 2003 (02.07.2003), claim 1; paragraph [0030] (Family: none)	5,6
Y	JP 2007-244606 A (Hisamitsu Pharmaceutical Co., Inc.), 27 September 2007 (27.09.2007), paragraphs [0019], [0035] (Family: none)	9,12,13

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

REFERENCES CITED IN THE DESCRIPTION

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

Patent documents cited in the description

- JP 2007244606 A **[0009]**
- JP 2006094895 A **[0009]**
- JP H10314305 A **[0009]**

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2001-104480
(43)Date of publication of application : 17.04.2001

(51)Int.Cl. A61M 5/24
A61M 5/315

(21)Application number : 11-283788
(22)Date of filing : 05.10.1999

(71)Applicant : DAIKYO SEIKO LTD
(72)Inventor : KAWACHI YASUSHI
SUDO MORIAKI

(54) RUBBER PISTON USED FOR SEALING PLASTIC PREFILLED SYRINGE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a rubber piston for a plastic prefilled syringe having a high liquid sealing property and having compatibility with a new drug having a high infiltrating property into fine clearances while preserving the technical levels such as the prevention of contamination of a drug with foreign matters, the suppression of medicinal component adsorption to constitition elements of a container, and the sliding property of a syringe as an important usability of syringe.
SOLUTION: In a rubber piston used for sealing a plastic prefilled syringe a part of the surface of which is covered with a thin film of a fluorine- containing resin, a thin film of a liquid polymer having affinity to the fluorine- containing resin is formed on the surface of the thin film.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 December 2010 (02.12.2010)

PCT

(10) International Publication Number
WO 2010/136492 A2

(51) **International Patent Classification**:
A61K 39/395 (2006.01) ***A61P 27/02*** (2006.01)
C07K 16/22 (2006.01) ***C07K 14/715*** (2006.01)
C07K 16/24 (2006.01)

(21) **International Application Number**:
PCT/EP2010/057246

(22) **International Filing Date**:
26 May 2010 (26.05.2010)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/181,887 28 May 2009 (28.05.2009) US

(71) **Applicant** *(for all designated States except US)*: **GLAXO GROUP LIMITED** [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford Middlesex UB6 0NN (GB).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **ADAMSON, Peter** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **ERTL, Peter, Franz** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **GERMASCHEWSKI, Volker** [DE/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **GOUGH, Gerald, Wayne** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **STEWARD, Michael** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB).

(74) **Agents**: **REDDISH, Anna** et al.; GlaxoSmithKline, Global Patents CN925.1, 980 Great West Road, Brentford Middlesex TW8 9GS (GB).

(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, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

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

(54) **Title**: ANTIGEN-BINDING PROTEINS

(57) **Abstract**: The invention relates to combinations of TNFα antagonists with VEGF antagonists for use in treating diseases of the eye, and provides antigen-binding proteins which bind to TNFα or a TNFα receptor and/or VEGF or a VEGF receptor.

WO 2010/136492 A2

Antigen-binding proteins

Background

Vision loss has become a major health problem for developed economies. Blindness or poor vision affects over 3 million US citizens over the age of 40 years and this increases significantly with age. For example, those aged 80 years old or greater comprise about 8% of the US population but nonetheless account for almost 70% of blindness. Eye diseases that are typically associated with age include age related macular degeneration (AMD), cataracts, diabetic macular edema, retinal vein occlusion (RVO) and glaucoma.

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world. There are two major clinical presentations of AMD. Atrophic (dry) AMD is characterised by the degeneration of retinal pigment epithelial (RPE) and neuroretina. The early stages of atrophic AMD are associated with the formation of drusen, under the RPE cell layer. Early atrophic AMD can progress to an end stage disease where the RPE degenerates completely and forms sharply demarcated areas of RPE atrophy in the region of the macula: "geographic atrophy". In this form of the disease, the degeneration of RPE results in the secondary death of macular rods and cones and in these cases this leads to the severe age-related vision loss. A proportion of AMD patients develop what can either be regarded as a different form or a further complication of the disease. Approximately 10-20% of AMD patients develop choroidal neovascularisation (CNV). When this occurs the form of the disease is known as '"wet AMD" and this can be associated with some of the most severe vision loss. In wet AMD, new choroidal vessels grow through breaks in Bruch's membrane and proliferate into and under the RPE and neuroretina. There are currently no definitive means of treatment for the very prevalent atrophic form of AMD nor to prevent the progression of early dry AMD either to geographic atrophy or to wet AMD, (Petrukhin K, Expert Opin Ther Targets (2007) 11: 625-639).

Diabetic macular edema (DME) is the most frequent cause of loss of reading vision in diabetic patients. The prevalence of DME in individuals who have had diabetes for 29 years or more is approximately 30% (Klein R et al Ophthalmology 1984: 91; 1464-1474). DME is associated with increased levels of IL-6, VEGF and other cytokines, with a generalised breakdown of the blood retinal barrier with leakage from abnormal retinal capillaries and microaneurysms developing in the sub retinal space. The goal

of current DME treatment is to reduce the edema and leakage leading to improved visual acquity. Good glycemic control and laser photocoagulation or antiangiogenic treatment aim to prevent or delay further deterioration of the central macular region of the diabetic eye. Intravitreal injection of corticosteroids have also been used.

Retinal vein occlusion occurs subsequent to obstruction of the blood flow through a retinal vein. This might be due to clot formation or pressure increases in closely associated retinal arteries due to diabetes, glaucoma or high blood pressure. The reduced blood flow out of the retina leads to a generalised increase in blood pressure in ocular blood vessels and reduced oxygen levels in the eye. This in turn leads to abnormal blood vessel growth, hemorraging and edema, tissue damage and vision loss. There are two main forms of RVO, branch retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO). Sudden blurring or loss of vision is the common feature of RVO. Intraocular corticosteroids have been used to treat RVO, albeit with the associated risk of cataract development and raised intraocular pressure (Kiernan DF et al Exp Opinion in Pharmacotherapy 2009 10(15) 2511-2525). The prevalence of RVO ranges from ~ 0.2% (CRVO) to ~0.7% (BRVO).

Uveitis predominantly affects people of working age and comprises an inflammation of the uveal tract (iris, ciliary body and choroid). Anterior uveitis is the most common form of uveitis making up about 75% of uveitis cases and it and mainly affects the iris and ciliary body. Uveitis is regarded as an autoimmune disease and whilst the etiology remains unknown an association with HLA-B27 is present in about 50% of cases. Inflammation involving the posterior uveal tract (i.e. the choroid) is known as posterior uveitis and secondary involvement of the retina is common. Uveitis is predominantly an inflammatory disease with infiltration of CD4 T-cells into the ocular compartment (Paroli MP et al 2007 17(6) 938-942 Eur J Ophthalmology). Corticosteriods are again the mainstay for treatment either given topically, periocularly or systemically.

TNF-α (Tumour Necrosis Factor-α) is a pro-inflammatory cytokine which has been associated with a number of ophthalmic inflammatory conditions (Theodossiadis *et al.*, Am. J. Ophthalmol. (2009) 147: 825-830).

VEGF (Vascular Endothelial Growth Factor) and VEGF-receptors are known to stimulate both choroidal and retinal vessel angiogenesis and regulate the vascular permeability of such vessels. (Gragoudas *et al.*, N. Engl. J. Med (2004) 351: 2805)

Neovascularisation and leakage are prominent features of the wet form of age-related macular degeneration. An aptamer, pegaptanib (Macugen™), which neutralises the VEGF-A isoform 165, and ranibizumab (Lucentis™) which blocks all isoforms of VEGF-A, have now been approved for use.

The inflammatory response also plays a significant pathophysiological role in neovascularisation (Sakuri *et al.,* Invest Ophthalmol Vis Sci (2003) 44: 5349-5354; Oh *et al*. Invest Ophthalmol Vis Sci (1999) 40: 1891-1898;Shi *et al*., Exp Eye Res (2006) 83: 1325-1334.

Literature references relating to TNFα antagonists include Olson *et al*., Arch Opthalmol (2007) 125: 1221-1224; Shi *et al*., Exp Eye Res (2006) 83: 1325-1334Kociok *et al*., Invest Ophthalmol Vis Sci (2006) 11: 5057-5065Markomichelakis *et al*. Am J Ophthalmol (2005) 139: 537-540.

Studies indicate that intravitreal injections of infliximab may elicit a severe intracocular inflammatory reaction that appears to be dose related. Such adverse events were not seen with adalimumab (Program 4247, Poster D913, Intravitreal TNF inhibitors in the Treatment of Refractory Diabetic Macular Edema: A Pilot Study from the Pan American Collaborative Retina Study Group and Program 4749, Poster D1087, Ocular and Systemic Safety of Intravitreal TNF Inhibitors: A Pilot Study From the Pan American Collaborative Retina Study Group, The Association for Research in Vision and Ophthalmology (ARVO) May 2-6 2010. Ft. Lauderdale USA).

There is a need for treatment regimes which are effective at preventing ophthalmic disease progression and provide improved vision for a wider group of patients.

Summary of invention

The present invention relates to the combination of a TNFα antagonist and a VEGF antagonist, specifically for use in treating diseases of the eye.

Both anti-VEGF and anti-TNF approaches have a basis in treating AMD, and mechanistically these modalities may not overlap, such that a patient who does not

respond successfully to an anti-VEGF approach therapy may respond to an anti-TNF treatment and vice versa.

The anti-inflammatory benefit of an anti-TNF combined with the anti-angiogenic activity of an anti-VEGF molecule will provide improved efficacy in treating such eye diseases.

The administration of a combination of an individual TNFα antagonist and an individual VEGF antagonist (i.e. separate TNFα and VEGF antagonist molecules) is covered by the present invention. In addition, the administration of a single construct with dual targeting functionality that acts as both a TNFα antagonist and a VEGF antagonist (i.e. able to bind to and inhibit, preferably block, the function of TNFα or a TNFα receptor, and bind to and inhibit, preferably block, the function of VEGF or a VEGF receptor) is covered by the present invention. The single construct may be based on an antibody scaffold or other such suitable scaffold. Receptor-Fc fusions are also considered part of the invention.

The present invention relates in particular to antigen binding proteins.

In particular, the present invention relates to a TNFα/VEGF dual targeting single construct wherein the TNFα antagonist portion is or is derived from a human anti-TNFα antibody. The TNFα antibody may be adalimumab or golimumab.

The present invention in particular relates to an antigen-binding protein comprising a protein scaffold which is linked to one or more epitope-binding domains wherein the antigen-binding protein has at least two antigen-binding sites at least one of which is from an epitope binding domain and at least one of which is from a paired V_H/V_L domain, and wherein at least one of the antigen-binding sites is capable of binding to TNFα or a TNFα Receptor e.g. TNFR1, and at least one of the antigen binding sites is capable of binding to VEGF or a VEGF Receptor, e.g. VEGFR2, for use in treating diseases of the eye.

A receptor-Fc fusion which is linked to one or more epitope-binding domains is also part of the invention e.g. a TNFα receptor-Fc fusion linked to a VEGF or VEGF receptor-binding domain, or a VEGF receptor-Fc fusion linked to a TNFα or a TNFα receptor-binding domain.

The present invention provides a dual targeting antigen binding molecule comprising a TNFα antagonist portion, a VEGF antagonist portion and a linker connecting said TNFα antagonist portion to said VEGF antagonist portion, wherein the TNFα antagonist portion comprises an amino acid sequence of any one of the TNFα antagonists listed in table 1; the VEGF antagonist portion comprises an amino acid sequence of any one of the VEGF antagonists listed in table 2; the linker is an amino acid sequence from 1 – 150 amino acids in length; and the dual targeting molecule is not DMS4000 or DMS4031. The linker may also be a non-peptide based linker, including, for example, polyethylene glycol (PEG) and PEG based linkers.

The invention also provides a polynucleotide sequence encoding an antigen binding protein of the invention e.g. a polynucleotide sequence encoding a heavy chain of any of the antigen-binding proteins described herein, and a polynucleotide encoding a light chain of any of the antigen-binding proteins described herein. Such polynucleotides represent the coding sequence which corresponds to the equivalent polypeptide sequences. However it will be understood that such polynucleotide sequences could be cloned into an expression vector along with a start codon, an appropriate signal sequence and a stop codon.

The invention also provides a recombinant transformed or transfected host cell comprising one or more polynucleotides encoding an antigen binding protein of the invention e.g. a heavy chain and a light chain of an antigen-binding protein described herein.

The invention further provides a method for the production of any of the antigen-binding proteins described herein which method comprises the step of culturing a host cell comprising at least one vector comprising a polynucleotide encoding an antigen binding protein of the invention, e.g. a first and second vector, said first vector comprising a polynucleotide encoding a heavy chain of an antigen-binding protein described herein and said second vector comprising a polynucleotide encoding a light chain of an antigen-binding protein described herein, in a suitable culture media, for example serum-free culture media.

The invention provides a pharmaceutical composition suitable for systemic delivery or topical delivery to the eye comprising an antigen-binding protein as described herein and a pharmaceutically acceptable carrier. The pharmaceutical composition of the invention may additionally comprise a further active agent.

The invention provides a TNFα antagonist selected from the group consisting of adalimumab, infliximab, etanercept, ESBA105, PEP1-5-19, PEP1-5-490, PEP1-5-493, an adnectin of SEQ ID NO:2, golimumab, certolizumab, ALK-6931, and an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31, for use in preventing or treating an eye disease, wherein the TNFα antagonist is to be administered in combination with a VEGF antagonist selected from the group consisting of bevacizumab, ranibizumab, r84, aflibercept, CT01, DOM15-10-11, DOM15-26-593, PRS-050, PRS-051, MP0012, CT-322, ESBA903, EPI-0030, EPI-0010 and DMS1571.

The invention also provides a VEGF antagonist selected from the group consisting of bevacizumab, ranibizumab, r84, aflibercept, CT01, DOM15-10-11, DOM15-26-593, PRS-050, PRS-051, MP0012, CT-322, ESBA903, EPI-0030, EPI-0010 and DMS1571, for use in preventing or treating an eye disease, wherein the VEGF antagonist is to be administered in combination with a TNFα antagonist selected from the group consisting of adalimumab, infliximab, etanercept, ESBA105, PEP1-5-19, PEP1-5-490, PEP1-5-493, an adnectin of SEQ ID NO:2, golimumab, certolizumab, ALK-6931, and an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31.

The invention also provides a dual targeting antigen binding molecule comprising a TNFα antagonist portion, a VEGF antagonist portion and a linker connecting said TNFα antagonist portion to said VEGF antagonist portion, wherein:

the TNFα antagonist portion comprises an amino acid sequence of any one of the TNFα antagonists listed in table 1;

the VEGF antagonist portion comprises an amino acid sequence of any one of the VEGF antagonists listed in table 2;

the linker is an amino acid sequence from 1 – 150 amino acids in length; and

the dual targeting molecule is not DMS4000 or DMS4031.

The invention also provides a dual targeting antigen binding molecule comprising a TNFα antagonist portion, a VEGF antagonist portion and a linker connecting said TNFα antagonist portion to said VEGF antagonist portion, wherein:

the TNFα antagonist portion comprises an amino acid sequence of any one of the TNFα antagonists listed in table 1;

the VEGF antagonist portion comprises an amino acid sequence of any one of the VEGF antagonists listed in table 2;

the linker is an amino acid sequence from 1 – 150 amino acids in length; and wherein the dual targeting antigen binding molecule is for use in preventing or treating a disease of the eye and is to be administered intravitreally every 4-6 weeks.

The invention also provides an antigen binding protein comprising the heavy chain sequence of SEQ ID NO:69, 70, 71 or 72 and the light chain sequence of SEQ ID NO:12.

A method of preventing or treating a patient afflicted with an eye disease comprising administering a prophylactically or therapeutically effective amount of a composition or dual targeting protein as disclosed herein systemically or topically to the eye of the patient is also provided.

Brief description of the figures

Figure 1 shows SDS-PAGE analysis of the anti-TNFα/anti-VEGF mAb-dAb, DMS4000.

Figure 2 shows SEC profile of the anti-TNFα/anti-VEGF mAb-dAb, DMS4000.

Figure 3 shows Anti-VEGF activity of DMS4000 .

Figure 4 shows Anti-TNFα activity of DMS4000.

Figure 5 shows (PK) properties of DMS4000.

Figure 6 shows the results of an ELISA and confirms that bispecific BPC1821 binds to both VEGFR2 and B7-1.

Figure 7 shows the results of an ELISA and confirms that bispecific BPC1825 shows binding to both VEGF and B7-1.

Figure 8 depicts a matrix for constructing dual-targeting antigen binding molecules of the invention.

Figure 9 shows BIAcore analysis for the PEP-DOM construct

Figure 10 shows BIAcore analysis for the PEP-DOM construct (close up of TNF/VEGF binding region of Figure 9 binding curve)

Figure 11 is a graphical representation of data presented in Table 10. All compounds were administered by intravitreal injection in a volume of 2µl. Black bars represent day 7 results. White bars represent day 14 results.

Figure 12 is a graphical representation of data presented in Table 11. All compounds were administered by intravitreal injection in a volume of 2µl. Black bars represent day 7 results. White bars represent day 14 results.

Figure 13 shows infrared (IR, upper left panel), autofluorescence (AF, lower left panel) and fluorescien angiography (FS, large panel) at 7 (FS 1st) and 14 days (FS 2nd) after laser PC - showing example images. 1. Vehicle treated eyes, 2. eyes treated with 2µg DMS1571 and 8. eyes treated with 30µg Enbrel™. It is notable that the CNV lesions appear more punctuate and less diffuse than lesions responding to treatment with DMS1571.

Figure 14 is a graphical representation of data presented in Table 12. All compounds were administered by intravitreal injection in a volume of 2µl

Figure 15 shows example photomicrographs of flat-mounted retinae stained with ED1 mab. Panels 1A-1B and panel Enbrel 8.4 show flat-mounts of retinas from eyes treated with anti-VEGF (DMS1571) (1A), Vehicle only (1B) or Enbrel (Enbrel 8.4). Macrophages, associated with laser burn site, visualised with ED1 (CD 68, black) X20. Panel 1D shows a Cryostat section (20µm) of retina showing macrophages (ED1+, black) associated with laser burn site which has penetrated to the inner nuclear layer (INL) of the retina. RGC, retinal ganglion cell layer; BV, blood vessel. x20.

Definitions

The term 'Protein Scaffold' as used herein includes but is not limited to an immunoglobulin (Ig) scaffold, for example an IgG scaffold, which may be a four chain or two chain antibody, or which may comprise only the Fc region of an antibody, or which may comprise one or more constant regions from an antibody, which constant regions may be of human or primate origin, or which may be an artificial chimera of human and primate constant regions. Such protein scaffolds may comprise antigen-binding sites in addition to the one or more constant regions, for example where the protein scaffold comprises a full IgG. Such protein scaffolds will be capable of being linked to other protein domains, for example protein domains which have antigen-binding sites, for example epitope-binding domains or ScFv domains.

The term 'receptor-Fc fusion' as used herein refers to a soluble ligand or extracellular domain of a receptor or cell surface protein linked to the Fc region of an antibody. Fragments of such soluble ligands or extracellular domains of a receptor or cell surface protein are included within this definition providing they retain the biological function of the full length protein, i.e. providing they retain antigen-binding ability. A "domain" is a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. An "antibody single variable domain" is a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

A "humanised antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al. Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al. Bio/Technology, 9:421 (1991)). A suitable human

acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanised antibodies – see for example EP-A-0239400 and EP-A-054951. In an embodiment, an antibody of the invention is a humanised antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antigen binding protein. These are the hypervariable regions of immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, all three light chain CDRs, all heavy and light chain CDRs, or at least two CDRs.

A "CDR variant" includes an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a partial alteration of the amino acid sequence (for example by no more than 10 amino acids), which modification permits the variant to retain the biological characteristics of the unmodified sequence. For example, the variant is a functional variant which binds to and neutralises IL-18. A partial alteration of the CDR amino acid sequence may be by deletion or substitution of one to several amino acids, or by addition or insertion of one to several amino acids, or by a combination thereof (for example by no more than 10 amino acids). The CDR variant may contain 1, 2, 3, 4, 5 or 6 amino acid substitutions, additions or deletions, in any combination, in the amino acid sequence. The CDR variant or binding unit variant may contain 1, 2 or 3 amino acid substitutions, insertions or deletions, in any combination, in the amino acid sequence. The substitutions in amino acid residues may be conservative substitutions, for example, substituting one hydrophobic amino acid for an alternative hydrophobic amino acid. For example leucine may be substituted with valine, or isoleucine.

The term "human antibody" refers to an antibody derived from human immunoglobulin gene sequences. These fully human antibodies provide an alternative to re-engineered, or de-immunized, rodent monoclonal antibodies (e.g. humanised antibodies) as a source of low immunogenicity therapeutic antibodies and they are normally generated using either phage display or transgenic mouse platforms In an embodiment, an antibody of the invention is a human antibody.

The phrase "immunoglobulin single variable domain" refers to an antibody variable domain (V_H, V_{HH}, V_L) that specifically binds an antigen or epitope independently of a different V region or domain. An immunoglobulin single variable domain can be present in a format (*e.g.,* homo- or hetero-multimer) with other, different variable regions or variable domains where the other regions or domains are not required for antigen binding by the single immunoglobulin variable domain (*i.e.*, where the immunoglobulin single variable domain binds antigen independently of the additional variable domains). A "domain antibody" or "dAb" is the same as an "immunoglobulin single variable domain" which is capable of binding to an antigen as the term is used herein. An immunoglobulin single variable domain may be a human antibody variable domain, but also includes single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and *Camelid* V_{HH} dAbs. Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanised according to standard techniques available in the art, and such domains are still considered to be "domain antibodies" according to the invention. As used herein "V_H includes camelid V_{HH} domains. NARV are another type of immunoglobulin single variable domain which were identified in cartilaginous fish including the nurse shark. These domains are also known as Novel Antigen Receptor variable region (commonly abbreviated to V(NAR) or NARV). For further details see Mol. Immunol. (2006) 44: 656-665 and US20050043519A.

The term "Epitope-binding domain" refers to a domain that specifically binds an antigen or epitope independently of a different V region or domain, this may be a domain antibody (dAb), for example a human, camelid or shark immunoglobulin single variable domain or it may be a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-

body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin); which have been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand.

CTLA-4 (Cytotoxic T Lymphocyte-associated Antigen 4) is a CD28-family receptor expressed on mainly CD4+ T-cells. Its extracellular domain has a variable domain-like Ig fold. Loops corresponding to CDRs of antibodies can be substituted with heterologous sequence to confer different binding properties. CTLA-4 molecules engineered to have different binding specificities are also known as Evibodies. For further details see Journal of Immunological Methods (2001) 248 (1-2): 31-45.

Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid β-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details see Biochim Biophys Acta (2000) 1482: 337-350, US7250297B1 and US20070224633.

An affibody is a scaffold derived from Protein A of *Staphylococcus aureus* which can be engineered to bind to antigen. The domain consists of a three-helical bundle of approximately 58 amino acids. Libraries have been generated by randomisation of surface residues. For further details see Protein Eng. Des. Sel. (2004) 17: 455-462 and EP1641818A1.

Avimers are multidomain proteins derived from the A-domain scaffold family. The native domains of approximately 35 amino acids adopt a defined disulphide bonded structure. Diversity is generated by shuffling of the natural variation exhibited by the family of A-domains. For further details see Nature Biotechnology (2205) 23(12): 1556 - 1561 and Expert Opinion on Investigational Drugs (June 2007) 16(6): 909-917.

A transferrin is a monomeric serum transport glycoprotein. Transferrins can be engineered to bind different target antigens by insertion of peptide sequences in a

permissive surface loop. Examples of engineered transferrin scaffolds include the Trans-body. For further details see J. Biol. Chem (1999) 274: 24066-24073.

Designed Ankyrin Repeat Proteins (DARPins) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two α-helices and a β-turn. They can be engineered to bind different target antigens by randomising residues in the first α-helix and a β-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. (2003) 332: 489-503; PNAS (2003) 100(4): 1700-1705; and J. Mol. Biol. (2007) 369: 1015-1028 and US20040132028A1.

Fibronectin is a scaffold which can be engineered to bind to antigen. Adnectins consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3). Three loops at one end of the β-sandwich can be engineered to enable an Adnectin to specifically recognize a therapeutic target of interest. For further details see Protein Eng. Des. Sel. (2005) 18: 435-444, US20080139791, WO2005056764 and US6818418B1.

Peptide aptamers are combinatorial recognition molecules that consist of a constant scaffold protein, typically thioredoxin (TrxA) which contains a constrained variable peptide loop inserted at the active site. For further details see Expert Opin. Biol. Ther. (2005) 5: 783-797.

Microbodies are derived from naturally occurring microproteins of 25-50 amino acids in length which contain 3-4 cysteine bridges – examples of microproteins include KalataB1 and conotoxin and knottins. The microproteins have a loop which can be engineered to include up to 25 amino acids without affecting the overall fold of the microprotein. For further details of engineered knottin domains, see WO2008098796.

Other epitope binding domains include proteins which have been used as a scaffold to engineer different target antigen binding properties including human γ-crystallin and human ubiquitin (affilins), kunitz type domains of human protease inhibitors, PDZ-domains of the Ras-binding protein AF-6, scorpion toxins (charybdotoxin), C-type lectin domain (tetranectins), as reviewed in Chapter 7 – Non-Antibody Scaffolds from Handbook of Therapeutic Antibodies (2007, edited by Stefan Dubel) and Protein

Science (2006) 15:14-27. Epitope binding domains of the present invention could be derived from any of these alternative protein domains.

A "dual variable domain immunoglobulin (DVD-Ig)" is a dual-specific, tetravalent immunoglobulin G (IgG)-like molecule (Wu *et al.* Nature Biotechnology (2007) 25: 1290-1297). A DVD-Ig can be defined as a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VDI-(XI)n-VD2-C-(X2)n, wherein VDI is a first variable domain, VD2 is a second variable domain, C is a constant domain, XI represents an amino acid or polypeptide (linker), X2 represents an Fc region and n is 0 or 1 (WO 2007024715). In the context of the present invention VDI binds to TNFα or a TNFα receptor, and VD2 binds to VEGF or a VEGF receptor, or vice versa.

As used herein, the terms "paired V_H domain", "paired V_L domain", and "paired V_H/V_L domain(s)" refer to antibody variable domains which specifically bind antigen only when paired with their partner variable domain. There is always one V_H and one V_L in any pairing, and the term "paired V_H domain" refers to the V_H partner, the term "paired V_L domain" refers to the V_L partner, and the term "paired V_H/V_L domain(s)" refers to the two domains together.

The term "antigen binding protein" as used herein refers to antibodies, antibody fragments, for example a domain antibody (dAb), ScFv, FAb, FAb_2, and other protein constructs, such as receptor-Fc fusions, which are capable of binding to TNFα and/or VEGF. Antigen binding molecules may comprise at least one Ig variable domain, for example antibodies, domain antibodies, multiples of domain antibodies e.g. dumbbells, dAb-dAb in-line fusions, Fab, Fab', F(ab')2, Fv, ScFv, diabodies, mAbdAbs, DVD-Igs, affibodies, heteroconjugate antibodies or bispecifics, including a bispecific antibody with a first specificity for TNFα or a TNFα receptor and a second specificity for VEGF or a VEGF receptor. In one embodiment the antigen binding molecule is an antibody. In another embodiment the antigen binding molecule is a dAb, i.e. an immunoglobulin single variable domain such as a V_H, V_{HH} or V_L that specifically binds an antigen or epitope independently of a different V region or domain. Antigen binding molecules may be capable of binding to two targets, i.e. they may be dual targeting proteins. Antigen binding molecules may be a combination of antibodies and antigen binding fragments such as for example, one or more domain antibodies and/or one or more ScFvs linked to a monoclonal antibody. Antigen binding molecules may also comprise a non-Ig domain for example a domain

which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin); which have been subjected to protein engineering in order to obtain binding to TNFα and/or VEGF. As used herein "antigen binding protein" will be capable of antagonising and/or neutralising human TNFα and/or VEGF. In addition, an antigen binding protein may block TNFα and/or VEGF activity by binding to TNFα and/or VEGF and preventing a natural ligand from binding and/or activating the receptor.

As used herein "VEGF antagonist" includes any compound capable of reducing and/or eliminating at least one activity of VEGF. By way of example, a VEGF antagonist may bind to VEGF and that binding may directly reduce or eliminate VEGF activity or it may work indirectly by blocking at least one ligand from binding the receptor.

As used herein "TNFα antagonist" includes any compound capable of reducing and/or eliminating at least one activity of TNFα. By way of example, a TNFα antagonist may bind to TNFα and that binding may directly reduce or eliminate TNFα activity or it may work indirectly by blocking at least one ligand from binding the receptor.

The term "specifically binds" as used in relation to antigen binding proteins means that the antigen binding protein binds to it's target protein(s) (e.g. TNFα, TNFR, BEGF, VEGFR) with no or insignificant binding to other (for example, unrelated) proteins. The term, however, does not exclude the fact that an antibody to a target protein in a given species (e.g. human) may also be cross-reactive with other forms of the target protein in other species (e.g. a non-human primate).

The term "KD" refers to the equilibrium dissociation constant. In one embodiment of the invention the antigen-binding site binds to antigen with a KD of at most 1mM, for example a KD of 10nM, 1nM, 500pM, 200pM, 100pM, to each antigen as measured by Biacore™. In one embodiment of the invention the antigen-binding site binds to antigen with a KD 10nM or less, 1nM or less, 500pM or less, 200pM or less, 100pM or less, to each antigen as measured by Biacore™.

As used herein, the term "antigen-binding site" refers to a site on a construct which is capable of specifically binding to antigen, this may be a single domain, for example an epitope-binding domain, or it may be paired V_H/V_L domains as can be found on a standard antibody. In some aspects of the invention single-chain Fv (ScFv) domains can provide antigen-binding sites.

The terms "mAb/dAb" and dAb/mAb" are used herein to refer to antigen-binding proteins of the present invention. The two terms can be used interchangeably, and are intended to have the same meaning as used herein.

The term "constant heavy chain 1" is used herein to refer to the constant domain of an immunoglobulin heavy chain, C_H1.

The term "constant light chain" is used herein to refer to the constant domain of an immunoglobulin light chain, C_L.

The term "library" refers to a mixture of heterogeneous polypeptides or nucleic acids. The library is composed of members, each of which has a single polypeptide or nucleic acid sequence. To this extent, "library" is synonymous with "repertoire."

A "universal framework" is a single antibody framework sequence corresponding to the regions of an antibody conserved in sequence as defined by Kabat ("Sequences of Proteins of Immunological Interest", US Department of Health and Human Services) or corresponding to the human germline immunoglobulin repertoire or structure as defined by Chothia and Lesk, J. Mol. Biol. (1987) 196: 910-917.

Detailed description of Invention

The present invention provides compositions comprising a TNFα antagonist and/or a VEGF antagonist suitable for use in the eye. The present invention also provides the combination of a TNFα antagonist and a VEGF antagonist, for use in preventing or treating diseases of the eye. The present invention also provides a method of preventing or treating diseases of the eye by administering a TNFα antagonist in combination with a VEGF antagonist. The TNFα antagonist and the VEGF antagonist may be administered separately, sequentially or simultaneously.

The administration of a combination of an individual TNFα antagonist and an individual VEGF antagonist (i.e. separate TNFα and VEGF antagonist molecules) is covered by the present invention. In addition, the administration of a single molecule or construct capable of binding to two or more antigens is covered by the present invention e.g. a molecule with dual targeting functionality (i.e. able to bind to and inhibit, preferably block, the function of TNFα or a receptor for TNFα, and bind to and inhibit, preferably block, the function of VEGF or a receptor for VEGF) that acts as both a TNFα antagonist and a VEGF antagonist, is covered by the present invention. For example, the invention provides a dual targeting molecule which is capable of binding to TNFα and VEGFR2, and so forth. In an embodiment the dual targeting molecule is capable of binding to a TNF receptor and a VEGF receptor.

The TNFα antagonist of the invention may inhibit signalling through a TNF receptor by 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98% or 100%. The VEGF antagonist of the invention may inhibit signalling through a VEGF receptor by 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98% or 100%.

In an embodiment the TNFα antagonist is a human antigen binding protein, in particular a human anti-TNFα antibody or fragment thereof, or a human anti-TNFR antibody or fragment thereof. In an embodiment the VEGF antagonist is a human antigen binding protein, in particular a human anti-VEGF antibody or fragment thereof, or a human anti-VEGFR antibody or fragment thereof. In an embodiment the antigen binding protein is a TNFα/VEGF dual targeting single construct wherein the TNFα antagonist portion is human. In a particular embodiment, the TNF antagonist is or is derived from adalimumab or golimumab.

The antagonists may be based on an antibody scaffold or other such suitable scaffold as described herein. Such antagonists may be antibodies or epitope binding domains for example dAbs. Receptor-Fc fusions are considered part of the invention.

The antagonists of the invention may be co-administered as a mixture of separate molecules which are administered at the same time (simultaneously) , or are administered within a specified period of each other (sequentially), for example within a month, a week or within 24 hours of each other, for example within 20 hours, or within 15 hours or within 12 hours, or within 10 hours, or within 8 hours, or within 6 hours, or within 4 hours, or within 2 hours, or within 1 hour, or within 30 minutes of

each other. The antagonists of the invention may be co-administered as separate formulations or as a single formulation, e.g. liposomes containing both antagonists.

TNFα antagonists within the scope of the invention, which may be administered in combination with a VEGF antagonist of the invention, or which may be used in generating dual targeting molecules of the invention, include those listed below in table 1.

Table 1: TNFα antagonists

Name	Format	SEQ ID NO
Adalimumab (Humira™)	Human mAb	10 (heavy chain) 12 (light chain)
Infliximab (Remicade™)	Chimaeric mAb	32 (heavy chain) 33 (light chain)
Etanercept (Enbrel™)	TNF Receptor-Fc fusion	34
ESBA105	Humanised scFv	38
PEP1-5-19	Human Vκ dAb	35
PEP1-5-490	Human Vk dAb	36
PEP1-5-493	Human Vk dAb	37
-	Adnectin	2
Golimumab (Simponi™)	Human mAb	-
Certolizumab (Cimiza™)	Humanised Fab (PEGylated)	-
ALK-6931	TNF Receptor-Fc(IgG1) fusion	-

In addition to the TNFα antagonists identified by name in Table 1, a TNFα antagonist according to the invention includes an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO: 31.

VEGF antagonists within the scope of the invention, which may be administered in combination with a TNFα antagonist of the invention, or which may be used in generating dual targeting molecules of the invention, include those listed below in table 2.

Table 2: VEGF antagonists

Name	Format	SEQ ID NO
Bevacizumab (Avastin™)	Humanised mAb	22 (heavy chain) 21 (light chain)
Ranibizumab (Lucentis™)	Humanised Fab	39 (heavy chain) 40 (light chain)
r84	Humanised mAb	41 (VH) 42 (VL)
Aflibercept (VEGF-Trap)	Receptor-Fc fusion	43
CT01	Adnectin	45
DOM15-10-11	Human Vκ dAb	44
DOM15-26-593	Human Vκ dAb	1
PRS-050	Anticalin	-
PRS-051	Anticalin	-
MP0112	Darpin	-
CT-322	Humanised scFv	-
ESBA903	Humanised scFv	-
EPI-0030	Humanised mAb	-
EPI-0010	Humanised mAb	-
DMS1571	Fc formatted version of DOM15-26-593 human Vκ dAb (exists as a dimer of this sequence)	65

The present invention provides an antigen-binding protein for use in treating diseases of the eye comprising a protein scaffold which is linked to one or more epitope-binding domains wherein the antigen-binding protein has at least two antigen-binding sites at least one of which is from an epitope binding domain and at least one of which is from a paired V_H/V_L domain and wherein at least one of the antigen-binding sites binds to TNFα, or a receptor for TNFα, and at least one of the antigen-binding sites binds to VEGF, or a receptor for VEGF.

Such antigen-binding proteins comprise a protein scaffold, for example an Ig scaffold such as IgG, for example a monoclonal antibody, which is linked to one or more epitope-binding domains, for example a domain antibody, wherein the binding protein has at least two antigen-binding sites, at least one of which is from an epitope

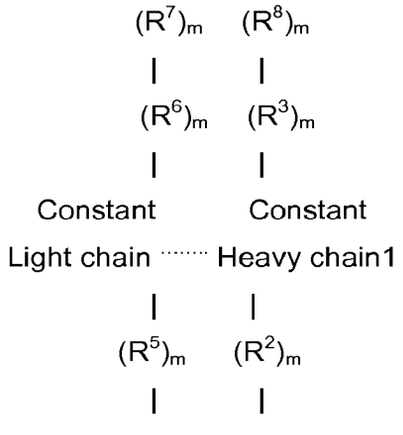
binding domain, and wherein at least one of the antigen-binding sites binds to TNFα, or a receptor for TNFα, at least one of the antigen-binding sites binds to VEGF, or a receptor for VEGF, and to methods of producing and uses thereof, particularly uses in ocular therapy.

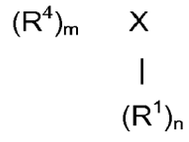
Such antigen-binding proteins of the present invention are also referred to as mAbdAbs.

In one embodiment the protein scaffold of the antigen-binding protein of the present invention is an Ig scaffold, for example an IgG scaffold or IgA scaffold. The IgG scaffold may comprise all the domains of an antibody (i.e. C_H1, C_H2, C_H3, V_H, V_L, C_L). The antigen-binding protein of the present invention may comprise an IgG scaffold selected from IgG1, IgG2, IgG3, IgG4 or IgG4PE.

The antigen-binding protein of the present invention has at least two antigen-binding sites, for example it has two binding sites, for example where the first binding site has specificity for a first epitope on an antigen and the second binding site has specificity for a second epitope on the same antigen. In a further embodiment there are 4 antigen-binding sites, or 6 antigen-binding sites, or 8 antigen-binding sites, or 10 or more antigen-binding sites. In one embodiment the antigen-binding protein has specificity for more than one antigen, for example two antigens, or for three antigens, or for four antigens.

In another aspect, the invention relates to an antigen-binding protein which is capable of binding to TNFα, or a TNFα receptor, and VEGF, or a VEGF receptor, comprising at least one homodimer comprising two or more structures of formula I:





(I)

wherein

X represents a constant antibody region comprising constant heavy domain 2 (C_H2) and constant heavy domain 3 (C_H3);

R^1, R^4 , R^7 and R^8 each represent an epitope-binding domain;

R^2 represents a domain selected from the group consisting of constant heavy chain 1 (C_H1), and an epitope-binding domain;

R^3 represents a domain selected from the group consisting of a paired V_H and an epitope-binding domain;

R^5 represents a domain selected from the group consisting of constant light chain (C_L), and an epitope-binding domain;

R^6 represents a domain selected from the group consisting of a paired V_L and an epitope-binding domain;

n represents an integer independently selected from: 0, 1, 2, 3 and 4;
m represents an integer independently selected from: 0 and 1,

wherein the Constant Heavy chain 1 (C_H1) and the Constant Light chain (C_L) domains are associated;

wherein at least one epitope binding domain is present;

and when R^3 represents a paired V_H domain, R^6 represents a paired V_L domain, so that the two domains are together capable of binding antigen.

In one embodiment R^6 represents a paired V_L and R^3 represents a paired V_H.

In a further embodiment either one or both of R^7 and R^8 represent an epitope binding domain.

In yet a further embodiment either one or both of R^1 and R^4 represent an epitope binding domain.

In one embodiment R^4 is present.

In one embodiment R^1, R^7 and R^8 represent an epitope binding domain.

In one embodiment R^1, R^7 and R^8, and R^4 represent an epitope binding domain.

In one embodiment $(R^1)_n$, $(R^2)_m$, $(R^4)_m$ and $(R^5)_m$ = 0, i.e. are not present, R^3 is a paired V_H domain, R^6 is a paired V_L domain, R^8 is a V_H dAb, and R^7 is a V_L dAb.

In another embodiment $(R^1)_n$, $(R^2)_m$, $(R^4)_m$ and $(R^5)_m$ are 0, i.e. are not present, R^3 is a paired V_H domain, R^6 is a paired V_L domain, R^8 is a V_H dAb, and $(R^7)_m$ = 0 i.e. not present.

In another embodiment $(R^2)_m$, and $(R^5)_m$ are 0, i.e. are not present, R^1 is a dAb, R^4 is a dAb, R^3 is a paired V_H domain, R^6 is a paired V_L domain, $(R^8)_m$ and $(R^7)_m$ = 0 i.e. not present.

In one embodiment of the present invention the epitope binding domain is a dAb.

In another aspect of the invention, the antigen binding protein is a bispecific antibody having a first specificity for TNFα or a TNFα receptor, and a second specificity for VEGF or a VEGF receptor.

In a further aspect of the invention, the antigen binding protein is a dual variable domain immunoglobulin (DVD-Ig).

In another aspect of the invention, the antigen binding protein is a dAb-dAb in-line fusion.

In another aspect of the invention, the antigen binding protein is a Receptor-Fc fusion, which may be linked to one or more epitope-binding domains. Receptor-Fc

fusions comprise an immunoglobulin scaffold i.e. they comprise the Fc portion of an antibody, which is linked to a soluble ligand or extracellular domain of a receptor or cell surface protein and one or more epitope binding domains. Such receptor-Fc-epitope binding domain fusions may also be referred to as receptor-Ig-epitope binding domain fusions. The Fc portion may be selected from antibodies of any isotype, for example IgG1, IgG2, IgG3, IgG4 or IgG4PE.

In one embodiment the antigen-binding proteins of the invention have specificity for VEGF, for example they comprise a receptor-Fc fusion linked to an epitope binding domain which binds to VEGF, for example a dAb, an anticalin, or an adnectin which binds to VEGF.

In one embodiment the antigen-binding proteins of the invention have specificity for VEGFR2, for example they comprise a receptor-Fc fusion linked to an epitope binding domain which binds to VEGFR2, for example a dAb or an adnectin which binds to VEGFR2.

In one embodiment the antigen-binding proteins of the invention have specificity for TNFα, for example they comprise a receptor-Fc fusion linked to an epitope binding domain which binds to TNFα, for example a dAb or an adnectin which binds to TNFα.

In an embodiment the antigen binding proteins of the invention have specificity for both TNFα or a TNFα receptor, and VEGF or a VEGF receptor, for example they comprise a TNFα receptor-Fc fusion linked to an epitope binding domain which binds to VEGF or a VEGF receptor. Another example, is an antigen binding protein that comprises a VEGF receptor-Fc fusion linked to an epitope binding domain which binds to TNFα or a TNFα receptor.

It will be understood that any of the antigen-binding proteins described herein will be capable of neutralising one or more antigens, for example they will be capable of neutralising TNFα and/or they will also be capable of neutralising VEGF.

The term "neutralises" and grammatical variations thereof as used throughout the present specification in relation to antigen-binding proteins of the invention means that a biological activity of the target is reduced, either totally or partially, in the presence of the antigen-binding proteins of the present invention in comparison to the activity of the target in the absence of such antigen-binding proteins.

Neutralisation may be due to but not limited to one or more of blocking ligand binding, preventing the ligand activating the receptor, down regulating the receptor or affecting effector functionality.

Levels of neutralisation can be measured in several ways, for example in an IL-8 secretion assay in MRC-5 cells which may be carried out for example as described in Example 1.3. The neutralisation of TNFα in this assay is measured by assessing the inhibition of IL-8 secretion in the presence of neutralising antigen-binding protein. Levels of neutralisation could also be measured in an assay which measures inhibition of ligand binding to receptor which may be carried out for example as described in Example 1.3. The neutralisation of VEGF, in this assay is measured by assessing the decreased binding between the ligand and its receptor in the presence of neutralising antigen-binding protein.

Other methods of assessing neutralisation, for example, by assessing the decreased binding between the ligand and its receptor in the presence of neutralising antigen-binding protein are known in the art, and include, for example, Biacore™ assays.

In an alternative aspect of the present invention there is provided antigen-binding proteins which have at least substantially equivalent neutralising activity to the antigen binding proteins exemplified herein.

The antigen-binding proteins of the invention have specificity for TNFα or TNFα receptor, for example they comprise an epitope-binding domain which is capable of binding to TNFα, and/or they comprise a paired V_H/V_L which binds to TNFα. The antigen-binding protein may comprise an antibody which is capable of binding to TNFα. The antigen-binding protein may comprise a dAb which is capable of binding to TNFα.

The antigen-binding protein of the present invention also has specificity for VEGF or a receptor for VEGF. In one embodiment the antigen-binding protein of the present invention is capable of binding TNFα and VEGF simultaneously.

It will be understood that any of the antigen-binding proteins described herein may be capable of binding two or more antigens simultaneously, for example, as determined by stochiometry analysis by using a suitable assay such as that described in Example 3.

Examples of such antigen-binding proteins include VEGF antibodies which have an epitope binding domain which is a TNFα antagonist, for example an anti-TNFα adnectin, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain. Examples include an antigen binding protein comprising the heavy chain sequence set out in SEQ ID NO: 20 or 22 and the light chain sequence set out in SEQ ID NO: 21, wherein one or both of the Heavy and Light chain further comprise one or more epitope-binding domains which bind to TNFα, for example an epitope binding domain selected from those set out in SEQ ID NO: 2 and SEQ ID NO: 17.

In one embodiment the antigen-binding protein will comprise an anti-VEGF antibody linked to an epitope binding domain which is a TNFα antagonist, wherein the anti-VEGF antibody has the same CDRs as the antibody which has the heavy chain sequence of SEQ ID NO:20 or 22, and the light chain sequence of SEQ ID NO: 21.

Examples of such antigen-binding proteins include TNFα antibodies which have an epitope binding domain which is a VEGF antagonist attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus. Examples include an antigen binding protein comprising the heavy chain sequence set out in SEQ ID NO: 10 and the light chain sequence set out in SEQ ID NO: 12 wherein one or both of the Heavy and Light chain further comprise one or more epitope-binding domains which is capable of antagonising VEGF, for example by binding to VEGF or to a VEGF receptor for example VEGFR2. Such epitope-binding domains can be selected from those set out in SEQ ID NO: 1, 18, 19, 23 or 44.

In one embodiment the antigen binding constructs of the present invention comprise the heavy chain sequence of SEQ ID NO: 14 and the light chain sequence of SEQ ID NO: 12, or the heavy chain sequence of SEQ ID NO: 15 and the light chain sequence of SEQ ID NO: 12, or the heavy chain sequence of SEQ ID NO: 24 and the light chain sequence of SEQ ID NO: 12.

In an embodiment, the antigen binding constructs of the present invention comprise an anti-TNFα binding protein as disclosed in WO0212502, US2007/0003548, US7250165, EP01309691, or WO0212500, all of which are herein incorporated by reference in their entirety.

In one embodiment the antigen-binding protein will comprise an anti-TNFα antibody linked to an epitope binding domain which is a VEGF antagonist, wherein the anti-TNFα antibody has the same CDRs as the antibody which has the heavy chain sequence of SEQ ID NO:10, and the light chain sequence of SEQ ID NO: 12.

Other examples of such antigen-binding proteins include anti-TNFα antibodies which have an anti-VEGF epitope binding domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain wherein the VEGF epitope binding domain is a VEGF dAb which is selected from any of the VEGF dAb sequences which are set out in WO2007080392 (which is incorporated herein by reference), in particular the dAbs which are set out in SEQ ID NO:117, 119, 123, 127-198, 539 and 540; or a VEGF dAb which is selected from any of the VEGF dAb sequences which are set out in WO2008149146 (which is incorporated herein by reference), in particular the dAbs which are described as DOM15-26-501, DOM15-26-555, DOM15-26-558, DOM15-26-589, DOM15-26-591, DOM15-26-594 and DOM15-26-595, or a VEGF dAb which is selected from any of the VEGF dAb sequences which are set out in WO2007066106 (which is incorporated herein by reference), or a VEGF dab which is selected from any of the VEGF dAb sequences which are set out in WO 2008149147 (which is incorporated herein by reference) or a VEGF dab which is selected from any of the VEGF dAb sequences which are set out in WO 2008149150 (which is incorporated herein by reference).

These specific sequences and related disclosures in WO2007080392, WO2008149146, WO2007066106, WO2008149147 and WO 2008149150 are incorporated herein by reference as though explicitly written herein with the express intention of providing disclosure for incorporation into claims herein and as examples of variable domains and antagonists for application in the context of the present invention.

Other examples of such antigen-binding constructs include anti-VEGF antibodies which have one or more anti-TNFalpha epitope binding domains, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain wherein the TNFalpha epitope binding domain is a TNF-alpha dAb which is selected from any of the TNFalpha dAbs disclosed in WO04003019 (which is incorporated herein by reference), in particular the dAbs which are described as TAR1-5-19, TAR1-5, and TAR1-27. These specific sequences and related disclosures in WO04003019 are incorporated herein by reference as though explicitly

written herein with the express intention of providing disclosure for incorporation into claims herein and as examples of variable domains and antagonists for application in the context of the present invention.

Other examples of such antigen-binding constructs include anti-VEGF antibodies which have one or more anti-TNFR1 epitope binding domains, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain wherein the TNFR1 epitope binding domain is a TNFR1 dAb which is selected from any of the TNFR1 dAb sequences in WO04003019 (which is incorporated herein by reference), in particular the dAbs which are described as TAR2-10, and TAR2-5; or a TNFR1 dAb which is selected from any of the TNFR1 dAb sequences in WO2006038027 (which is incorporated herein by reference), in particular the dAbs which are set out in SEQ ID NO: 32-98, 167-179, 373-401, 431, 433-517 and 627; or a TNFR1 dAb which is selected from any of the TNFR1 dAb sequences in WO2008149144 (which is incorporated herein by reference), in particular the dAbs which are described as DOM1h-131-511, DOM1h-131-201, DOM1h-131-202, DOM1h-131-203, DOM1h-131-204, DOM1h-131-205; or a TNFR1 dAb which is selected from any of the TNFR1 dAb sequences in WO2008149148 (which is incorporated herein by reference), in particular the dAb which is described as DOM1h-131-206.

These specific sequences and related disclosures in WO2006038027 and WO2008149144 are incorporated herein by reference as though explicitly written herein with the express intention of providing disclosure for incorporation into claims herein and as examples of variable domains and antagonists for application in the context of the present invention.

Further examples of antigen-binding proteins include TNFR2-Ig fusions linked to an epitope binding domain with a specificity for VEGFR2, for example an anti- VEGFR2 adnectin, linked to the c-terminus or the n-terminus of the TNFR2-Ig fusion, for example an antigen-binding protein comprising the TNFR2-Ig sequence set out in SEQ ID NO:34 which further comprises one or more epitope-binding domains which bind to VEGFR2, for example the adnectin set out in SEQ ID NO:18.

Other examples of such antigen-binding proteins include TNFR2-Ig fusions linked to an epitope binding domain with a specificity for VEGF for example an anti- VEGF dAb or anti-VEGF anticalin, linked to the c-terminus or the n-terminus of the TNFR2-

Ig fusion, for example a Receptor-Fc-epitope binding domain fusion comprising the TNFR2-Ig sequence set out in SEQ ID NO:34, which further comprises one or more epitope-binding domains which bind to VEGF, for example the dAb set out in SEQ ID NO:1, or the anticalin set out in SEQ ID NO:19.

Throughout this specification, amino acid residues in variable domain sequences and full length antibody sequences are numbered according to the Kabat numbering convention. Similarly, the terms "CDR", "CDRL1", "CDRL2", "CDRL3", "CDRH1", "CDRH2", "CDRH3" used in the Examples follow the Kabat numbering convention. For further information, see Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987).

However, although we use the Kabat numbering convention for amino acid residues in variable domain sequences and full length antibody sequences throughout this specification, it will be apparent to those skilled in the art that there are alternative numbering conventions for amino acid residues in variable domain sequences and full length antibody sequences. There are also alternative numbering conventions for CDR sequences, for example those set out in Chothia et al. (1989) Nature 342: 877-883. The structure and protein folding of the antibody may mean that other residues are considered part of the CDR sequence and would be understood to be so by a skilled person.

Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods. The minimum overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the "minimum binding unit". The minimum binding unit may be a sub-portion of a CDR.

Antigen binding proteins with CDR variants are also considered part of the invention. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/or the c-terminus and/or n-terminus of the light chain and/or the n-terminus or c-terminus of the receptor-Fc or receptor-Fc-dAb fusion..

In one embodiment of the present invention there is provided an antigen-binding protein according to the invention described herein and comprising a constant region such that the antibody or receptor-Fc fusion has reduced ADCC and/or complement activation or effector functionality. In one such embodiment the heavy chain constant region may comprise a naturally disabled constant region of IgG2 or IgG4 isotype or a mutated IgG1 constant region. Examples of suitable modifications are described in EP0307434. One example comprises the substitutions of alanine residues at positions 235 and 237 (EU index numbering, Kabat *et al.*, (1983) "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services).

In an embodiment, the Fc portion of the antigen binding protein is functionally disabled. Such Fc disablement may provide the antigen binding protein with an improved safety profile.

The invention also provides a method of reducing CDC function of antigen-binding proteins by positioning of the epitope binding domain on the heavy chain of the antibody, in particular, by positioning the epitope binding domain on the c-terminus of the heavy chain.

In one embodiment the antigen-binding proteins of the present invention will retain Fc functionality for example will be capable of one or both of ADCC and CDC activity.

The antigen-binding proteins of the invention may have some effector function. For example if the Immunoglobulin scaffold contains an Fc region derived from an antibody with effector function, for example if the Immunoglobulin scaffold comprises CH2 and CH3 from IgG1. Levels of effector function can be varied according to known techniques, for example by mutations in the CH2 domain, for example wherein the IgG1 CH2 domain has one or more mutations at positions selected from 239 and 332 and 330, for example the mutations are selected from S239D and I332E and A330L such that the antibody has enhanced effector function, and/or for example altering the glycosylation profile of the antigen-binding protein of the invention such that there is a reduction in fucosylation of the Fc region.

In one embodiment, the antigen-binding proteins comprise an epitope-binding domain which is a domain antibody (dAb), for example the epitope binding domain may be a human V_H or human V_L, or a camelid V_{HH} or a shark dAb (NARV).

In one embodiment the antigen-binding proteins comprise an epitope-binding domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin); which have been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand.

The antigen-binding proteins of the present invention may comprise a protein scaffold attached to an epitope binding domain which is an adnectin, for example an IgG scaffold with an adnectin attached to the c-terminus of the heavy chain, or it may comprise a protein scaffold attached to an adnectin, for example an IgG scaffold with an adnectin attached to the n-terminus of the heavy chain, or it may comprise a protein scaffold attached to an adnectin, for example an IgG scaffold with an adnectin attached to the c-terminus of the light chain, or it may comprise a protein scaffold attached to an adnectin, for example an IgG scaffold with an adnectin attached to the n-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a CTLA-4, for example an IgG scaffold with a CTLA-4 attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a CTLA-4 attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with CTLA-4 attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with CTLA-4 attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a lipocalin, for example an IgG scaffold with a lipocalin attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a lipocalin attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a lipocalin attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a lipocalin attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is an SpA, for example an IgG scaffold with an SpA attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an SpA attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an SpA attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with an SpA attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is an affibody, for example an IgG scaffold with an affibody attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affibody attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affibody attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with an affibody attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is an affimer, for example an IgG scaffold with an affimer attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affimer attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affimer attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with an affimer attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a GroEl, for example an IgG scaffold with a GroEl attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroEl attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroEl attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a GroEl attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a transferrin, for example an IgG scaffold with a transferrin attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a transferrin attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a transferrin

attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a transferrin attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a GroES, for example an IgG scaffold with a GroES attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroES attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroES attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a GroES attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a DARPin, for example an IgG scaffold with a DARPin attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a DARPin attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a DARPin attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a DARPin attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a peptide aptamer, for example an IgG scaffold with a peptide aptamer attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a peptide aptamer attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a peptide aptamer attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a peptide aptamer attached to the c-terminus of the light chain.

In one embodiment of the present invention there are four epitope binding domains, for example four domain antibodies, two of the epitope binding domains may have specificity for the same antigen, or all of the epitope binding domains present in the antigen-binding protein may have specificity for the same antigen.

Protein scaffolds of the present invention may be linked to epitope-binding domains by the use of linkers. Similarly receptor-Fc fusions of the present invention may be linked to epitope binding domains by the use of linkers. Also VDI and VD2 domains of DVD-Igs may be linked together by means of linkers, and so forth. Examples of

suitable linkers include amino acid sequences which may be from 1 amino acid to 150 amino acids in length, or from 1 amino acid to 140 amino acids, for example, from 1 amino acid to 130 amino acids, or from 1 to 120 amino acids, or from 1 to 80 amino acids, or from 1 to 50 amino acids, or from 1 to 20 amino acids, or from 1 to 10 amino acids, or from 5 to 18 amino acids. Such sequences may have their own tertiary structure, for example, a linker of the present invention may comprise a single variable domain. The size of a linker in one embodiment is equivalent to a single variable domain. Suitable linkers may be of a size from 1 to 20 angstroms, for example less than 15 angstroms, or less than 10 angstroms, or less than 5 angstroms.

In one embodiment of the present invention at least one of the epitope binding domains is directly attached to the Ig scaffold with a linker comprising from 1 to 150 amino acids, for example 1 to 20 amino acids, for example 1 to 10 amino acids.

Such linkers may be selected from any one of those set out in SEQ ID NO: 3-8, SEQ ID NO:25, or SEQ ID NO:66-68, or multiples of such linkers. For example, the linker may be 'TVAAPS', or the linker may be 'GGGGS', or multiples of such linkers.

In an embodiment of the invention the linker is 'STG' (SEQ ID NO:25).

A linker can be any linker as herein described with one or two amino acid changes. Linkers of use in the antigen-binding proteins of the present invention may comprise alone or in addition to other linkers, one or more sets of GS residues, for example 'GSTVAAPS' or 'TVAAPSGS' or 'GSTVAAPSGS', or multiples of such linkers. In an embodiment the linker comprises or consists of 'GSTVAAPSGS'.

In an embodiment the linker comprises or consists of GS(TVAAPSGS) x2 (e.g. 'GSTVAAPSGSTVAAPSGS' SEQ ID NO:66). In an embodiment the linker comprises or consists of GS(TVAAPSGS) x 3 (e.g. 'GSTVAAPSGSTVAAPSGSTVAAPSGS' SEQ ID NO:67). In an embodiment the linker comprises or consists of GS(TVAAPSGS) x 4 (e.g. 'GSTVAAPSGSTVAAPSGS TVAAPSGSTVAAPSGS' SEQ ID NO:68).

In one embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(PAS)_n(GS)_m$'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(GGGGS)_{n\ or\ p}(GS)_m$'. In another embodiment the epitope

binding domain is linked to the Ig scaffold by the linker '$(TVAAPS)_{n\ or\ p}(GS)_m$'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(GS)_m(TVAAPSGS)_{n\ or\ p}$'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(GS)_m(TVAAPS)_p(GS)_m$'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(PAVPPP)_n(GS)_m$'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(TVSDVP)_n(GS)_m$'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '$(TGLDSP)_n(GS)_m$'. In all such embodiments, n = 1-10, and m = 0-4, and p=2-10.

Examples of such linkers include $(PAS)_n(GS)_m$ wherein n=1 and m=1 (SEQ ID NO:145), $(PAS)_n(GS)_m$ wherein n=2 and m=1 (SEQ ID NO:146), $(PAS)_n(GS)_m$ wherein n=3 and m=1 (SEQ ID NO:147), $(PAS)_n(GS)_m$ wherein n=4 and m=1, $(PAS)_n(GS)_m$ wherein n=2 and m=0, $(PAS)_n(GS)_m$ wherein n=3 and m=0, $(PAS)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(GGGGS)_n(GS)_m$ wherein n=1 and m=1, $(GGGGS)_n(GS)_m$ wherein n=2 and m=1, $(GGGGS)_n(GS)_m$ wherein n=3 and m=1, $(GGGGS)_n(GS)_m$ wherein n=4 and m=1, $(GGGGS)_n(GS)_m$ wherein n=2 and m=0 (SEQ ID NO:148), $(GGGGS)_n(GS)_m$ wherein n=3 and m=0 (SEQ ID NO:149), $(GGGGS)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(GS)_m(TVAAPS)_p$ wherein p=1 and m=1, $(GS)_m(TVAAPS)_p$ wherein p=2 and m=1, $(GS)_m(TVAAPS)_p$ wherein p=3 and m=1, $(GS)_m(TVAAPS)_p$ wherein p=4 and m=1), $(GS)_m(TVAAPS)_p$ wherein p=5 and m=1, or $(GS)_m(TVAAPS)_p$ wherein p=6 and m=1.

Examples of such linkers include $(TVAAPS)_n(GS)_m$ wherein n=1 and m=1, $(TVAAPS)_n(GS)_m$ wherein n=2 and m=1 (SEQ ID NO:150), $(TVAAPS)_n(GS)_m$ wherein n=3 and m=1 (SEQ ID NO:151), $(TVAAPS)_n(GS)_m$ wherein n=4 and m=1, $(TVAAPS)_n(GS)_m$ wherein n=2 and m=0, $(TVAAPS)_n(GS)_m$ wherein n=3 and m=0, $(TVAAPS)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(GS)_m(TVAAPSGS)_n$ wherein n=1 and m=1, $(GS)_m(TVAAPSGS)_n$ wherein n=2 and m=1 (SEQ ID NO:66), $(GS)_m(TVAAPSGS)_n$ wherein n=3 and m=1 (SEQ ID NO:67), or $(GS)_m(TVAAPSGS)_n$ wherein n=4 and m=1 (SEQ ID NO:68), $(GS)_m(TVAAPSGS)_n$ wherein n=5 and m=1 (SEQ ID NO:152),

$(GS)_m(TVAAPSGS)_n$ wherein n=6 and m=1 (SEQ ID NO:153), $(GS)_m(TVAAPSGS)_n$ wherein n=1 and m=0 (SEQ ID NO:8), $(GS)_m(TVAAPSGS)_n$ wherein n=2 and m=10, $(GS)_m(TVAAPSGS)_n$ wherein n=3 and m=0, or $(GS)_m(TVAAPSGS)_n$ wherein n=0. Examples of such linkers include $(TVAAPSGS)_p(GS)_m$ wherein p=2 and m=1, $(TVAAPSGS)_p(GS)_m$ wherein p=3 and m=1 , $(TVAAPSGS)_p(GS)_m$ wherein p=4 and m=1, $(TVAAPSGS)_p(GS)_m$ wherein p=2 and m=0, $(TVAAPSGS)_p(GS)_m$ wherein p=3 and m=0, $(TVAAPSGS)_p(GS)_m$ wherein p=4 and m=0.

Examples of such linkers include $(PAVPPP)_n(GS)_m$ wherein n=1 and m=1 (SEQ ID NO:154), $(PAVPPP)_n(GS)_m$ wherein n=2 and m=1 (SEQ ID NO:155), $(PAVPPP)_n(GS)_m$ wherein n=3 and m=1 (SEQ ID NO:156), $(PAVPPP)_n(GS)_m$ wherein n=4 and m=1, $(PAVPPP)_n(GS)_m$ wherein n=2 and m=0, $(PAVPPP)_n(GS)_m$ wherein n=3 and m=0, $(PAVPPP)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(TVSDVP)_n(GS)_m$ wherein n=1 and m=1 (SEQ ID NO:157), $(TVSDVP)_n(GS)_m$ wherein n=2 and m=1 (SEQ ID NO:158), $(TVSDVP)_n(GS)_m$ wherein n=3 and m=1 (SEQ ID NO:159), $(TVSDVP)_n(GS)_m$ wherein n=4 and m=1, $(TVSDVP)_n(GS)_m$ wherein n=2 and m=0, $(TVSDVP)_n(GS)_m$ wherein n=3 and m=0, $(TVSDVP)_n(GS)_m$ wherein n=4 and m=0.

Examples of such linkers include $(TGLDSP)_n(GS)_m$ wherein n=1 and m=1 (SEQ ID NO:160), $(TGLDSP)_n(GS)_m$ wherein n=2 and m=1 (SEQ ID NO:161), $(TGLDSP)_n(GS)_m$ wherein n=3 and m=1 (SEQ ID NO:162), $(TGLDSP)_n(GS)_m$ wherein n=4 and m=1, $(TGLDSP)_n(GS)_m$ wherein n=2 and m=0, $(TGLDSP)_n(GS)_m$ wherein n=3 and m=0, $(TGLDSP)_n(GS)_m$ wherein n=4 and m=0.

In another embodiment there is no linker between the epitope binding domain and the Ig scaffold. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker 'TVAAPS'. In another embodiment the epitope binding domain, is linked to the Ig scaffold by the linker 'TVAAPSGS'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker 'GS'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker 'ASTKGPT'.

In one embodiment, the antigen-binding protein of the present invention comprises at least one antigen-binding site, for example at least one epitope binding domain, which is capable of binding human serum albumin.

In one embodiment, there are at least 3 antigen-binding sites, for example there are 4, or 5 or 6 or 8 or 10 antigen-binding sites and the antigen-binding protein is capable of binding at least 3 or 4 or 5 or 6 or 8 or 10 antigens, for example it is capable of binding 3 or 4 or 5 or 6 or 8 or 10 antigens simultaneously.

The invention also provides the antigen-binding proteins disclosed herein for use in medicine, for example for use in the manufacture of a medicament for treating a disease of the eye (alternatively referred to herein as an 'eye disease'), for example diabetic macula edema (DME), cystoid macula edema, uveitis, AMD (Age related macular degeneration), choroidal neovascular AMD, geographic atrophy, diabetic retinopathy, retinal vein occlusion (BRVO and/or CRVO) and other maculopathies and ocular vasculopathies. In an embodiment, the disease to be treated is AMD. In another embodiment, the disease to be treated is DME.

The invention provides a method of treating a patient suffering from a disease of the eye, for example diabetic macula edema, cystoid macula edema, uveitis, AMD (Age related macular degeneration), choroidal neovascular AMD, geographic atrophy, diabetic retinopathy, retinal vein occlusion (BRVO and/or CRVO) and other maculopathies and ocular vasculopathies comprising administering a therapeutic amount of an antigen-binding protein of the invention.

The antigen-binding proteins of the invention may be used for the treatment of a disease of the eye, for example diabetic macula edema, cystoid macula edema, uveitis, AMD (Age related macular degeneration), choroidal neovascular AMD, geographic atrophy, diabetic retinopathy, retinal vein occlusion (BRVO and/or CRVO) and other maculopathies and ocular vasculopathies or any other disease associated with the over production of TNFα and/or VEGF.

In a particular embodiment the disease is AMD, specifically choroidal neovascular AMD.

Protein scaffolds of use in the present invention include full monoclonal antibody scaffolds comprising all the domains of an antibody, an Fc portion of a conventional antibody, or protein scaffolds of the present invention may comprise a non-conventional antibody structure, such as a monovalent antibody or an Fc portion of a non-conventional antibody structure. Such monovalent antibodies may comprise a

paired heavy and light chain wherein the hinge region of the heavy chain is modified so that the heavy chain does not homodimerise, such as the monovalent antibody described in WO2007059782. Other monovalent antibodies may comprise a paired heavy and light chain which dimerises with a second heavy chain which is lacking a functional variable region and C_H1 region, wherein the first and second heavy chains are modified so that they will form heterodimers rather than homodimers, resulting in a monovalent antibody with two heavy chains and one light chain such as the monovalent antibody described in WO2006015371. Such monovalent antibodies can provide the protein scaffold of the present invention to which epitope binding domains can be linked. The Fc region of such monovalent antibodies can provide the Immunoglobulin scaffold of the present invention to which soluble ligands, extracellular domains of a receptor or cell surface protein and epitope binding domains can be linked. In such a monovalent structure it is possible to have a soluble ligand or extracellular domain of a receptor or cell surface protein linked to the first heavy chain and one or more epitope binding domains linked to the second heavy chain.

Epitope-binding domains of use in the present invention are domains that specifically bind an antigen or epitope independently of a different V region or domain, this may be a domain antibody or may be a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin); which have been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand. In one embodiment this may be an domain antibody or other suitable domains such as a domain selected from the group consisting of CTLA-4, lipocallin, SpA, an Affibody, an avimer, GroEl, transferrin, GroES and fibronectin. In one embodiment this may be selected from a dAb, an Affibody, an ankyrin repeat protein (DARPin) and an adnectin. In another embodiment this may be selected from an Affibody, an ankyrin repeat protein (DARPin) and an adnectin. In another embodiment this may be a domain antibody, for example a domain antibody selected from a human, camelid or shark (NARV) domain antibody.

Epitope-binding domains can be linked to the protein scaffold at one or more positions. These positions include the C-terminus and the N-terminus of the protein scaffold, for example at the C-terminus of the heavy chain and/or the C-terminus of the light chain of an IgG, or for example the N-terminus of the heavy chain and/or the N-terminus of the light chain of an IgG.

In one embodiment, a first epitope binding domain is linked to the protein scaffold and a second epitope binding domain is linked to the first epitope binding domain, for example where the protein scaffold is an IgG scaffold, a first epitope binding domain may be linked to the c-terminus of the heavy chain of the IgG scaffold, and that epitope binding domain can be linked at its c-terminus to a second epitope binding domain, or for example a first epitope binding domain may be linked to the c-terminus of the light chain of the IgG scaffold, and that first epitope binding domain may be further linked at its c-terminus to a second epitope binding domain, or for example a first epitope binding domain may be linked to the n-terminus of the light chain of the IgG scaffold, and that first epitope binding domain may be further linked at its n-terminus to a second epitope binding domain, or for example a first epitope binding domain may be linked to the n-terminus of the heavy chain of the IgG scaffold, and that first epitope binding domain may be further linked at its n-terminus to a second epitope binding domain.

When the epitope-binding domain is a domain antibody, some domain antibodies may be suited to particular positions within the scaffold.

Domain antibodies of use in the present invention can be linked at the C-terminal end of the heavy chain and/or the light chain of conventional IgGs. In addition some dAbs can be linked to the C-terminal ends of both the heavy chain and the light chain of conventional antibodies.

Epitope-binding domains can be linked to the Receptor-Fc fusion at one or more positions. These positions include the C-terminus and the N-terminus of the Receptor-Fc fusion. For example they may be linked directly to the Fc portion of the Receptor-Fc fusion, or they may be linked to the soluble ligand or extracellular domain of a receptor or cell surface protein portion of the Receptor-Fc fusion. Where the soluble ligand or extracellular domain of a receptor or cell surface protein is linked to the N-terminus of the Fc portion, the epitope-binding domain may be linked

directly to the c-terminus of the Fc portion or to the N-terminus of the soluble ligand or extracellular domain of a receptor or cell surface protein.

In one embodiment, a first epitope binding domain is linked to the Receptor-Fc fusion and a second epitope binding domain is linked to the first epitope binding domain, for example a first epitope binding domain may be linked to the c-terminus of the Receptor-Fc fusion, and that epitope binding domain can be linked at its c-terminus to a second epitope binding domain, or for example a first epitope binding domain may be linked to the n-terminus of the Receptor-Fc fusion, and that first epitope binding domain may be further linked at its n-terminus to a second epitope binding domain, When the epitope-binding domain is a domain antibody, some domain antibodies may be suited to particular positions within the scaffold.

In constructs where the N-terminus of dAbs are fused to an antibody constant domain (either C_H3 or C_L), a peptide linker may help the dAb to bind to antigen. Indeed, the N-terminal end of a dAb is located closely to the complementarity-determining regions (CDRS) involved in antigen-binding activity. Thus a short peptide linker acts as a spacer between the epitope-binding, and the constant domain of the protein scaffold, which may allow the dAb CDRs to more easily reach the antigen, which may therefore bind with high affinity.

The surroundings in which dAbs are linked to the IgG will differ depending on which antibody chain they are fused to. When fused at the C-terminal end of the antibody light chain of an IgG scaffold, each dAb is expected to be located in the vicinity of the antibody hinge and the Fc portion. It is likely that such dAbs will be located far apart from each other. In conventional antibodies, the angle between Fab fragments and the angle between each Fab fragment and the Fc portion can vary quite significantly. It is likely that – with mAbdAbs – the angle between the Fab fragments will not be widely different, whilst some angular restrictions may be observed with the angle between each Fab fragment and the Fc portion.

When fused at the C-terminal end of the antibody heavy chain of an IgG scaffold, each dAb is expected to be located in the vicinity of the C_H3 domains of the Fc portion. This is not expected to impact on the Fc binding properties to Fc receptors (e.g. FcγRI, II, III an FcRn) as these receptors engage with the C_H2 domains (for the FcγRI, II and III class of receptors) or with the hinge between the C_H2 and C_H3 domains (e.g. FcRn receptor). Another feature of such antigen-binding proteins is

that both dAbs are expected to be spatially close to each other and provided that flexibility is provided by provision of appropriate linkers, these dAbs may even form homodimeric species, hence propagating the 'zipped' quaternary structure of the Fc portion, which may enhance stability of the construct.

Such structural considerations can aid in the choice of the most suitable position to link an epitope-binding domain, for example a dAb, on to a protein scaffold, for example an antibody or on to a Receptor-Fc fusion.

The size of the antigen, its localization (in blood or on a cell surface), its quaternary structure (monomeric or multimeric) can vary. Conventional antibodies are naturally designed to function as adaptor constructs due to the presence of the hinge region, wherein the orientation of the two antigen-binding sites at the tip of the Fab fragments can vary widely and hence adapt to the molecular feature of the antigen and its surroundings. In contrast dAbs linked to an antibody or other protein scaffold, for example a protein scaffold which comprises an antibody with no hinge region, may have less structural flexibility either directly or indirectly.

Understanding the solution state and mode of binding at the dAb is also helpful. Evidence has accumulated that *in vitro* dAbs can predominantly exist in monomeric, homo-dimeric or multimeric forms in solution (Reiter *et al.*, J Mol Biol (1999) 290: 685-698; Ewert *et al.*, J Mol Biol (2003) 325: 531-553, Jespers *et al.*, J Mol Biol (2004) 337: 893-903; Jespers *et al.*, Nat Biotechnol (2004) 22: 1161-1165; Martin *et al.*, Protein Eng. (1997) 10: 607-614; Sepulvada *et al.*, J Mol Biol (2003) 333: 355-365). This is fairly reminiscent to multimerisation events observed *in vivo* with Ig domains such as Bence-Jones proteins (which are dimers of immunoglobulin light chains (Epp *et al.*, Biochemistry (1975) 14: 4943-4952; Huan *et al.*, Biochemistry (1994) 33: 14848-14857; Huang *et al.*, Mol immunol (1997) 34: 1291-1301) and amyloid fibers (James *et al.* J Mol Biol. (2007) 367: 603-8).

For example, it may be desirable to link domain antibodies that tend to dimerise in solution to the C-terminal end of the Fc portion in preference to the C-terminal end of the light chain or the N-terminal end of the Receptor-Fc fusion as linking to the C-terminal end of the Fc will allow those dAbs to dimerise in the context of the antigen-binding protein of the invention.

The antigen-binding proteins of the present invention may comprise antigen-binding sites specific for a single antigen, or may have antigen-binding sites specific for two or more antigens, or for two or more epitopes on a single antigen, or there may be antigen-binding sites each of which is specific for a different epitope on the same or different antigens.

In particular, the antigen-binding proteins of the present invention may be useful in treating diseases associated with TNFα and VEGF for example diseases of the eye, for example diabetic macula edema, cystoid macula edema, uveitis, AMD (Age related macular degeneration), choroidal neovascular AMD, geographic atrophy, diabetic retinopathy, retinal vein occlusion (BRVO and/or CRVO) and other maculopathies and ocular vasculopathies.

Particular TNFα antagonists and VEGF antagonists which may be administered in combination for the treatment of any of the aforementioned diseases of the eye, in particular AMD, are as follows.

In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is adalimumab and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is infliximab and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is etanercept and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is ESBA105 and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is PEP1-5-19 and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is PEP1-5-490 and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is PEP1-5-493 and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is EPI-0010. . In an embodiment, the TNFα antagonist is the adnectin of SEQ ID NO:2 and the VEGF antagonist is DMS1571

In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is ESBA903. In an

embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is golimumab and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is certolizumab and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is CT-322. In an embodiment, the TNFα

antagonist is ALK-6931 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is EPI-0010. In an embodiment, the TNFα antagonist is ALK-6931 and the VEGF antagonist is DMS1571.

In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain or SEQ ID NO:31 and the VEGF antagonist is bevacizumab. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain or SEQ ID NO:31 and the VEGF antagonist is ranibizumab. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is r84. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is aflibercept. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is CT01. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is DOM15-10-11. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is DOM15-26-593. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is PRS-050. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is PRS-051. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is MP0112. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is CT-322. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is ESBA903. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is EPI-0030. In an embodiment, the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is EPI-0010. In an embodiment,

the TNFα antagonist is an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31 and the VEGF antagonist is DMS1571.

Each of the above combinations may also be used to generate dual targeting molecules of the invention. Particular and non-limiting examples of dual targeting molecules of the invention are as follows: Fc enabled DMS4000 (SEQ ID NO:14 and SEQ ID NO:12), Fc disabled DMS4000 (SEQ ID NO:47 and SEQ ID NO: 12), DMS4031 (SEQ ID NO: 16 and SEQ ID NO:12), DOM-PEP in-line fusion (SEQ ID NO:62), PEP-DOM in-line fusion (SEQ ID NO: 64), a dual targeting molecule having a heavy chain selected from SEQ ID NO:69-72 and a light chain of SEQ ID NO:12, and those listed in SED ID NO:72-140.

The antigen-binding proteins of the present invention may be produced by transfection of a host cell with an expression vector comprising the coding sequence for the antigen-binding protein of the invention. An expression vector or recombinant plasmid is produced by placing these coding sequences for the antigen-binding protein in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antigen-binding protein light or heavy chain. In certain embodiments this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the antigen-binding protein may reside on a single vector, for example in two expression cassettes in the same vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antigen-binding protein of the invention. The antigen-binding protein which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other antigen-binding proteins.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors may be used. One vector, pUC19, is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antigen-binding proteins of the present invention. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, cells from various strains of E. coli may be used for replication of the cloning vectors and other steps in the construction of antigen-binding proteins of this invention.

Suitable host cells or cell lines for the expression of the antigen-binding proteins of the invention include mammalian cells such as NS0, Sp2/0, CHO (e.g. DG44), COS, HEK, a fibroblast cell (e.g., 3T3), and myeloma cells, for example they may be expressed in a CHO or a myeloma cell. Human cells may be used, thus enabling the

molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook et al., cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs or other embodiments of the present invention (see, e.g., Plückthun, A., Immunol. Rev. (1992) 130: 151-188). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host, or in alternative embodiments the molecule may express in the bacterial host and then be subsequently re-folded. For example, various strains of E. coli used for expression are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Streptomyces, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. Drosophila and Lepidoptera and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering (1986) 8: 277-298, Plenum Press and references cited therein.

The general methods by which the vectors may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the antigen-binding protein of the invention from such host cell may all be conventional techniques. Typically, the culture method of the present invention is a serum-free culture method, usually by culturing cells serum-free in suspension. Likewise, once produced, the antigen-binding proteins of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention. For example, preparation of altered antibodies are described in WO 99/58679 and WO 96/16990.

Yet another method of expression of the antigen-binding proteins may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

In a further aspect of the invention there is provided a method of producing an antibody of the invention which method comprises the step of culturing a host cell transformed or transfected with a vector encoding the light and/or heavy chain of the antibody of the invention and recovering the antibody thereby produced.

In accordance with the present invention there is provided a method of producing an antigen-binding protein of the present invention which method comprises the steps of;

(a) providing a vector comprising a polynucleotide encoding the antigen-binding protein
(b) transforming a mammalian host cell (e.g. CHO) with said vector;
(c) culturing the host cell of step (b) under conditions conducive to the secretion of the antigen-binding protein from said host cell into said culture media;
(d) recovering the secreted antigen-binding protein of step (c).

In accordance with the present invention there is provided a method of producing an antigen-binding protein of the present invention which method comprises the steps of;

(a) providing a first vector encoding a heavy chain of the antigen-binding protein;
(b) providing a second vector encoding a light chain of the antigen-binding protein;
(c) transforming a mammalian host cell (e.g. CHO) with said first and second vectors;
(d) culturing the host cell of step (c) under conditions conducive to the secretion of the antigen-binding protein from said host cell into said culture media;
(e) recovering the secreted antigen-binding protein of step (d).

Once expressed by the desired method, the antigen-binding protein is then examined for in vitro activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the antigen-binding protein to its target. Additionally, other in vitro assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antigen-binding protein in the body despite the usual clearance mechanisms.

The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient. It is envisaged that repeated dosing (e.g. once a week or once every two weeks) over an extended time period (e.g. four to six months) maybe required to achieve maximal therapeutic efficacy.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the eye of the host. Systemic administration may be sufficient to deliver effective amounts of the antigen-binding proteins and pharmaceutical compositions of the invention via passive, e.g. intravenous or subcutaneous, administration. The antigen-binding proteins and pharmaceutical compositions of the invention may also be delivered more locally to the eye either by topical application e.g. eye drops or a gel, intravitreal injection, intracameral or periocular administration, i.e. subsclerally via either retrobulbar, peribulbar, subtenon or subconjunctival injection or via delivery to the inferior, superior or lateral rectus muscle. Other routes of local administration may allow the antigen-binding proteins and pharmaceutical compositions of the invention to reach the posterior segment of the eye more readily at lower doses. Topical application has been described to allow penetrance of antibody fragments to the posterior of the eye in the rabbit model, (Williams KA *et al.*, (2005)). Intravitreal injection of antibody fragments or full monoclonal antibodies has been described and is well-tolerated for AMD patients for the products ranibizumab and bevacizumab.

In an embodiment, the TNF antagonist and the VEGF antagonist are both administered intravitreally. In an embodiment, the VEGF antagonist is administered intravitreally and the TNF antagonist, in particular ESBA105, is administered by a means other than topically e.g. also intravitreally or subconjunctivally. In an

embodiment the TNF antagonist is administered intravitreally and the VEGF antagonist is administered topically.

It can be useful to target the delivery of the antigen binding protein into particular regions of the eye such as the surface of the eye, or to the tear ducts or lachrymal glands or there can be intra-ocular delivery (e.g. to the anterior or posterior chambers of the eye, such as the vitreous humour) and to ocular structures such as the iris, ciliary body, lachrymal gland. Hence the invention further provides a method of delivering a composition directly to the eye which comprises administering said composition to the eye by a method selected from: intra-ocular injection, topical delivery (e.g. eye drops), peri-ocular administration and use of a slow release formulation.

It can also be useful if the antigen binding protein is delivered to the eye e.g. by topical delivery (e.g. as eye drops), along with an ocular penetration enhancer e.g. sodium caprate, or with a viscosity enhancer e.g. Hydroxypropylmethylcellulose (HPMC). Accordingly the invention further provides compositions comprising (a) antigen binding protein of the invention and also (b) an ocular penetration enhancer and /or (c) a viscosity enhancer e.g. for topical delivery to the eye.

Delivery of the antigen-binding proteins and pharmaceutical compositions of the invention may also be administered by an intravitreal implant. Retrobulbar and peribulbar injections can be achieved with special 23 to 26 gauge needles and are less invasive than intravitreal injections. Subtenon injection places the composition in contact with the sclera for a longer period which could aid penetration to the posterior eye. Injection of proteins just beneath the conjuctiva has been described in rabbit models and this allows molecules to diffuse more directly across the sclera to reach the posterior segment of the eye.

Sustained release drug delivery systems may also be used which allow for release of material over a longer time-frame into or around the eye so that dosing could be less frequent. Such systems include micelles, gels, hydrogels, nanoparticles, microcapsules or implants that can be filled or coated with therapeutic compositions. These may be delivered into the vitreous of the eye by injection or by any of the other previously described less invasive routes, i.e. through the periocular or sub-scleral routes. Examples of such sustained release systems and local delivery routes include thermo-sensitive slow release hydrogels for subscleral administration or

intravitreal administration of a nanoparticle based formulation that targets to the posterior retina and RPE layer (Janoira KG, *et al.*, (2007); Birch DG (2007)). Many other combinations of delivery system and local administration route are possible and could be considered for compositions of the antigen-binding proteins, and pharmaceutical compositions of the invention.

In a particular embodiment, an antigen binding protein of the invention is administered intravitreally by intravitreal injection. In a particular embodiment, an antigen protein of the invention, in particular a dual targeting construct, is administered intravitreally every 4-8 weeks, preferably every 6-8 weeks. In a particular embodiment, an antigen binding protein is administered by subconjunctival injection. In a particular embodiment, an antigen binding protein of the invention is administered topically. In another embodiment, an antigen binding protein of the invention is administered via a sustained release drug delivery system. In a particular embodiment, an antigen binding protein of the invention is administered via intravenous injection. In a particular embodiment, an antigen binding protein of the invention is administered via subcutaneous injection.

In a particular embodiment of the invention, the antigen binding protein is DMS4000 or an antigen binding protein consisting of a heavy chain sequence of SEQ ID NO:69, 70, 71 or 72 and a light chain sequence of SEQ ID NO:12, which is to be administered by intravitreal injection every 4-8 weeks.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antigen-binding protein of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the antigen-binding protein, may be buffered at physiological pH, in a form ready for injection. The compositions for parenteral administration will commonly comprise a solution of the antigen-binding protein of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier, for example an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions may be made sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antigen-binding protein of the

invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 200 mg, e.g. about 50 ng to about 30 mg or more, or about 5 mg to about 25 mg, of an antigen-binding protein of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 or about 5 mg to about 25 mg of an antigen-binding protein of the invention per ml of Ringer's solution. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania. For the preparation of intravenously administrable antigen-binding protein formulations of the invention see Lasmar U and Parkins D "The formulation of Biopharmaceutical products", Pharma. Sci.Tech.today, page 129-137, Vol.3 (3rd April 2000); Wang, W "Instability, stabilisation and formulation of liquid protein pharmaceuticals", Int. J. Pharm 185 (1999) 129-188; Stability of Protein Pharmaceuticals Part A and B ed Ahern T.J., Manning M.C., New York, NY: Plenum Press (1992); Akers,M.J. "Excipient-Drug interactions in Parenteral Formulations", J.Pharm Sci 91 (2002) 2283-2300; Imamura, K *et al.,* "Effects of types of sugar on stabilization of Protein in the dried state", J Pharm Sci 92 (2003) 266-274; Izutsu, Kkojima, S. "Excipient crystalinity and its protein-structure-stabilizing effect during freeze-drying", J Pharm. Pharmacol, 54 (2002) 1033-1039; Johnson, R, "Mannitol-sucrose mixtures-versatile formulations for protein lyophilization", J. Pharm. Sci, 91 (2002) 914-922;Ha,E Wang W, Wang Y.j. "Peroxide formation in polysorbate 80 and protein stability", J. Pharm Sci, 91, 2252-2264,(2002) the entire contents of which are incorporated herein by reference and to which the reader is specifically referred.

In one embodiment the therapeutic agent of the invention, when in a pharmaceutical preparation, is present in unit dose forms. The appropriate therapeutically effective dose will be determined readily by those of skill in the art. Suitable doses may be calculated for patients according to their weight, for example suitable doses may be in the range of 0.00001 to 20mg/kg, for example 0.0001 to 20mg/kg, for example 0.1 to 20mg/kg, for example 1 to 20mg/kg or for example 1 to 15mg/kg, for example 10

to 15mg/kg. To effectively treat conditions of use in the present invention in a human, suitable doses may be within the range of 0.0001 to 1000 mg, for example 0.001 to 1000mg, for example 0.01 to 500mg, for example 500mg, for example 0.1 to 100mg, or 0.1 to 80mg, or 0.1 to 60mg, or 0.1 to 40mg, or for example 1 to 100mg, or 1 to 50mg, of an antigen-binding protein of this invention, which may be administered parenterally, for example subcutaneously, intravenously or intramuscularly; or topically. Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

Where the therapeutic agent is to be administered directly into the eye, e.g. by intravitreal injection, it is preferable that the dosage should be such that the total amount of protein administered to each human eye does not exceed 2 mg. In an embodiment the total amount of protein administered to a single human eye is approximately 2 mg. In an embodiment the total amount of protein administered to a single human eye is approximately 1.8 mg. In an embodiment the total amount of protein administered to a single human eye is approximately 1.6 mg. In an embodiment the total amount of protein administered to a single human eye is approximately 1.4 mg. In an embodiment the total amount of protein administered to a single human eye is approximately 1.2 mg. In an embodiment the total amount of protein administered to a single human eye is approximately 1.0 mg. In an embodiment, the total amount of protein administered to a single human eye is less than 2.0 mg, less than 1.8 mg, less than 1.6 mg, less than 1.4 mg, less than 1.2 mg, or less than 1.0 mg.

The antigen-binding proteins described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

There are several methods known in the art which can be used to find epitope-binding domains of use in the present invention.

The term "library" refers to a mixture of heterogeneous polypeptides or nucleic acids. The library is composed of members, each of which has a single polypeptide or nucleic acid sequence. To this extent, "library" is synonymous with "repertoire." Sequence differences between library members are responsible for the diversity present in the library. The library may take the form of a simple mixture of

polypeptides or nucleic acids, or may be in the form of organisms or cells, for example bacteria, viruses, animal or plant cells and the like, transformed with a library of nucleic acids. In one example, each individual organism or cell contains only one or a limited number of library members. Advantageously, the nucleic acids are incorporated into expression vectors, in order to allow expression of the polypeptides encoded by the nucleic acids. In a one aspect, therefore, a library may take the form of a population of host organisms, each organism containing one or more copies of an expression vector containing a single member of the library in nucleic acid form which can be expressed to produce its corresponding polypeptide member. Thus, the population of host organisms has the potential to encode a large repertoire of diverse polypeptides.

A "universal framework" is a single antibody framework sequence corresponding to the regions of an antibody conserved in sequence as defined by Kabat ("Sequences of Proteins of Immunological Interest", US Department of Health and Human Services) or corresponding to the human germline immunoglobulin repertoire or structure as defined by Chothia and Lesk, J. Mol. Biol. (1987) 196: 910-917. There may be a single framework, or a set of such frameworks, which has been found to permit the derivation of virtually any binding specificity though variation in the hypervariable regions alone.

Amino acid and nucleotide sequence alignments and homology, similarity or identity, as defined herein are in one embodiment prepared and determined using the algorithm BLAST 2 Sequences, using default parameters (Tatusova, T. A. *et al.*, FEMS Microbiol Lett, (1999) 174: 187-188).

When a display system (*e.g.*, a display system that links coding function of a nucleic acid and functional characteristics of the peptide or polypeptide encoded by the nucleic acid) is used in the methods described herein, e.g. in the selection of a dAb or other epitope binding domain, it is frequently advantageous to amplify or increase the copy number of the nucleic acids that encode the selected peptides or polypeptides. This provides an efficient way of obtaining sufficient quantities of nucleic acids and/or peptides or polypeptides for additional rounds of selection, using the methods described herein or other suitable methods, or for preparing additional repertoires (*e.g.*, affinity maturation repertoires). Thus, in some embodiments, the methods of selecting epitope binding domains comprises using a display system (*e.g.*, that links coding function of a nucleic acid and functional characteristics of the

peptide or polypeptide encoded by the nucleic acid, such as phage display) and further comprises amplifying or increasing the copy number of a nucleic acid that encodes a selected peptide or polypeptide. Nucleic acids can be amplified using any suitable methods, such as by phage amplification, cell growth or polymerase chain reaction.

In one example, the methods employ a display system that links the coding function of a nucleic acid and physical, chemical and/or functional characteristics of the polypeptide encoded by the nucleic acid. Such a display system can comprise a plurality of replicable genetic packages, such as bacteriophage or cells (bacteria). The display system may comprise a library, such as a bacteriophage display library. Bacteriophage display is an example of a display system.

A number of suitable bacteriophage display systems (*e.g.*, monovalent display and multivalent display systems) have been described. (See, *e.g.*, Griffiths *et al.*, U.S. Patent No. 6,555,313 B1 (incorporated herein by reference); Johnson *et al.*, U.S. Patent No. 5,733,743 (incorporated herein by reference); McCafferty *et al.*, U.S. Patent No. 5,969,108 (incorporated herein by reference); Mulligan-Kehoe, U.S. Patent No. 5,702,892 (Incorporated herein by reference); Winter, G. *et al.*, Annu. Rev. Immunol. *(1994) 12*: 433-455; Soumillion, P. *et al.,* Appl. Biochem. Biotechnol. (1994) 47(2-3): 175-189; Castagnoli, L. *et al.,* Comb. Chem. High Throughput Screen *(2001)* 4(2): 121-133) The peptides or polypeptides displayed in a bacteriophage display system can be displayed on any suitable bacteriophage, such as a filamentous phage (*e.g.*, fd, M13, F1), a lytic phage (*e.g.*, T4, T7, lambda), or an RNA phage (*e.g.*, MS2), for example.

Generally, a library of phage that displays a repertoire of peptides or phagepolypeptides, as fusion proteins with a suitable phage coat protein (*e.g.*, fd pIII protein), is produced or provided. The fusion protein can display the peptides or polypeptides at the tip of the phage coat protein, or if desired at an internal position. For example, the displayed peptide or polypeptide can be present at a position that is amino-terminal to domain 1 of pIII. (Domain 1 of pIII is also referred to as N1.) The displayed polypeptide can be directly fused to pIII (*e.g.*, the N-terminus of domain 1 of pIII) or fused to pIII using a linker. If desired, the fusion can further comprise a tag (*e.g.*, myc epitope, His tag). Libraries that comprise a repertoire of peptides or polypeptides that are displayed as fusion proteins with a phage coat protein can be produced using any suitable methods, such as by introducing a library of phage

vectors or phagemid vectors encoding the displayed peptides or polypeptides into suitable host bacteria, and culturing the resulting bacteria to produce phage (*e.g.*, using a suitable helper phage or complementing plasmid if desired). The library of phage can be recovered from the culture using any suitable method, such as precipitation and centrifugation.

The display system can comprise a repertoire of peptides or polypeptides that contains any desired amount of diversity. For example, the repertoire can contain peptides or polypeptides that have amino acid sequences that correspond to naturally occurring polypeptides expressed by an organism, group of organisms, desired tissue or desired cell type, or can contain peptides or polypeptides that have random or randomized amino acid sequences. If desired, the polypeptides can share a common core or scaffold. For example, all polypeptides in the repertoire or library can be based on a scaffold selected from protein A, protein L, protein G, a fibronectin domain, an anticalin, CTLA4, a desired enzyme (*e.g.*, a polymerase, a cellulase), or a polypeptide from the immunoglobulin superfamily, such as an antibody or antibody fragment (*e.g.*, an antibody variable domain). The polypeptides in such a repertoire or library can comprise defined regions of random or randomized amino acid sequence and regions of common amino acid sequence. In certain embodiments, all or substantially all polypeptides in a repertoire are of a desired type, such as a desired enzyme (*e.g.*, a polymerase) or a desired antigen-binding fragment of an antibody (*e.g.*, human V_H or human V_L). In some embodiments, the polypeptide display system comprises a repertoire of polypeptides wherein each polypeptide comprises an antibody variable domain. For example, each polypeptide in the repertoire can contain a V_H, a V_L or an Fv (*e.g.*, a single chain Fv).

Amino acid sequence diversity can be introduced into any desired region of a peptide or polypeptide or scaffold using any suitable method. For example, amino acid sequence diversity can be introduced into a target region, such as a complementarity determining region of an antibody variable domain or a hydrophobic domain, by preparing a library of nucleic acids that encode the diversified polypeptides using any suitable mutagenesis methods (*e.g.*, low fidelity PCR, oligonucleotide-mediated or site directed mutagenesis, diversification using NNK codons) or any other suitable method. If desired, a region of a polypeptide to be diversified can be randomized. The size of the polypeptides that make up the repertoire is largely a matter of choice and uniform polypeptide size is not required. The polypeptides in the repertoire may have at least tertiary structure (i.e. form at least one domain).

Selection/Isolation/Recovery

An epitope binding domain or population of domains can be selected, isolated and/or recovered from a repertoire or library (*e.g.*, in a display system) using any suitable method. For example, a domain is selected or isolated based on a selectable characteristic (*e.g.*, physical characteristic, chemical characteristic, functional characteristic). Suitable selectable functional characteristics include biological activities of the peptides or polypeptides in the repertoire, for example, binding to a generic ligand (*e.g.*, a superantigen), binding to a target ligand (*e.g.*, an antigen, an epitope, a substrate), binding to an antibody (*e.g.*, through an epitope expressed on a peptide or polypeptide), and catalytic activity. (See, *e.g.*, Tomlinson *et al.*, WO 99/20749; WO 01/57065; WO 99/58655.)

In some embodiments, the protease resistant peptide or polypeptide is selected and/or isolated from a library or repertoire of peptides or polypeptides in which substantially all domains share a common selectable feature. For example, the domain can be selected from a library or repertoire in which substantially all domains bind a common generic ligand, bind a common target ligand, bind (or are bound by) a common antibody, or possess a common catalytic activity. This type of selection is particularly useful for preparing a repertoire of domains that are based on a parental peptide or polypeptide that has a desired biological activity, for example, when performing affinity maturation of an immunoglobulin single variable domain. Selection based on binding to a common generic ligand can yield a collection or population of domains that contain all or substantially all of the domains that were components of the original library or repertoire. For example, domains that bind a target ligand or a generic ligand, such as protein A, protein L or an antibody, can be selected, isolated and/or recovered by panning or using a suitable affinity matrix. Panning can be accomplished by adding a solution of ligand (*e.g.*, generic ligand, target ligand) to a suitable vessel (*e.g.*, tube, petri dish) and allowing the ligand to become deposited or coated onto the walls of the vessel. Excess ligand can be washed away and domains can be added to the vessel and the vessel maintained under conditions suitable for peptides or polypeptides to bind the immobilized ligand. Unbound domains can be washed away and bound domains can be recovered using any suitable method, such as scraping or lowering the pH, for example.

Suitable ligand affinity matrices generally contain a solid support or bead (*e.g.*, agarose) to which a ligand is covalently or noncovalently attached. The affinity matrix can be combined with peptides or polypeptides (*e.g.*, a repertoire that has

been incubated with protease) using a batch process, a column process or any other suitable process under conditions suitable for binding of domains to the ligand on the matrix. Domains that do not bind the affinity matrix can be washed away and bound domains can be eluted and recovered using any suitable method, such as elution with a lower pH buffer, with a mild denaturing agent (*e.g.*, urea), or with a peptide or domain that competes for binding to the ligand. In one example, a biotinylated target ligand is combined with a repertoire under conditions suitable for domains in the repertoire to bind the target ligand. Bound domains are recovered using immobilized avidin or streptavidin (*e.g.*, on a bead).

In some embodiments, the generic or target ligand is an antibody or antigen binding fragment thereof. Antibodies or antigen binding fragments that bind structural features of peptides or polypeptides that are substantially conserved in the peptides or polypeptides of a library or repertoire are particularly useful as generic ligands. Antibodies and antigen binding fragments suitable for use as ligands for isolating, selecting and/or recovering protease resistant peptides or polypeptides can be monoclonal or polyclonal and can be prepared using any suitable method.

LIBRARIES/REPERTOIRES

Libraries that encode and/or contain epitope binding domains can be prepared or obtained using any suitable method. A library can be designed to encode domains based on a domain or scaffold of interest (*e.g.*, a domain selected from a library) or can be selected from another library using the methods described herein. For example, a library enriched in domains can be prepared using a suitable polypeptide display system.

Libraries that encode a repertoire of a desired type of domain can readily be produced using any suitable method. For example, a nucleic acid sequence that encodes a desired type of polypeptide (*e.g.*, an immunoglobulin variable domain) can be obtained and a collection of nucleic acids that each contain one or more mutations can be prepared, for example by amplifying the nucleic acid using an error-prone polymerase chain reaction (PCR) system, by chemical mutagenesis (Deng *et al., J. Biol. Chem.*, 269:9533 (1994)) or using bacterial mutator strains (Low *et al., J. Mol. Biol.*, 260:359 (1996)).

In other embodiments, particular regions of the nucleic acid can be targeted for diversification. Methods for mutating selected positions are also well known in the art

and include, for example, the use of mismatched oligonucleotides or degenerate oligonucleotides, with or without the use of PCR. For example, synthetic antibody libraries have been created by targeting mutations to the antigen binding loops. Random or semi-random antibody H3 and L3 regions have been appended to germline immunobulin V gene segments to produce large libraries with unmutated framework regions (Hoogenboom and Winter (1992) supra; Nissim *et al.* (1994) supra; Griffiths *et al.* (1994) supra; DeKruif *et al.* (1995) supra). Such diversification has been extended to include some or all of the other antigen binding loops (Crameri *et al.* Nature Med. (1996) 2: 100; Riechmann *et al.* Bio/Technology (1995) 13: 475; Morphosys, WO 97/08320, supra). In other embodiments, particular regions of the nucleic acid can be targeted for diversification by, for example, a two-step PCR strategy employing the product of the first PCR as a "mega-primer." (See, *e.g.*, Landt, O. *et al.,* Gene (1990) 96: 125-128) Targeted diversification can also be accomplished, for example, by SOE PCR. (See, *e.g.*, Horton, R.M. *et al.,* Gene (1989) *77*: 61-68)

Sequence diversity at selected positions can be achieved by altering the coding sequence which specifies the sequence of the polypeptide such that a number of possible amino acids (*e.g.*, all 20 or a subset thereof) can be incorporated at that position. Using the IUPAC nomenclature, the most versatile codon is NNK, which encodes all amino acids as well as the TAG stop codon. The NNK codon may be used in order to introduce the required diversity. Other codons which achieve the same ends are also of use, including the NNN codon, which leads to the production of the additional stop codons TGA and TAA. Such a targeted approach can allow the full sequence space in a target area to be explored.

Some libraries comprise domains that are members of the immunoglobulin superfamily (*e.g.,* antibodies or portions thereof). For example the libraries can comprise domains that have a known main-chain conformation. (See, *e.g.*, Tomlinson et al., WO 99/20749.)

Libraries can be prepared in a suitable plasmid or vector. As used herein, vector refers to a discrete element that is used to introduce heterologous DNA into cells for the expression and/or replication thereof. Any suitable vector can be used, including plasmids (*e.g.*, bacterial plasmids), viral or bacteriophage vectors, artificial chromosomes and episomal vectors. Such vectors may be used for simple cloning and mutagenesis, or an expression vector can be used to drive expression of the

library. Vectors and plasmids usually contain one or more cloning sites (*e.g.*, a polylinker), an origin of replication and at least one selectable marker gene. Expression vectors can further contain elements to drive transcription and translation of a polypeptide, such as an enhancer element, promoter, transcription termination signal, signal sequences, and the like. These elements can be arranged in such a way as to be operably linked to a cloned insert encoding a polypeptide, such that the polypeptide is expressed and produced when such an expression vector is maintained under conditions suitable for expression (*e.g.*, in a suitable host cell).

Cloning and expression vectors generally contain nucleic acid sequences that enable the vector to replicate in one or more selected host cells. Typically in cloning vectors, this sequence is one that enables the vector to replicate independently of the host chromosomal DNA and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 micron plasmid origin is suitable for yeast, and various viral origins (*e.g.* SV40, adenovirus) are useful for cloning vectors in mammalian cells. Generally, the origin of replication is not needed for mammalian expression vectors, unless these are used in mammalian cells able to replicate high levels of DNA, such as COS cells.

Cloning or expression vectors can contain a selection gene also referred to as selectable marker. Such marker genes encode a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will therefore not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics and other toxins, *e.g.* ampicillin, neomycin, methotrexate or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients not available in the growth media.

Suitable expression vectors can contain a number of components, for example, an origin of replication, a selectable marker gene, one or more expression control elements, such as a transcription control element (*e.g.*, promoter, enhancer, terminator) and/or one or more translation signals, a signal sequence or leader sequence, and the like. Expression control elements and a signal or leader sequence, if present, can be provided by the vector or other source. For example,

the transcriptional and/or translational control sequences of a cloned nucleic acid encoding an antibody chain can be used to direct expression.

A promoter can be provided for expression in a desired host cell. Promoters can be constitutive or inducible. For example, a promoter can be operably linked to a nucleic acid encoding an antibody, antibody chain or portion thereof, such that it directs transcription of the nucleic acid. A variety of suitable promoters for procaryotic (*e.g.*, the β-lactamase and lactose promoter systems, alkaline phosphatase, the tryptophan (trp) promoter system, lac, tac, T3, T7 promoters for *E. coli*) and eucaryotic (*e.g.*, simian virus 40 early or late promoter, Rous sarcoma virus long terminal repeat promoter, cytomegalovirus promoter, adenovirus late promoter, EG-1a promoter) hosts are available.

In addition, expression vectors typically comprise a selectable marker for selection of host cells carrying the vector, and, in the case of a replicable expression vector, an origin of replication. Genes encoding products which confer antibiotic or drug resistance are common selectable markers and may be used in procaryotic (*e.g.*, β-lactamase gene (ampicillin resistance), *Tet* gene for tetracycline resistance) and eucaryotic cells (*e.g.*, neomycin (G418 or geneticin), gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes). Dihydrofolate reductase marker genes permit selection with methotrexate in a variety of hosts. Genes encoding the gene product of auxotrophic markers of the host (*e.g.*, *LEU2, URA3, HIS3*) are often used as selectable markers in yeast. Use of viral (*e.g.*, baculovirus) or phage vectors, and vectors which are capable of integrating into the genome of the host cell, such as retroviral vectors, are also contemplated.

Suitable expression vectors for expression in prokaryotic (*e.g.*, bacterial cells such as *E. coli*) or mammalian cells include, for example, a pET vector (*e.g.*, pET-12a, pET-36, pET-37, pET-39, pET-40, Novagen and others), a phage vector (*e.g.*, pCANTAB 5 E, Pharmacia), pRIT2T (Protein A fusion vector, Pharmacia), pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, pEF-1 (Invitrogen, Carlsbad, CA), pCMV-SCRIPT, pFB, pSG5, pXT1 (Stratagene, La Jolla, CA), pCDEF3 (Goldman, L.A., *et al.*, *Biotechniques*, *21*:1013-1015 (1996)), pSVSPORT (GibcoBRL, Rockville, MD), pEF-Bos (Mizushima, S., *et al.*, Nucleic Acids Res. (1990) 18: 5322) and the like. Expression vectors which are suitable for use in various expression hosts, such as prokaryotic cells (*E. coli*), insect cells (*Drosophila* Schnieder S2 cells, Sf9), yeast (*P.*

methanolica, *P. pastoris*, *S. cerevisiae*) and mammalian cells (eg, COS cells) are available.

Some examples of vectors are expression vectors that enable the expression of a nucleotide sequence corresponding to a polypeptide library member. Thus, selection with generic and/or target ligands can be performed by separate propagation and expression of a single clone expressing the polypeptide library member. As described above, a particular selection display system is bacteriophage display. Thus, phage or phagemid vectors may be used, for example vectors may be phagemid vectors which have an *E. coli*. origin of replication (for double stranded replication) and also a phage origin of replication (for production of single-stranded DNA). The manipulation and expression of such vectors is well known in the art (Hoogenboom and Winter (1992) supra; Nissim *et al.* (1994) supra). Briefly, the vector can contain a β-lactamase gene to confer selectivity on the phagemid and a lac promoter upstream of an expression cassette that can contain a suitable leader sequence, a multiple cloning site, one or more peptide tags, one or more TAG stop codons and the phage protein pIII. Thus, using various suppressor and non-suppressor strains of *E. coli* and with the addition of glucose, iso-propyl thio-β-D-galactoside (IPTG) or a helper phage, such as VCS M13, the vector is able to replicate as a plasmid with no expression, produce large quantities of the polypeptide library member only or produce phage, some of which contain at least one copy of the polypeptide-pIII fusion on their surface.

Antibody variable domains may comprise a target ligand binding site and/or a generic ligand binding site. In certain embodiments, the generic ligand binding site is a binding site for a superantigen, such as protein A, protein L or protein G. The variable domains can be based on any desired variable domain, for example a human VH (*e.g.*, V_H1a, V_H1b, V_H2, V_H3, V_H4, V_H5, V_H6), a human Vλ (*e.g.*, VλI, VλII, VλIII, VλIV, VλV, VλVI or Vκ1) or a human Vκ (*e.g.*, Vκ2, Vκ3, Vκ4, Vκ5, Vκ6, Vκ7, Vκ8, Vκ9 or Vκ10).

A still further category of techniques involves the selection of repertoires in artificial compartments, which allow the linkage of a gene with its gene product. For example, a selection system in which nucleic acids encoding desirable gene products may be selected in microcapsules formed by water-in-oil emulsions is described in WO99/02671, WO00/40712 and Tawfik & Griffiths Nature Biotechnol (1998) 16(7):

652-6. Genetic elements encoding a gene product having a desired activity are compartmentalised into microcapsules and then transcribed and/or translated to produce their respective gene products (RNA or protein) within the microcapsules. Genetic elements which produce gene product having desired activity are subsequently sorted. This approach selects gene products of interest by detecting the desired activity by a variety of means.

Characterisation of the epitope binding domains.

The binding of a domain to its specific antigen or epitope can be tested by methods which will be familiar to those skilled in the art and include ELISA. In one example, binding is tested using monoclonal phage ELISA.

Phage ELISA may be performed according to any suitable procedure: an exemplary protocol is set forth below.

Populations of phage produced at each round of selection can be screened for binding by ELISA to the selected antigen or epitope, to identify "polyclonal" phage antibodies. Phage from single infected bacterial colonies from these populations can then be screened by ELISA to identify "monoclonal" phage antibodies. It is also desirable to screen soluble antibody fragments for binding to antigen or epitope, and this can also be undertaken by ELISA using reagents, for example, against a C- or N-terminal tag (see for example Winter *et al*. Ann. Rev. Immunology (1994) 12: 433-55 and references cited therein.

The diversity of the selected phage monoclonal antibodies may also be assessed by gel electrophoresis of PCR products and probing (Marks *et al*. 1991, *supra*; Nissim *et al*. 1994 *supra*), (Tomlinson *et al.*, 1992) J. Mol. Biol. 227, 776) or by sequencing of the vector DNA or restriction digets analysis with a frequent cutter such as BSTNI.

Structure of dAbs

In the case that the dAbs are selected from V-gene repertoires selected for instance using phage display technology as herein described, then these variable domains comprise a universal framework region, such that is they may be recognised by a specific generic ligand as herein defined. The use of universal frameworks, generic ligands and the like is described in WO99/20749.

Where V-gene repertoires are used variation in polypeptide sequence may be located within the structural loops of the variable domains. The polypeptide sequences of either variable domain may be altered by DNA shuffling or by mutation in order to enhance the interaction of each variable domain with its complementary pair. DNA shuffling is known in the art and taught, for example, by Stemmer, 1994, Nature 370: 389-391 and U.S. Patent No. 6,297,053, both of which are incorporated herein by reference. Other methods of mutagenesis are well known to those of skill in the art.

Scaffolds for use in Constructing dAbs

i. Selection of the main-chain conformation

The members of the immunoglobulin superfamily all share a similar fold for their polypeptide chain. For example, although antibodies are highly diverse in terms of their primary sequence, comparison of sequences and crystallographic structures has revealed that, contrary to expectation, five of the six antigen binding loops of antibodies (H1, H2, L1, L2, L3) adopt a limited number of main-chain conformations, or canonical structures (Chothia and Lesk J. Mol. Biol. (1987) 196: 901; Chothia *et al.* Nature (1989) 342: 877). Analysis of loop lengths and key residues has therefore enabled prediction of the main-chain conformations of H1, H2, L1, L2 and L3 found in the majority of human antibodies (Chothia *et al.* J. Mol. Biol. (1992) 227: 799; Tomlinson *et al.* EMBO J. (1995) 14: 4628; Williams *et al.* J. Mol. Biol. (1996) 264: 220). Although the H3 region is much more diverse in terms of sequence, length and structure (due to the use of D segments), it also forms a limited number of main-chain conformations for short loop lengths which depend on the length and the presence of particular residues, or types of residue, at key positions in the loop and the antibody framework (Martin *et al.* J. Mol. Biol. (1996) 263: 800; Shirai *et al.* FEBS Letters (1996) 399: 1).

The dAbs are advantageously assembled from libraries of domains, such as libraries of V_H domains and/or libraries of V_L domains. In one aspect, libraries of domains are designed in which certain loop lengths and key residues have been chosen to ensure that the main-chain conformation of the members is known. Advantageously, these are real conformations of immunoglobulin superfamily molecules found in nature, to minimise the chances that they are non-functional, as discussed above. Germline V gene segments serve as one suitable basic framework for constructing antibody or T-cell receptor libraries; other sequences are also of use. Variations may occur at a low

frequency, such that a small number of functional members may possess an altered main-chain conformation, which does not affect its function.

Canonical structure theory is also of use to assess the number of different main-chain conformations encoded by ligands, to predict the main-chain conformation based on ligand sequences and to chose residues for diversification which do not affect the canonical structure. It is known that, in the human V_κ domain, the L1 loop can adopt one of four canonical structures, the L2 loop has a single canonical structure and that 90% of human V_κ domains adopt one of four or five canonical structures for the L3 loop (Tomlinson *et al.* (1995) supra); thus, in the V_κ domain alone, different canonical structures can combine to create a range of different main-chain conformations. Given that the $V\lambda$ domain encodes a different range of canonical structures for the L1, L2 and L3 loops and that V_κ and $V\lambda$ domains can pair with any V_H domain which can encode several canonical structures for the H1 and H2 loops, the number of canonical structure combinations observed for these five loops is very large. This implies that the generation of diversity in the main-chain conformation may be essential for the production of a wide range of binding specificities. However, by constructing an antibody library based on a single known main-chain conformation it has been found, contrary to expectation, that diversity in the main-chain conformation is not required to generate sufficient diversity to target substantially all antigens. Even more surprisingly, the single main-chain conformation need not be a consensus structure - a single naturally occurring conformation can be used as the basis for an entire library. Thus, in a one particular aspect, the dAbs possess a single known main-chain conformation.

The single main-chain conformation that is chosen may be commonplace among molecules of the immunoglobulin superfamily type in question. A conformation is commonplace when a significant number of naturally occurring molecules are observed to adopt it. Accordingly, in one aspect, the natural occurrence of the different main-chain conformations for each binding loop of an immunoglobulin domain are considered separately and then a naturally occurring variable domain is chosen which possesses the desired combination of main-chain conformations for the different loops. If none is available, the nearest equivalent may be chosen. The desired combination of main-chain conformations for the different loops may be created by selecting germline gene segments which encode the desired main-chain conformations. In one example, the selected germline gene segments are frequently

expressed in nature, and in particular they may be the most frequently expressed of all natural germline gene segments.

In designing libraries the incidence of the different main-chain conformations for each of the six antigen binding loops may be considered separately. For H1, H2, L1, L2 and L3, a given conformation that is adopted by between 20% and 100% of the antigen binding loops of naturally occurring molecules is chosen. Typically, its observed incidence is above 35% (i.e. between 35% and 100%) and, ideally, above 50% or even above 65%. Since the vast majority of H3 loops do not have canonical structures, it is preferable to select a main-chain conformation which is commonplace among those loops which do display canonical structures. For each of the loops, the conformation which is observed most often in the natural repertoire is therefore selected. In human antibodies, the most popular canonical structures (CS) for each loop are as follows: H1 - CS 1 (79% of the expressed repertoire), H2 - CS 3 (46%), L1 - CS 2 of V_κ(39%), L2 - CS 1 (100%), L3 - CS 1 of V_κ(36%) (calculation assumes a κ:λ ratio of 70:30, Hood *et al.*, Cold Spring Harbor Symp. Quant. Biol. (1967) 48: 133). For H3 loops that have canonical structures, a CDR3 length (Kabat *et al.* (1991) *Sequences of proteins of immunological interest*, U.S. Department of Health and Human Services) of seven residues with a salt-bridge from residue 94 to residue 101 appears to be the most common. There are at least 16 human antibody sequences in the EMBL data library with the required H3 length and key residues to form this conformation and at least two crystallographic structures in the protein data bank which can be used as a basis for antibody modelling (2cgr and 1tet). The most frequently expressed germline gene segments that this combination of canonical structures are the V_H segment 3-23 (DP-47), the J_H segment JH4b, the V_κ segment O2/O12 (DPK9) and the J_κ segment J_κ1. V_H segments DP45 and DP38 are also suitable. These segments can therefore be used in combination as a basis to construct a library with the desired single main-chain conformation.

Alternatively, instead of choosing the single main-chain conformation based on the natural occurrence of the different main-chain conformations for each of the binding loops in isolation, the natural occurrence of combinations of main-chain conformations is used as the basis for choosing the single main-chain conformation. In the case of antibodies, for example, the natural occurrence of canonical structure combinations for any two, three, four, five, or for all six of the antigen binding loops can be determined. Here, the chosen conformation may be commonplace in naturally

occurring antibodies and may be observed most frequently in the natural repertoire. Thus, in human antibodies, for example, when natural combinations of the five antigen binding loops, H1, H2, L1, L2 and L3, are considered, the most frequent combination of canonical structures is determined and then combined with the most popular conformation for the H3 loop, as a basis for choosing the single main-chain conformation.

Diversification of the canonical sequence

Having selected several known main-chain conformations or a single known main-chain conformation, dAbs can be constructed by varying the binding site of the molecule in order to generate a repertoire with structural and/or functional diversity. This means that variants are generated such that they possess sufficient diversity in their structure and/or in their function so that they are capable of providing a range of activities.

The desired diversity is typically generated by varying the selected molecule at one or more positions. The positions to be changed can be chosen at random or they may be selected. The variation can then be achieved either by randomisation, during which the resident amino acid is replaced by any amino acid or analogue thereof, natural or synthetic, producing a very large number of variants or by replacing the resident amino acid with one or more of a defined subset of amino acids, producing a more limited number of variants.

Various methods have been reported for introducing such diversity. Error-prone PCR (Hawkins *et al.*, J. Mol. Biol. (1992) 226: 889), chemical mutagenesis (Deng *et al.*, J. Biol. Chem. (1994) 269: 9533) or bacterial mutator strains (Low *et al.*, J. Mol. Biol. *(1996)* 260: 359) can be used to introduce random mutations into the genes that encode the molecule. Methods for mutating selected positions are also well known in the art and include the use of mismatched oligonucleotides or degenerate oligonucleotides, with or without the use of PCR. For example, several synthetic antibody libraries have been created by targeting mutations to the antigen binding loops. The H3 region of a human tetanus toxoid-binding Fab has been randomised to create a range of new binding specificities (Barbas *et al.*, Proc. Natl. Acad. Sci. USA (1992) 89: 4457). Random or semi-random H3 and L3 regions have been appended to germline V gene segments to produce large libraries with unmutated framework regions (Hoogenboom & Winter J. Mol. Biol. (1992) 227: 381; Barbas *et al.,* Proc. Natl. Acad. Sci. USA (1992) 89: 4457; Nissim *et al.,* EMBO J. (1994) 13: 692;

Griffiths *et al.* EMBO J. (1994) 13: 3245; De Kruif *et al,* J. Mol. Biol. (1995) 248: 97). Such diversification has been extended to include some or all of the other antigen binding loops (Crameri *et al*. Nature Med. *(1996)* 2: 100; Riechmann *et al*. Bio/Technology (1995) 13: 475; Morphosys, WO97/08320, supra).

Since loop randomisation has the potential to create approximately more than 10^{15} structures for H3 alone and a similarly large number of variants for the other five loops, it is not feasible using current transformation technology or even by using cell free systems to produce a library representing all possible combinations. Even for some of the largest libraries constructed in excess of 6×10^{12} different antibodies, using technologies such as ribosomal display, only a fraction of the potential diversity would be represented in a library of this design (He and Taussig, Nucleic Acid Research 1997 25(24): 5132).

In a one embodiment, only those residues which are directly involved in creating or modifying the desired function of the molecule are diversified. For many molecules, the function will be to bind a target and therefore diversity should be concentrated in the target binding site, while avoiding changing residues which are crucial to the overall packing of the molecule or to maintaining the chosen main-chain conformation.

In one aspect, libraries of dAbs are used in which only those residues in the antigen binding site are varied. These residues are extremely diverse in the human antibody repertoire and are known to make contacts in high-resolution antibody/antigen complexes. For example, in L2 it is known that positions 50 and 53 are diverse in naturally occurring antibodies and are observed to make contact with the antigen. In contrast, the conventional approach would have been to diversify all the residues in the corresponding Complementarity Determining Region (CDR1) as defined by Kabat *et al*. (1991, supra), some seven residues compared to the two diversified in the library. This represents a significant improvement in terms of the functional diversity required to create a range of antigen binding specificities.

In nature, antibody diversity is the result of two processes: somatic recombination of germline V, D and J gene segments to create a naive primary repertoire (so called germline and junctional diversity) and somatic hypermutation of the resulting rearranged V genes. Analysis of human antibody sequences has shown that diversity in the primary repertoire is focused at the centre of the antigen binding site whereas

somatic hypermutation spreads diversity to regions at the periphery of the antigen binding site that are highly conserved in the primary repertoire (see Tomlinson *et al.*, J. Mol. Biol. (1996) 256: 813). This complementarity has probably evolved as an efficient strategy for searching sequence space and, although apparently unique to antibodies, it can easily be applied to other polypeptide repertoires. The residues which are varied are a subset of those that form the binding site for the target. Different (including overlapping) subsets of residues in the target binding site are diversified at different stages during selection, if desired.

In the case of an antibody repertoire, an initial 'naive' repertoire is created where some, but not all, of the residues in the antigen binding site are diversified. As used herein in this context, the term "naive" or "dummy" refers to antibody molecules that have no pre-determined target. These molecules resemble those which are encoded by the immunoglobulin genes of an individual who has not undergone immune diversification, as is the case with fetal and newborn individuals, whose immune systems have not yet been challenged by a wide variety of antigenic stimuli. This repertoire is then selected against a range of antigens or epitopes. If required, further diversity can then be introduced outside the region diversified in the initial repertoire. This matured repertoire can be selected for modified function, specificity or affinity.

It will be understood that the sequences described herein include sequences which are substantially identical, for example sequences which are at least 90% identical, for example which are at least 91%, or at least 92%, or at least 93%, or at least 94% or at least 95%, or at least 96%, or at least 97% or at least 98%, or at least 99% identical to the sequences described herein.

For nucleic acids, the term "substantial identity" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, or at least about 98% to 99.5% of the nucleotides. Alternatively, substantial identity exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

For nucleotide and amino acid sequences, the term "identical" indicates the degree of identity between two nucleic acid or amino acid sequences when optimally aligned and compared with appropriate insertions or deletions. Alternatively, substantial identity exists when the DNA segments will hybridize under selective hybridization

conditions, to the complement of the strand.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions, times 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence encoded by SEQ ID NO: 14, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in the polypeptide sequence encoded by SEQ ID NO: 14 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the polypeptide

sequence encoded by SEQ ID NO: 14, or:

$$na \leq xa - (xa \bullet y),$$

wherein na is the number of amino acid alterations, xa is the total number of amino acids in the polypeptide sequence encoded by SEQ ID NO: 14, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of xa and y is rounded down to the nearest integer prior to subtracting it from xa.

Examples

Example 1

1.1 Generation of a Dual Targeting anti-TNFα/anti-VEGF mAbdAb (DMS4000)

An anti-TNFα/anti-VEGF mAbdAb (designated DMS4000) was produced by fusion of a dAb to the C-terminus of the mAb (adalimumab) heavy chain. For construction of the heavy chain expression cassette, vector DNA encoding the heavy chain of an alternative mAbdAb was taken as a starting point. The dAb portion was excised using the restriction enzymes SalI and HindIII. DOM15-26-593, an anti-VEGF dAb, was amplified by PCR (using primers coding SalI and HindIII ends) and ligated into the vector backbone from which the dAb had been excised using the same restriction sites, resulting in a linker of 'STG' (serine, threonine, glycine) between the mAb and the dAb.

Sequence verified clones (SEQ ID NO:11 and 13 for light and heavy chains respectively) were selected and large scale DNA preparations were made and the anti-TNFα/anti-VEGF mAbdAb was expressed in mammalian HEK293-6E cells (National Research Council Canada) using transient transfection techniques by co-transfection of light and heavy chains (SEQ ID NO:12 and 14).

The sequence of the anti-TNFα/anti-VEGF mAbdAb heavy chain was further modified to have a codon optimised sequence for the anti VEGF dAb, and incorporate L235A and G237A mutations (Kabat numbering) to disable the FC effector function (DMS4000 mAbdAb heavy chain Fc disabled SEQ ID NO 46 and 47).

1.2 Purification and SEC analysis of the Dual Targeting anti-TNFα/anti-VEGF mAbdAb (DMS4000)

The anti-TNFα/anti-VEGF mAbdAb (designated DMS4000) was purified from clarified expression supernatant using Protein-A affinity chromatography according to established protocols. Concentrations of purified samples were determined by spectrophotometry from measurements of light absorbance at 280nm. SDS-PAGE analysis (figure 1) of the purified sample shows non-reduced sample running at ~170kDa whilst reduced sample shows two bands running at ~25 and ~60kDa corresponding to light chain and dAb-fused heavy chain respectively.

For size exclusion chromatography (SEC) analysis the anti-TNFα/anti-VEGF mAbdAb was applied onto a Superdex-200 10/30 HR column (attached to an Akta Express FPLC system) pre-equilibrated and running in PBS at 0.5ml/min. The SEC profile shows a single species running as a symmetrical peak (figure 2).

1.3 Binding Affinities of the Dual Targeting anti-TNFα/anti-VEGF mAbdAb (DMS4000)

VEGF Receptor Binding Assay.

This assay measures the binding of $VEGF_{165}$ to VEGF R2 (VEGF receptor) and the ability of test molecules to block this interaction. ELISA plates were coated overnight with VEGF receptor (R&D Systems, Cat No: 357-KD-050) (0.5µg/ml final concentration in 0.2M sodium carbonate bicarbonate pH9.4), washed and blocked with 2% BSA in PBS. VEGF (R&D Systems, Cat No: 293-VE-050) and the test molecules (diluted in 0.1%BSA in 0.05% Tween 20™ PBS) were pre-incubated for one hour prior to addition to the plate (3ng/ml VEGF final concentration). Binding of VEGF to VEGF receptor was detected using biotinylated anti-VEGF antibody (0.5µg/ml final concentration) (R&D Systems, Cat No: BAF293) and a peroxidase conjugated anti-biotin secondary antibody (1:5000 dilution) (Stratech, Cat No: 200-032-096) and visualised at OD450 using a colorimetric substrate (Sure Blue TMB peroxidase substrate, KPL) after stopping the reaction with an equal volume of 1M HCl.

MRC-5/TNFα Assay

The ability of test molecules to prevent human TNFα binding to human TNFR1 and neutralise IL-8 secretion was determined using human lung fibroblast MRC-5 cells. A

dilution series of test samples was incubated with TNFα (500pg/ml) (Peprotech) for 1 hour. This was then diluted 1 in 2 with a suspension of MRC-5 cells (ATCC, Cat.# CCL-171) ($5x10^3$ cells/well). After an overnight incubation, samples were diluted 1 in 10, and IL-8 release was determined using an IL-8 ABI 8200 cellular detection assay (FMAT) where the IL-8 concentration was determined using anti-IL-8 (R&D systems, Cat# 208-IL) coated polystyrene beads, biotinylated anti-IL-8 (R&D systems, Cat# BAF208) and streptavidin Alexafluor 647 (Molecular Probes, Cat#S32357). The assay readout was localised fluorescence emission at 647nm and unknown IL-8 concentrations were interpolated using an IL-8 standard curve included in the assay.

Binding affinities to VEGF and TNFα were determined as described as set out above. Assay data were analysed using GraphPad Prism. Potency values were determined using a sigmoidal dose response curve and the data fitted using the best fit model. Anti-VEGF potency (Figure 3) of this mAbdAb was calculated to be 57pM whilst the control, an anti-VEGF mAb, gave an EC50 value of 366pM. In the anti-TNFα bioassay (Figure 4) the potency was 10pM whilst an anti-TNFα control mAb produced an EC50 of 22pM. In conclusion, assay data shows that this dual targeting mAbdAb is potent against both antigens (TNFα and VEGF).

1.4 Rat PK of the Dual Targeting anti-TNFα/anti-VEGF mAbdAb (DMS4000)

This molecule was tested for its *in vivo* pharmacokinetic properties in the rat. The anti-TNFα/anti-VEGF mAbdAb was administered i.v. to three rats, and serum samples collected over a period of 10 days (240 hours). The concentration of drug remaining at various time points post-dose was assessed by ELISA against both TNFα & VEGF. The results are shown in Figure 5.

The PK parameters confirmed that this molecule had *in vivo* pharmacokinetic properties that compared with those of an anti-TNFα mAb. The shorter observed $t_{1/2}\beta$ for the VEGF component is not considered to be significant and may be an assay artefact. Further details are shown in Table 3.

Table 3

Antigen	Half Life (hr)	Cmax (µg/mL)	AUC (0-inf) (hr* µg/mL)	Clearance (mL/hr/kg)	% AUC Extrapolated
TNFα	180.1	89.9	7286.3	0.7	35.8
VEGF	94.2	102.8	4747.1	1.1	14.3

1.5 Generation of an alternative anti-TNFα/anti-VEGF mAbdAb (DMS4031)

An alternative anti-TNFα/anti-VEGF mAbdAb (designated DMS4031) was constructed in a similar way to that described above in Example 1.1, using the same anti-TNFα mAb (adalimumab) linked to a VEGF dAb on the C-terminus of the heavy chain using an STG linker. The anti-VEGF dAb used in this case was DOM15-10-11. This molecule was expressed in mammalian HEK293-6E cells (National Research Council Canada) using transient transfection techniques by co-transfection of light and heavy chains (SEQ ID NO:12 and 16). This molecule expressed to give a mAbdAb of similar expression levels to that described in Example 1.2, however when tested for potency in the same VEGF assay as described in Example 1.3 it was found to have undetectable levels of inhibition of VEGF binding to VEGF receptor in this assay.

Example 2

Biacore analysis of dual targeting anti-TNFα/anti-VEGF mAbdAbs

The test mAbdAb was subjected to BIAcore analysis to determine kinetic association and dissociation constants for binding to their corresponding antigens. Analysis was performed on BIAcore™ 3000 instrument. The temperature of the instrument was set to 25°C. HBS-EP buffer was used as running buffer. Experimental data were collected at the highest possible rate for the instrument. One flow cell on a research grade CM5 chip was coated with protein A using standard amine coupling chemistry according to manufacturer's instructions, and a second flow cell was treated equally but buffer was used instead of protein A to generate a reference surface. The flow cell coated with protein A was then used to capture mAbdAbs. Antigen was injected as a series 2x serial dilutions as detailed in table 2. Several dilutions were run in duplicate. Injections of buffer alone instead of ligand were used for background subtraction. Samples were injected in random order using the kinetics Wizard

inherent to the instrument software. The surface was regenerated at the end of each cycle by injecting 10mM Glycine, pH 1.5. Both data processing and kinetic fitting were performed using BIAevaluation software 4.1. Data showing averages of duplicate results (from the same run) is shown in Table 4. The multiple values shown for DMS4031 represent two experiments run on separate occasions. The value of 787nM probably overestimates the affinity due to the concentrations of ligand analysed

Table 4

Molecule number	Antigen	Ka [1/Ms]	Kd [1/s]	KD [pM]	Top concentration (nM)	# dilutions
DMS4000	TNFα	3.65E+05	4.16E-05	112	10	6
DMS4000	VEGF	9.19E+05	4.78E-04	520	2.5	5

Example 3

Stoichiometry assessment of antigen binding proteins (using Biacore™)

This example is prophetic. It provides guidance for carrying out an additional assay in which the antigen binding proteins of the invention can be tested.

Anti-human IgG is immobilised onto a CM5 biosensor chip by primary amine coupling. Antigen binding proteins are captured onto this surface after which a single concentration of TNFα or VEGF is passed over, this concentration is enough to saturate the binding surface and the binding signal observed reached full R-max. Stoichiometries are then calculated using the given formula:

Stoich=Rmax * Mw (ligand) / Mw (analyte)* R (ligand immobilised or captured)

Where the stoichiometries are calculated for more than one analyte binding at the same time, the different antigens are passed over sequentially at the saturating antigen concentration and the stoichometries calculated as above. The work can be carried out on the Biacore 3000, at 25°C using HBS-EP running buffer.

Example 4

Design and Construction of CTLA4-Ig fused to anti-VEGFR2 adnectin via a GS linker (BPC1821)

A codon-optimised DNA sequence encoding CTLA4-Ig (a HindIII site at the N-terminus and BamHI site at the C-terminus were included to facilitate cloning) was constructed and cloned into a mammalian expression vector (pTT expression vector from the National Research Council Canada with a modified multiple cloning site (MCS)) containing the CT01 adnectin. This allowed the adnectin to be fused onto the C-terminus of the CTLA4-Ig via a GS linker. The resulting antigen binding protein was named BPC1821. The DNA and protein sequences of BPC1821 are given in SEQ I.D. No. 26 and 27 respectively.

The expression plasmid encoding BPC1821 was transiently transfected into HEK 293-6E cells (National Research Council Canada) using 293fectin (Invitrogen, 12347019). A tryptone feed was added to the cell culture after 24 hours and the supernatant was harvested after 96 hours. BPC1821 was purified using a Protein A column before being tested in a binding assay.

Example 5

VEGFR2 and B7-1 Binding ELISA (BPC1821)

A 96-well high binding plate was coated with 0.4µg/ml of recombinant human VEGFR2 Fc Chimera (R&D Systems, 357-KD-050) in PBS and stored overnight at 4°C. The plate was washed twice with Tris-Buffered Saline with 0.05% of Tween-20. 200µL of blocking solution (5% BSA in DPBS buffer) was added to each well and the plate was incubated for at least 1hour at room temperature. Another wash step was then performed. BPC1821 and two negative control antibodies (Sigma I5154 and the bispecific IGF1R–VEGFR2 antigen binding construct BPC1801 – heavy chain SEQ ID NO:163 and light chain SEQ ID NO:164) were successively diluted across the plate in blocking solution. After 1 hour incubation, the plate was washed. Recombinant human B7-1 Fc Chimera (RnD Systems, 140-B1-100) was biotinylated using the ECL biotinylation module from GE Healthcare. The labelling was performed at a quarter of the kit recommended level. The biotinylated B7-1 was diluted in blocking solution to 1µg/mL and 50µL was added to each well. The plate was incubated for one hour then washed. ExtrAvidin peroxidase (Sigma, E2886) was

diluted 1 in 1000 in blocking solution and 50µL was added to each well. After another wash step, 50µl of OPD SigmaFast substrate solution was added to each well and the reaction was stopped 15 minutes later by addition of 25µL of 3M sulphuric acid. Absorbance was read at 490nm using the VersaMax Tunable Microplate Reader (Molecular Devices) using a basic endpoint protocol.

Figure 6 shows the results of the ELISA and confirms that bispecific BPC1821 shows binding to both VEGFR2 and B7-1. The negative control antibodies do not show binding to both VEGFR2 and B7-1. Control concentrations were diluted from starting concentrations of 2 µg/ml.

Example 6

Design and Construction of CTLA4-Ig fused to an anti-VEGF dAb via a GS linker (BPC1825)

The DNA plasmid containing the CTLA4-Ig fused to the anti-VEGFR2 adnectin was used as a base plasmid to construct a CTLA4-Ig-anti-VEGF dAb bispecific. The vector was prepared by digesting the base plasmid with BamHI and EcoRI to remove the adnectin sequence. DNA sequences encoding the anti-VEGF dAb were restricted with BamHI and EcoRI and ligated into the vector. The resulting CTLA4-Ig-anti-VEGF dAb bispecific was named BPC1825, where the dAb was fused onto the C-terminus of the CTLA4-Ig via a GS linker. The DNA and protein sequences of BPC1825 are given in SEQ ID NO:28 and 29, respectively.

The expression plasmid encoding BPC1825 was transiently transfected into HEK 293-6E cells (National Research Council Canada) using 293fectin (Invitrogen, 12347019). A tryptone feed was added to each cell culture after 24 hours and supernatants were harvested after 96 hours. The supernatants were used as the test articles in binding assays.

Example 7

VEGF and B7-1 Binding ELISA (BPC1825)

A 96-well high binding plate was coated with 0.4µg/ml of human VEGF165 (in-house material) in PBS and stored overnight at 4°C. The plate was washed twice with Tris-Buffered Saline with 0.05% of Tween-20. 200µL of blocking solution (5% BSA in

DPBS buffer) was added to each well and the plate was incubated for at least 1hour at room temperature. Another wash step was then performed. BPC1825 and two negative control antibodies (Sigma I5154 and BPC1824 – a CTLA4-Ig-anti-IL-13 dAb fusion – SEQ ID NO:165) were successively diluted across the plate in blocking solution. After 1 hour incubation, the plate was washed. Recombinant human B7-1 Fc Chimera (RnD Systems, 140-B1-100) was biotinylated using the ECL biotinylation module from GE Healthcare. The labelling was performed at a quarter of the kit recommended level. The biotinylated B7-1 was diluted in blocking solution to 1µg/mL and 50µL was added to each well. The plate was incubated for one hour then washed. ExtrAvidin peroxidase (Sigma, E2886) was diluted 1 in 1000 in blocking solution and 50µL was added to each well. After another wash step, 50µl of OPD SigmaFast substrate solution was added to each well and the reaction was stopped 15 minutes later by addition of 25µL of 3M sulphuric acid. Absorbance was read at 490nm using the VersaMax Tunable Microplate Reader (Molecular Devices) using a basic endpoint protocol.

Figure 7 shows the results of the ELISA and confirms that bispecific BPC1825 shows binding to both VEGF and B7-1. The negative control antibodies do not show binding to both VEGF and B7-1. Concentration of Sigma I5154 IgG was diluted from a starting concentration of 2 µg/ml.

Example 8

Design and construction of a TNFα receptor Fc fusion fused to a VEGF dAb via an STG or TVAAPPSTG linker

A codon-optimised DNA sequence encoding a human TNFα receptor Fc fusion (etanercept) was constructed and cloned into a mammalian expression vector (pTT5) along with the DOM15-26-593 anti VEGF dAb from the DMS4000 construct.

The Receptor Fc was flanked with additional sequences to provide an N-terminal Campath1 signal peptide, and provide either an STG linker or TVAAPSTVAAPSTVAAPSTVAAPSTG linker at the C-terminus for fusion to the dAb. The flanking sequences included an AgeI restriction site and a SalI restriction site to facilitate cloning into the vector with the dAb. The resulting antigen binding proteins were named EtanSTG593 and EtanTV4593, respectively. The DNA and protein

sequences of EtanSTG593 are given in SEQ ID No:48 and 49, respectively, and of EtanTV4593 are given in SEQ ID No: 50 and 51 respectively.

Example 9

EtanSTG593 and EtanTV4593 purification and VEGF and TNFα binding Analysis

The EtanSTG593 and EtanTV4593 plasmids were independently expressed in HEK 293-6E cells (National Research Council Canada) using 293Fectin (Invitrogen) for transfection. EtanSTG593 and EtanTV4593 were harvested after 5 days, and purified by MAb Select Sure (GE Healthcare) affinity chromatography to give batch samples M4004 and M4005 respectively. The proteins were formulated in F1 buffer (0.1M Citrate pH6, 10% PEG300, 5% Sucrose) or ET buffer (10mM Tris pH7.4, 4% D-Mannitol, 1% Sucrose). The proteins were further purified by Size Exclusion Chromotography on a HiLoad Superdex S200 10/300 GL column (GE Healthcare) to reduce the level of aggregates.

Binding analysis was carried out on a ProteOn XPR36 machine (BioRad TM). Protein A was immobilised on a GLM chip by primary amine coupling. The constructs to be tested were captured on this Protein A surface. The analytes, TNFα and VEGF were used at 256 nM, 64 nM, 16 nM, 4 nM and 1nM. 0 nM (i.e. buffer alone)TNFα and VEGF was used to double reference binding curves.

The novel six by six flowcell set up of the ProteOn allows up to six constructs to be captured at the same time and also allows six concentrations of analyte to be flowed over the captured antibody(s), in all generating 36 interactions per cycle.

To regenerate the Protein A surface, 50 mM NaOH was used, this removed captured construct(s) and allowed another capture and binding cycle to begin. The data obtained was fitted to 1:1 model inherent to the ProteOn analysis software. The run was carried out using HBS-EP as running buffer and at a temperature of 25°C.

Table 5: VEGF Binding Results

Construct	Ka [1/Ms]	Kd [1/s]	KD(nM)
M4004 F1	1.18E+05	1.01E-04	0.850
M4005 F1	3.18E+05	1.85E-05	0.058
M4004 ET	1.24E+05	7.84E-05	0.631
M4005 ET	4.54E+05	4.44E-05	0.098

Table 6: TNFα Binding Results

Construct	Ka [1/Ms]	Kd [1/s]	KD(nM)
M4004 F1	5.10E+06	1.22E-04	0.024
M4005 F1	4.95E+06	1.05E-04	0.021
M4004 ET	4.81E+06	1.15E-04	0.024
M4005 ET	4.87E+06	1.38E-04	0.028

Example 10 – prophetic example

10.1 Generating dual-targeting antigen binding proteins

A dual-targeting antigen binding construct can be engineered by introducing physical linkages between two previously identified antigen binding proteins e.g. antibody fragments or whole monoclonal antibodies. The physical linkages may be introduced by encoding genetic linker sequences between the two moieties. The nature of the linker in terms of length and amino acid composition may have a bearing on the properties of one or both of the moieties in the bispecific agent. In the event of having multiple antibodies or antibody fragments for generating bispecifics, an empirical approach may be adopted to identify an optimum combination of leads.

Individual binding moieties such as mAbs, FAbs, ScFvs, dAbs etc. against defined targets can be identified and developed in isolation using a variety of well documented *in vivo* (for example: Harlow, E and Lane, D (1998) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press) and *in vitro* (for example: Barbas III, CF et al (2001) Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press) techniques to deliver agents with known properties of potency, efficacy and biophysical behaviour. From these individual agents a number of different bispecific opportunities arise which are only limited by the degree of complexity of the molecular engineering required to create them. The desired molecular architecture is normally determined by the nature of the condition to be treated. For example, for chronic dosing a molecular format that delivers an intrinsically long *in vivo* half life is to be favoured. This can be most readily achieved by the inclusion of the Fc region of an IgG antibody which delivers long terminal half life by virtue of salvage recycling pathways. Thus a mAb or other Fc-based bispecific is a frequently employed format.

To develop a mAb-based dual targeting molecule one potential approach is to append an antibody fragment to a full IgG. At a molecular level, this can be done by introducing a restriction site at one of the termini of the mAb chain and inserting an antibody fragment such that the mAb chain is extended with an additional functional unit. The nature of the linker between the functional units may need to be varied to optimise the overall properties of the bispecific. If a range of different antibody fragments are available that address the same target, these may be directly compared with one another using this approach. Bispecifics of this nature will normally be expressed in mammalian cells, typically HEK293 cells transiently but CHO cells for stable cells lines and large-scale manufacturing. For TNF/VEGF bispecifics, an anti-TNFα mAb may be linked to a VEGF binding protein such as an antibody fragment in this manner, or alternatively an anti-VEGF mAb may be linked to an anti-TNFα binding protein. For example, TNFα and VEGF antagonists that may be utilised in this way are listed in table 1 and 2, respectively. In such an exercise, if all possible reagents are available, all potential combinations would be tested.

Non mAb-based bispecifics can be made by linking two antibody fragments or other proteins which bind antigens in a generally analogous manner together as a genetic fusion. The junction of the two units is normally represented by a linker of a length and sequence composition that may be determined empirically. Such molecules allow freedom of molecular engineering due to their modular, single chain nature and afford the possibility of expression in systems other than mammalian cells.

Figure 8 shows a matrix of possible dual targeting constructs that may be used in accordance with the invention. Sequences of a number of the possible dual targeting constructs shown in Figure 8 are given in SEQ ID NO:73-140. In these specific dual targeting molecules a 'TVAAPS' linker (SED ID NO:4) is used to link the component parts, with the exception of heavy chains in DVD-Igs, DVD-Fabs fusions with N-terminal ScFvs (SEQ ID NO:116-118) and fusions with N-terminal VH dAbs (SEQ ID NO:133, 134) where the linker is 'ASTKGPS' (SEQ ID NO:6). SEQ ID NO:73-140 are exemplary only and the skilled person would realise that other linkers and constructions are possible.

Table 7: abbreviations used in Figure 8

IgG	Immunoglobulin G
mAb	Monoclonal antibody
FAb	Fragment for antigen binding
ScFv	Single chain variable fragment
dAb	Domain antibody
VHH	Camelid single domain antibody
A/C	Anticalin
Dpn	Darpin
Axn	Adnectin
DVD-Ig	Dual variable domain IgG
Fc	IgG CH2-CH3 region
Rec	Receptor
PEG	Polyethylene glycol

10.2 Testing the dual-targeting antigen binding proteins for required characteristics

Potency/Affinity: A fundamental property of a bispecific molecule suitable for further development is a kinetic binding affinity (usually determined by a form of surface plasmon resonance (SPR), for example BIAcore) for antigen which, in turn, would be used to predict a minimum pharmacologically effective concentration after a given therapeutic dose based upon prior knowledge of antigen concentration and availability. The affinity may also be predicted to be related to neutralisation potency, an attribute normally assessed by an *in vitro* assay that determines the concentration of compound that mediates a particular pharmacological effect. This may be the inhibition of a receptor/ligand binding event or the stimulation/inhibition of a downstream response pathway. For example, the potency of a TNF antagonist may be assessed by the extent to which it prevents the production of other cytokines that are regulated by TNF. A common form of this would be the reduction in the secretion of IL8 from MRC-5 cells in response to TNF. For a VEGF antagonist, the extent to which receptor phosphorylation is reduced is a direct consequence of the inhibitory potency of anti-VEGF agent, whilst the reduction in proliferation of HUVEC cells is a

biological correlate of this effect. As with the kinetic affinity, the bispecific would be required to demonstrate target potency for both antigens.

Biophysics: Because conventional mAbs are known to have good expression, biophysical and pharmacokinetic profiles, any developable bispecific molecule would be required to demonstrate similar characteristics. Expression level would be determined during transient and stable cell culture and would be required to be in the same normal range as conventional therapeutic antibodies. The bispecific would need to be amenable to similar purification processes to mAbs (for example protein-A capture) and other down stream processing (DSP) steps that are required in the production of clinical grade material. The purified protein would need to demonstrate a clean, symmetrical size exclusion chromatography (SEC) profile, stability at high (>25mg/ml) protein concentrations in biocompatible buffers and resistance to a range of stress conditions (temperature, pH, freeze-thaw, deamidation conditions etc).

Pharmacokinetics (PK)/Pharmacodynamics (PD): The pharmacokinetic profile of a bispecific antigen binding protein is required to be consistent with the nature of the targets and the disease setting. In the majority of cases, antibodies are positively differentiated by virtue of their long serum half life and this is usually the desired profile. PK as normally assessed in both rodent and primate species and the terminal half life ($t_{1/2}\beta$) of the bispecific should be comparable with that of antibody agents against the same targets (it is assumed that the bispecific will reflect the more rapidly cleared species in the event of the two activities being metabolised at radically different rates). PK assays for bispecific molecules ideally measure the two activities in a single assay (a bridging assay), thereby providing confidence that the residual drug in the circulation is intact and fully bifunctional (for example, TNF is immobilized on a plate, the samples containing drug are added to the plate and the amount of bispecific present assayed by the addition of, for example, biotinylated VEGF which is itself detected by an anti-biotin agent). Other *in vivo* analyses on bispecific compounds would include the testing in models of disease under the proviso that such models exist and that the cross-reactivity of the bispecific with the host species is well understood. For a TNF/VEGF bispecific this may include inflammatory conditions where the inflammation is exacerbated by increased vascular leakage or a vascular proliferative condition where the activation of macrophages in the local environment exacerbates the disease state. In primates, such models may also allow the derivation of certain pharmacodynamic markers of activity that may play a role in the calculation of dose etc.

Safety: The relative novelty of bispecific formats (even if the component parts and targets are precedented) raises issues of safety and tolerability. As with any biological drug, the full range of toxicology tests would be required, with an increased emphasis on any hypothetical concerns related to the bispecific molecular format. This may include additional unanticipated pharmacology or the potential for increased immunogenicity. The latter possibility may be addressed using *in silico* tools to look for T-cell epitopes which could be used to construct a risk profile for this aspect of the molecule.

Non mAb-based bispecific formats (for example direct fusion of two antibody or antibody-like fragments) can be judged on many of the same criteria of affinity, potency and biophysical behaviour, although some attributes, in particular PK, may vary with different molecular format. Such molecules may also be produced in different expression systems (for example, in prokaryotic cells), which may in itself create different requirements especially with regards to purification, DSP and safety studies.

Example 11

TNF/VEGF dAb-dAb in-line fusions (ILF)

Detailed below is a method for constructing dAb-dAb in-line fusions in order to make a TNF-VEGF bispecific. However, as described above in Example 10, the same approach could be used to generate any other bispecific based upon antibodies or antibody fragments with similar target specificities.

Bispecific molecules that have the potential to inhibit both TNFα and VEGF were constructed by the genetic fusion of two single Domain Antibodies (dAbs) into a dAb-dAb in-line fusion (ILF). To construct these molecules, independently selected dAbs against the two targets were isolated by phage display and high affinity and potency against the targets was achieved by rounds of affinity maturation using a range of suitable techniques. The final molecules that were selected for the ILFs were DOM15-26-593 (anti-VEGF) (SEQ ID NO:1) and PEP1-5-19 (anti-TNFα) (SEQ ID NO:35).

DOM15-26-593 is a VH dAb with a monomeric affinity for human VEGF-A of approximately 1nM. PEP1-5-19 is a Vk dAb with a monomeric affinity for human TNFα of approximately 8nM. Two different ILF constructs were made, one with the DOM15-26-593 dAb at the amino terminus (abbreviated below as "DOM-PEP"), and one with the PEP1-5-19 dAb in this location ("PEP-DOM"). The two dAbs in the ILFs were separated by a short linker that was derived from a sequence naturally associated with the C terminus of a VH or a Vk dAb. Hence the ILF with the VH dAb at the N-terminus included the linker "ASTKGPS" (SEQ ID NO:6 - the natural extension from VH into CH1), and the ILF with the PEP1-5-19 at the N-terminus included the linker sequence "TVAAPS" (SEQ ID NO:4 - the natural extension from Vk into Ck).

To make the ILFs, the mammalian transient expression vector pTT5 (NRC, Canada) was modified to include a secretion signal and appropriate cloning sites. These were as detailed below in table 8. To make the DOM-PEP construct, individual fragments corresponding to the DOM15-26-593 dAb and PEP1-5-19 domain dAb were amplified with the respective gene specific primers as described below. Linker sequences and restriction sites were incorporated within the primer sequences.

Table 8

N.B. restriction sites are underlined in DNA sequences

Primer	Sequence 5' - 3'	Comments
AVG18	attatgggatccaccggcgaggtgcagctgttggtgt (SEQ ID NO:52)	forward primer for DOM15-26-593 (DOM-PEP, has BamHI site)
AVG19	gctggggcccttggtgctagcgctcgagacggtgaccagg (SEQ ID NO:53)	reverse primer for DOM15-26-593 (DOM-PEP, has NheI site)
AVG26	ctcgagcgctagcaccaagggccccagcgacatccagatgaccc (SEQ ID NO:54)	forward primer for PEP (DOM-PEP, has NheI site)
AVG21	ttatgtcaagcttttaccgtttgatttccaccttggt (SEQ ID NO:55)	reverse primer for PEP (DOM-PEP, has HindIII site)
AVG22	attatgggatccaccggcgacatccagatgacccagtctcc (SEQ ID NO:56)	forward primer for PEP (PEP-DOM, has BamHI site)
AVG36	gcgccgccaccgtacgtttgatttccaccttggtccc (SEQ ID NO:57)	reverse primer for PEP (PEP-DOM, has BsiWI site)

AVG37	caaacgtacggtggcggcgccgagcgaggtgcagctgttggtgtc (SEQ ID NO:58)	forward primer for DOM15-26-593 (PEP-DOM, has BsiWI site but short overhang for digest)
AVG25	ttatgtcaagctttagctcgagacggtgaccag (SEQ ID NO:59)	reverse primer for DOM15-26-593 (PEP-DOM, has HindIII site)
AVG24	ggtggaaatcaaacgtacggtggcggcgccgagcga (SEQ ID NO:60)	forward primer for DOM15-26-593 (PEP-DOM, has BsiWI site appropriate overhang for subsequent digest)

DOM15-26-593 for the DOM-PEP construct was amplified with AVG18 and AVG19 and PEP1-5-19 for the DOM-PEP construct was amplified with AVG26 and AVG21. After purification the PCR fragments were digested with BamHI and NheI, and NheI and HindIII respectively and the fragments purified. They were then added to a 3-fragment ligation with a modified form of the vector pTT5 which contained a multiple cloning site that allowed the insertion of a BamHI-HindIII fragment downstream of a eukaryotic promoter. Ligations, transformations and analysis of resulting colonies was done using standard techniques, with nucleotide sequence analysis confirming that the resulting vector contained an insert with a sequence as laid out in SEQ ID NO:61, predicting a translation product shown in SEQ ID NO:62.

For the PEP-DOM construct, the PEP1-5-19 dAb was amplified with AVG22 and AVG36 and the DOM15-26-593 dAb with AVG37 & AVG25. These fragments were digested with BamHI and BsiWI (PEP) and BsiWI and HindIII (DOM), respectively. The DOM fragment was found to digest poorly and this was attributed to the short overhang on the 5' end of the primer. The PCR product was therefore re-amplified with AVG25 and AVG24 to extend the overhang, the digest was repeated and the fragment added to a 3-fragment ligation along with digested PEP insert and the pTT5 vector as described above. Ligations, transformations and analysis of resulting colonies was done using standard techniques, with nucleotide sequence analysis confirming that the resulting vector contained an insert with a sequence as laid out in SEQ ID NO:63, predicting a translation product shown in SEQ ID NO:64.

The sequenced clones were prepared for transfection by DNA maxiprep and DNA transfected into HEK293-6E cells (National Research Council Canada) using standard methodology. After clarification of the culture medium, the recombinant protein was harvested from transfected cell supernatant by protein-A affinity chromatography and purified material buffer exchanged into PBS and quantified. The ability of these proteins to bind both TNFα and VEGF was then assessed by surface plasmon resonance (SPR) as described below.

Using a number of monoclonal antibodies (alternatively protein A or protein L could be used) believed to bind to either VH or Vk dAbs away from the dAb CDR regions, the DOM-PEP and PEP-DOM proteins were captured on the sensor surface via the mAbs, the TNF and VEGF ligands were flowed over the captured bispecific and the binding characteristics analysed. The analysis determined that when the compounds were captured with either one of 2 different anti-Vk dAbs tested the binding of the TNF ligand was impaired, suggesting that this capture antibody was sterically interfering with the ligand binding. Further analysis was therefore restricted to the bispecific captured with an anti-VH dAb monoclonal antibody.

Approximately 1600 response units (RUs) of the anti-VH monoclonal were captured on a protein-A surface and the test compounds passed over the complex. The experimental set up was designed to provide a qualitative rather than quantitative measure of the binding activities therefore estimations of kinetics etc. were not possible. The clearest data was obtained for the PEP-DOM protein, where the two dAbs were both clearly able to bind to the ligands independently and simultaneously as evidenced by the additive binding curves (Figures 9 & 10).

Closer analysis of the binding events in the curve in Figure 9 demonstrates the binding of both ligands to the PEP-DOM protein.

The possibility of DOM-PEP binding both TNFα and VEGF is also seen (data not shown).

Example 12

An *in vivo* study: Laser-Induced Choroidal Neovascularisation (CNV) in rats: testing DMS1571 (VEGF-dab) and Enbrel™ separately

Rationale

Results obtained in a previous experiment showed that the anti-VEGF antagonist, DMS1571 (an Fc formatted version of the DOM 15-26-593 anti-VEGF dAb, which exists as a dimer of SEQ ID NO:65), is efficacious in the rat laser-induced choroidal neovascularization (CNV) model. The aim of this experiment was to further evaluate the dose-ranging of this molecule in the rat CNV model and, in addition, to undertake a dose ranging study of a TNFα antagonist (Enbrel™) in the same model. DMS4000 was also tested in this the study.

Methodology

Animals

12-week old Dark Agouti (DA) rats (Harlon Olac) were used in these studies. Prior to procedures animals were surgically anesthetized by intraperitoneal injection of a mixture of Ketamine (37.5%, Dodge Animal Health Ltd.), Dormitor (25%, Pfizer Animal Health, Kent) and sterile water (Pfizer Animal Health, Exton, PA) at 0.175 ml/100g and pupils were dilated with a combination of topical 1% tropicamide (Alcon Laboratories, Fort Worth, TX) and 2.5% phenylephrine (Akorn, Inc., Decatur, IL). All animal experiments conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Experimental CNV

Experimental CNV was induced unilaterally in groups of 2-4 month old female DA rats by rupturing Bruch's membrane using laser light photocoagulation (PC). Dye laser PC was performed using a diode-pumped, 532 nm argon laser (Novus Omni Coherent Inc., Santa Clara, CA) attached to a slit lamp funduscope, and a handheld planoconcave contact lens (Moorfields Eye Hospital, London, UK) applied to the cornea to neutralize ocular power. Eight lesions (532 nm, 150 mW, 0.15 second, 100 µm diameter) were made in a peripapillary distributed and standardized fashion centered on the optic nerve at 500µm radius (at 1-1.5 mm from optic disc) and avoiding major vessels in each eye. The morphologic end point of the laser injury was identified as the temporary appearance of a cavitation bubble, a sign associated with the disruption of Bruch's membrane (for background reference, general methods are disclosed in Campos, Amaral, Becerra, & Fariss, 2006 A novel imaging technique

for experimental choroidal neovascularization. Invest Ophthalmol Vis Sci, 47(12), 5163-5170, which is herein incorporated by reference in its entirety). Laser spots that did not result in the formation of a cavitation bubble were excluded from the studies.

In vivo imaging

In vivo image data of CNV and associated leakage was generated using confocal high-resolution Scanning Laser Ophthalmoscope (SLO) Fluorescein Angiography (FA) (0.3ml 5% intra-abdominally injected Fluorescein Sodium, FS obtained from Moorfields Eye Hospital, London, UK) at 7 days after lesion generation followed by a second imaging session 14 days post-procedure. Time points were chosen based on previous historical control studies on the time course of changes in intensity and area of fluorescein staining in angiograms taken after laser PC in non-treated rats. These historical studies showed that fluorescein staining was first observed 4 days after PC and that the intensity of the staining then rapidly increased reaching its peak approximately 14 days after photocoagulation (for general background on methodology see Kamizuru *et al.*, 2001; Monoclonal antibody-mediated drug targeting to choroidal neovascularization in the rat. Invest Ophthalmol Vis Sci, 42(11), 2664-2672; Takehana *et al.*, 1999 Suppression of laser-induced choroidal neovascularization by oral tranilast in the rat. Invest Ophthalmol Vis Sci, 40(2), 459-466, which are herein incorporated by reference in their entirity). Further assessment was not undertaken as the time course of experimental CNV in these studies indicated that fluorescein leakage begins to decrease approximately 5 weeks after photocoagulation. Baseline reflectance (at 488nm and 790nm) and autofluorescence (ex. 488nm, em. >498nm) images were made prior to injection of FS to help locate lesions in FA images. The arterio-venous phase was recorded immediately after FS injection. Fluorescein angiograms were thereafter recorded one minute after injection and again four minutes after injection, the latter 4 min data sets being used for statistical analysis.

Evaluation and statistical analysis of image data

The effect of drug treatment was evaluated by quantitative assessment of late-phase (4 ±1 minutes after FS injection) fluorescein angiography. Leakage was defined as the presence of hyperfluorescent areas corresponding with lesions in reflectance images. Prior to quantification the gain and brightness of all images used in analysis were normalized. The intensity and area of leakage in late-phase fluorescein angiography was quantified by multiplying the diameter of leakage (µm) with the mean pixel brightness value (0 to 1) in that area. Unpaired t-tests were used to

compare results between test groups. Values of $P < 0.05$ were considered statistically significant. Data are shown as means ± SEM unless otherwise noted. Before image analysis was performed identification was scrambled and quantification was undertaken in masked fashion.

Immunohistochemichal detection of macrophages in rat CNV lesions

Eyes which had previously been subject to fluorescence angiography in CNV studies were immediately enucleated and fixed in 4% p-formaldehyde. The eye-cup was then prepared from the treated eye of each animal and flat-mounted following four butterfly incisions. The macrophage content of vascular lesions determined by immunohistochemical staining using ED1 mAb and subsequently quantitated by counting ED1 positive cells - ED1 (CD68) mAb (catalogue number MCA341 Serotech, Kidlington, Oxford, UK)

Treatments

The table below (table 9) shows the treatments given to each experimental group

Number	Compound	Total Dose µg	Concentration mg/ml	Volume µl	Administration
1	Vehicle A – 50mM NaAcetate pH 5.5, 104mM NaCl, 0.025% Tween 80	N/A	N/A	2	intravitreal
2	DMS1571 in vehicle A	2	1	2	Intravitreal
3	DMS1571 in vehicle A	1	0.5	2	Intravitreal
4	DMS1571 in vehicle A	0.5	0.25	2	Intravitreal
5	DMS1571 in vehicle A	0.2	0.1	2	Intravitreal
6	DMS1571 in vehicle A	0.1	0.05	2	intravitreal
7	Vehicle B – 4% Mannitol, 1% sucrose, 10mM TrisHCL pH 7.4	N/A	N/A	2	Intravitreal
8	Enbrel™ in vehicle B	30	15	2	Intravitreal

9	Enbrel™ in vehicle B	10	5	2	Intravitreal
10	Enbrel™ in vehicle B	3	1.5	2	Intravitreal
11	Enbrel™ in vehicle B	1	0.5	2	Intravitreal
12	Enbrel™ in vehicle B	0.3	0.15	2	Intravitreal
13	DMS4000 in vehicle C	2	1	2	Intravitreal
14	Vehicle C – 100mM NaCitrate pH6, 10% PEG300, 5% sucrose	N/A	N/A	2	intravitreal

In each case, compounds were administered by intravitreal injection immediately prior to laser PC.

Results of laser-induced CNV studies

High-magnification fluorescein angiography was performed at two time points, at 7 days and 14 days after PC, on the treated eyes. Images were graded for choroidal leakage associated with experimental CNV and other vascular abnormalities related to the treatment noted. Images were recorded in both near-infrared reflectance (IR) and auto-fluorescence mode (AF). IR images were used to locate lesions in the retina prior to injecting the fluorescein contrast agent. All images were recorded at the level of the RPE (retinal pigment epithelium).

Effect of DMS1571 (VEGF-Dab) and Enbrel™ in rat CNV

Table 10: DMS1571

	1.0	2.0	3.0	4.0	5.0	6.0
$mean_{07}$	49.7	38.9	37.5	43.1	53.3	55.8
$mean_{14}$	53.0	36.7	43.5	39.6	49.1	48.9

	1	2	3	4	5	6
SEM_{07}	2.189	1.623	1.738	2.877	2.761	2.952
SEM_{14}	1.967	1.613	3.054	1.834	2.503	4.389

Mean +/- SEM for CNV leakage assessed at 7 and 14 days for DMS1571
1.0-vehicle, 2.0-2µg DMS1571, 3.0-1µg DMS1571, 4.0-0.5µg DMS1571, 5.0-0.2µg DMS1571, 6.0-0.1µg DMS1571. Agents were injected immediately prior to induction of laser injury. N=5 animals per group in all cases. All compounds were administered by intravitreal injection in a volume of 2µl.

Figure 11 is a graphical representation of data presented in Table 10. All compounds were administered by intravitreal injection in a volume of 2µl. Black bars represent day 7 results. White bars represent day 14 results.

Table 11: Enbrel™

	7.0	8.0	9.0	10.0	11.0	12.0
$mean_{07}$	43.7	37.0	42.9	46.0	45.3	38.3
$mean_{14}$	45.2	37.4	45.2	41.4	40.8	45.4
	7	8	9	10	11	12
SEM_{07}	3.934	1.247	1.649	1.912	2.294	1.917
SEM_{14}	2.469	1.302	1.167	1.794	1.246	2.268

Mean +/- SEM for CNV leakage assessed at 7 and 14 days for test Enbrel™
7.0-vehicle, 8.0-30µg Enbrel™, 9.0-10ug Enbrel™l, 10.0-3µg enbrel. 11.0-1µg Enbrel™, 12.0-0.3µg Enbrel™. Agents were injected immediately prior to induction of laser injury. N=5 animals per group in all cases. All compounds were administered by intravitreal injection in a volume of 2µl.

Figure 12 is a graphical representation of data presented in Table 11. All compounds were administered by intravitreal injection in a volume of 2µl. Black bars represent day 7 results. White bars represent day 14 results.

Figure 13 shows infrared (IR, upper left panel), autofluorescence (AF, lower left panel) and fluorescien angiography (FS, large panel) at 7 days (FS 1st) and 14 days (FS 2nd) after laser PC - showing example images. 1. Vehicle treated eyes, 2. eyes treated with 2µg DMS1571 and 8. eyes treated with 30µg Enbrel™. It is notable that the CNV lesions appear more punctuate and less diffuse than lesions responding to treatment with DMS1571. Arrows indicate neovascularisations indicated in both control and Enbrel™ treated animals but not in DMS1571 animals.

Table 12: DMS4000

	13.0	14.0
$mean_{07}$	33.4	36.8
$mean_{14}$	35.7	42.5
	13	14
SEM_{07}	1.808	2.559
SEM_{14}	1.241	2.131

Mean +/- SEM for CNV leakage assessed at 7 and 14 days for DMS4000

13.0-2µg DMS4000, 14.0-vehicle, Agents were injected immediately prior to induction of laser injury. N=5 animals per group in all cases. All compounds were administered by intravitreal injection in a volume of 2µl.

Figure 14 is a graphical representation of data presented in Table 12. All compounds were administered by intravitreal injection in a volume of 2µl.

Effect of DMS1571 (VEGF-dab) and Enbrel™ on macrophage content of rat CNV lesions

Table 13 – Quantitation of ED1 positive cells (macrophages) in CNV lesions

Group	**Macrophage (ED1 positive) content of CNV lesions**	
Vehicle (group 1)	35.2 (mean)	5.9 (SEM)
DMS1571 (group 2)	29.3	4.1
Enbrel (group 8)	16.2*	1.37

* $p<0.0016$ vs control, n=5 eyes in each case

Figure 15 shows example photomicrographs of flat-mounted retinae stained with ED1 mab. Panels 1A-1B and panel Enbrel 8.4 show flat-mounts of retinas from eyes treated with anti-VEGF (DMS1571) (1A), Vehicle only (1B) or Enbrel (Enbrel 8.4). Macrophages, associated with laser burn site, visualised with ED1 (CD 68, black) X20. Panel 1D shows a Cryostat section (20µm) of retina showing macrophages (ED1+, black) associated with laser burn site which has penetrated to the inner nuclear layer (INL) of the retina. RGC, retinal ganglion cell layer; BV, blood vessel. x20.

Conclusions

The results illustrate that DMS1571 is effective in significantly attenuating CNV disease. The strong and robust effect is noted at doses above 1µg with the 0.5µg dose showing a sub-maximal effect and at doses less than 0.5µg the therapeutic is ineffective. The experiments show that doses at 30µg of Enbrel™ are also effective in the model and lower doses ineffective. The finding that both VEGF inhibitors, as exemplified by DMS1571, and inhibitors of TNFα, as exemplified by Enbrel™, are able to independently attenuate choroidal neovascular disease in a rodent model suggests that a single therapeutic entity comprising both VEGF and TNFα capabilities, as exemplified by DMS4000, would be useful in the treatment of choroidal neovascular AMD. It is observed that DMS4000 (in which the TNFα binding function is not compatible with binding rat TNFalpha) performs equally well in the rat CNV model as DMS1571 at an equivalent dose.

It is notable from the fluorescence angiography pictures when comparing the DMS1571 treated eyes with the Enbrel™ treated eyes that the Enbrel™ eyes have a distinctive patterning in which the lesions appear more punctuate and less diffuse when compared to DMS1571 treated eyes. These differences in lesion patterning are highly suggestive of independent mechanisms of action of the DMS1571 (VEGF antagonist) and Enbrel™ (TNFα antagonist) therapeutics. This assertion is further supported by the finding that in the Enbrel™ treated group significantly fewer macrophages are recuitred to the CNV vascular lesions.

Example 13 An *in vivo* study: Laser-Induced Choroidal Neovascularisation (CNV) in rats: testing DMS1571 (VEGF-dab) and Enbrel™ in combination

The methods used in this example were essentially the same as those given in Example 12.

Table 14 below shows the treatments given to each experimental group.

Identification	Compound	Total Dose µg	Concentration mg/ml	Total Volume µl	Administration
A	DMS1571	2	1	2	intravitreal

B	DMS1571 plus Enbrel™	2 DMS1571 30 Enbrel™	2 DMS1571 30 Enbrel™	2#	intravitreal
C	Vehicle*	N/A	N/A	2	intravitreal
D	DMS1571	0.5 DMS1571	0.25	2	intravitreal
E	DMS1571 plus Enbrel™	0.5 DMS1571 30 Enbrel™	0.5 DMS1571 30 Enbrel™	2#	intravitreal

* Vehicle - 50mM NaAcetate 10mM TrisHCL pH7.4, 104mM NaCl, 0.025% Tween 80, 4% mannitol, 1% sucrose

in cases where both DMS1571 and Enbrel™ are being administered together, 1µl of each is administered

Table 15: Effect of DMS1571 (VEGF-dab) and Enbrel™ in rat CNV

Identification	7 day mean	7 days SEM	14 day mean	14 day SEM
A	104.26	5.27	85.24	4.90
B	95.89	5.56	106.91	5.45
C	98.45	6.81	91.25	5.16
D	101.82	4.77	105.31	3.61
E	104.61	6.32	113.91	4.46

Example 14 – DME model – prophetic example

It is envisaged that antigen binding proteins disclosed herein will be effective in treating and/or preventing Diabetic Macular Edema (DME). This may be verified in a diabetic macula edema model in which DME and retinal vascular leak is observed following initiation of hyperglycemia as in Ishida, T. Usui and K. Yamashiro *et al*.

(VEGF164 is proinflammatory in the diabetic retina, Invest Ophthalmol Vis Sci 44 (2003), pp. 2155–2162).

Sequences

Protein or polynucleotide description	SEQ ID NO:	
	DNA	Amino acid
anti-VEGF dAb DOM15-26-593		1
Anti-TNFα adnectin		2
G4S Linker		3
Linker		4
Linker		5
Linker		6
Linker		7
Linker		8
Signal peptide sequence		9
Anti-TNFα mAb (adalimumab) Heavy Chain		10
Anti-TNFα mAb (adalimumab) Light Chain	11	12
Anti-TNFα mAb (adalimumab)-DOM15-26-593 Heavy Chain (DMS4000 mAbdAb heavy chain)	13	14
DOM 15-26-anti-TNFα mAb (adalimumab) Heavy Chain	-	15
Anti-TNFα mAb (adalimumab)-DOM15-10-11 Heavy Chain (DMS4031 mAbdAb heavy chain)	-	16
Anti-TNFR1 dAb (DOM1h-131-206)		17
Anti-VEGFR2 adnectin		18
Anti-VEGF anticalin		19
Alternative Anti-VEGF antibody Heavy chain		20
Anti-VEGF antibody (bevacizumab) Light chain		21
Alternative Anti-VEGF antibody (bevacizumab) Heavy chain		22
anti-VEGF dAb DOM15-26		23
DOM15-26-593-Anti-TNFα mAb (adalimumab) Heavy Chain		24
Linker		25
BPC1821 (CTLA4-Ig fused to anti-VEGFR2 adnectin via a GS linker)	26	27

BPC1825 (CTLA4-Ig fused to an anti-VEGF dAb via a GS linker)	28	29
Anti-TNFα mAb heavy chain		30
Anti-TNFα mAb light chain		31
Anti-TNFα mAb (Infliximab) Heavy chain		32
Anti-TNFα mAb (Infliximab) Light chain		33
TNFR-Fc fusion (Etanercept)		34
Anti-TNFαVk dAb (PEP1-5-19)		35
Anti-TNFα Vk dAb (PEP1-5-490)		36
Anti-TNFα Vk dAb (PEP1-5-493)		37
Anti-TNFα scFv (ESBA105)		38
Anti-VEGF Fab (ranibizumab) Heavy Chain		39
Anti-VEGF Fab (ranibizumab) Light Chain		40
Anti-VEGF Vk dAb (DOM15-10-11)		44
Anti-VEGF antibody (R84) Heavy chain		41
Anti-VEGF antibody (R84) light chain		42
VEGFR1/2 hybrid – Fc fusion (aflibercept - VEGF-Trap)		43
CT01		45
Anti-TNFα mAb (adalimumab)-DOM15-26-593 Heavy Chain FC disabled (DMS4000 mAbdAb heavy chain Fc disabled)	46	47
EtanSTG593	48	49
EtanTV4593	50	51
AVG18 primer	52	
AVG19 primer	53	
AVG26 primer	54	
AVG21 primer	55	
AVG22 primer	56	
AVG36 primer	57	
AVG37 primer	58	
AVG25 primer	59	
AVG24 primer	60	
DOM15-26-593 - PEP1-5-19 in-line fusion	61	62

PEP1-5-19-DOM15-26-593 in-line fusion	63	64
DMS1571 – a myc tagged Fc formatted version of the DOM 15-26-593 anti-VEGF dAb (exists as a dimer of this sequence)		65
Linker		66
Linker		67
Linker		68
Anti-TNFα mAb (adalimumab) Fc disabled-DOM15-26-593 Heavy Chain with GSTVAAPSGS linker	141	69
Anti-TNFα mAb (adalimumab) Fc disabled -DOM15-26-593 Heavy Chain with GS(TVAAPSGS) x2 linker	142	70
Anti-TNFα mAb (adalimumab) Fc disabled -DOM15-26-593 Heavy Chain with GS(TVAAPSGS) x3 linker	143	71
Anti-TNFα mAb (adalimumab) Fc disabled –DOM15-26-593 Heavy Chain with GS(TVAAPSGS) x4 linker	144	72
Etanercept-DOM15-26-593		73
Etanercept-DOM15-10-11		74
Etanercept-VEGF anticalin		75
Infliximab-bevacizumab DVD-Ig heavy chain		76
Infliximab-bevacizumab DVD-Ig light chain		77
Infliximab-r84 DVD-Ig heavy chain		78
Infliximab-r84 DVD-Ig light chain		79
Infliximab-ranibizumab DVD-Fab		80
Infliximab-ranibizumab DVD-Fab		81
Infliximab-DOM15-26-593 mAb-dAb heavy chain		82
Infliximab-DOM15-10-11 mAb-dAb heavy chain		83
Infliximab-VEGF anticalin heavy chain		84
Infliximab-DOM15-26-593 mAb-dAb light chain		85
Infliximab-DOM15-10-11 mAb-dAb light chain		86
Infliximab-VEGF anticalin light chain		87
Adalimumab-bevacizumab DVD-Ig heavy chain		88
Adalimumab-bevacizumab DVD-Ig light chain		89
Adalimumab-r84 DVD-Ig heavy chain		90
Adalimumab-r84 DVD-Ig light chain		91
Adalimumab-ranibizumab DVD-Fab		92

Adalimumab-ranibizumab DVD-Fab		93
Adalimumab-VEGF anticalin heavy chain		94
Adalimumab-DOM15-26-593 mAb-dAb light chain		95
Adalimumab-DOM15-10-11 mAb-dAb light chain		96
Adalimumab-VEGF anticalin light chain		97
anti- TNFα mAb -bevacizumab DVD-Ig heavy chain		98
anti- TNFα mAb -bevacizumab DVD-Ig light chain		99
anti- TNFα mAb -r84 DVD Ig heavy chain		100
anti- TNFα mAb -r84 DVD-Ig light chain		101
anti- TNFα mAb -ranibizumab DVD-Fab heavy chain		102
anti- TNFα mAb -ranibizumab DVD-Fab light chain		103
anti- TNFα mAb -DOM15-26-593 mAb-dAb heavy chain		104
anti- TNFα mAb -DOM15-10-11 mAb-dAb heavy chain		105
anti- TNFα mAb -VEGF anticalin heavy chain		106
anti- TNFα mAb -DOM15-26-593 mAb-dAb light chain		107
anti- TNFα mAb -DOM15-10-11 mAb-dAb light chain		108
anti- TNFα mAb -VEGF anticalin light chain		109
ESBA105-bevacizumab DVD-Ig heavy chain		110
ESBA105-bevacizumab DVD-Ig light chain		111
ESBA105-r84 DVD-Ig heavy chain		112
ESBA105-r84 DVD-Ig light chain		113
ESBA105-ranibizumab DVD-Fab heavy chain		114
ESBA105-ranibizumab DVD-Fab light chain		115
ESBA105-DOM15-26-593 scFv-VH dAb		116
ESBA105-DOM15-10-11 scFv-Vk dAb		117
ESBA105-VEGF anticalin		118
PEP1-5-19-DOM15-10-11 dAb-dAb		119
PEP1-5-19-VEGF anticalin		120
Anti-TNF adnectin-DOM15-26-593		121
Anti-TNF adnectin-DOM15-10-11		122
Anti-TNF adnectin-VEGF anticalin		123
Bevacizumab-ESBA105 mAb-scFv, heavy chain		124

Bevacizumab-ESBA105 mAb-scFv, light chain		125
Bevacizumab-PEP1-5-19 mAb-dAb heavy chain		126
Bevacizumab-PEP1-5-19 mAb-dAb light chain		127
Bevacizumab-TNF adnectin heavy chain		128
Bevacizumab-TNF adnectin light chain		129
Aflibercept-ESBA105		130
Aflibercept-PEP1-5-19		131
Aflibercept-TNF adnectin		132
DOM15-26-593-ESBA105 dAb-scFv		133
DOM15-26-593-TNF adnectin		134
DOM15-10-11-ESBA105 dAb-scFv		135
DOM15-10-11-PEP1-5-19 dAb-dAb		136
DOM15-10-11-TNF adnectin		137
VEGF anticalin-ESBA105		138
VEGF anticalin-PEP1-5-19		139
VEGF anticalin-TNF adnectin		140
Linker		145
Linker		146
Linker		147
Linker		148
Linker		149
Linker		150
Linker		151
Linker		152
Linker		153
Linker		154
Linker		155
Linker		156
Linker		157
Linker		158
Linker		159
Linker		160

Linker		161
Linker		162
BPC1801 (bispecific IGF1R-VEGFR2) heavy chain		163
BPC1801 (bispecific IGF1R-VEGFR2) light chain		164
BPC1824 (CTLA4-Ig-anti-IL-13 dAb fusion)		165

SEQ ID NO:1
EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:2
VSDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTATISGL
KPGVDDTITVYAVTNHHMPLRIFGPISINHRT

SEQ ID NO:3
GGGGS

SEQ ID NO:4
TVAAPS

SEQ ID NO:5
ASTKGPT

SEQ ID NO:6
ASTKGPS

SEQ ID NO:7
GS

SEQ ID NO:8
TVAAPSGS

SEQ ID NO:9
MGWSCIILFLVATATGVHS

SEQ ID NO:10
EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK

SEQ ID NO:11
GATATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCTCTGTGGGCGATAGAGTGACCAT
CACCTGCCGGGCCAGCCAGGGCATCAGAAACTACCTGGCCTGGTATCAGCAGAAGCCTGGCA
AGGCCCCTAAGCTGCTGATCTACGCCGCCAGCACCCTGCAGAGCGGCGTGCCCAGCAGATTC

AGCGGCAGCGGCTCCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCCGAGGACGT
GGCCACCTACTACTGCCAGCGGTACAACAGAGCCCCTTACACCTTCGGCCAGGGCACCAAGG
TGGAGATCAAGCGTACGGTGGCCGCCCCCAGCGTGTTCATCTTCCCCCCCAGCGATGAGCAG
CTCAAGAGCGGCACCGCCAGCGTGGTGTGTCTGCTGAACAACTTCTACCCCCGGGAGGCCAA
AGTGCAGTGGAAAGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGC
AGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTAC
GAGAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAA
GAGCTTCAACCGGGGCGAGTGC

SEQ ID NO:12

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:13

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCCGGCAGAAGCCTGAGACTGAG
CTGTGCCGCCAGCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTGAGGCAGGCCCCTG
GCAAGGGCCTGGAGTGGGTGTCCGCCATCACCTGGAATAGCGGCCACATCGACTACGCCGAC
AGCGTGGAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGAT
GAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGTGTCCTACCTGAGCA
CCGCCAGCAGCCTGGACTACTGGGGCCAGGGCACCCTGGTGACAGTCTCGAGCGCTAGCACC
AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCTGTGACCGTGTCCTGGAATAGCGGAG
CCCTGACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTG
AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA
CCACAAGCCCAGCAACACCAAAGTGGACAAGAAAGTGGAGCCCAAGAGCTGCGATAAGACCC
ACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGCGGACCTAGCGTGTTCCTGTTCCCC
CCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGA
TGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACA
ACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGC
CCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGG
TCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
CAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGC
TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG
GCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGTCGACCGGTGA
GGTGCAGCTGTTGGTGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGTCTCTCCT
GTGCAGCCTCCGGATTCACCTTTAAGGCTTATCCGATGATGTGGGTCCGCCAGGCTCCAGGG
AAGGGTCTAGAGTGGGTTTCAGAGATTTCGCCTTCGGGTTCTTATACATACTACGCAGACTC
CGTGAAGGGCCGGTTCACCATCTCCCGCGACAATTCCAAGAACACGCTGTATCTGCAAATGA

ACAGCCTGCGTGCCGAGGACACCGCGGTATATTACTGTGCGAAAGATCCTCGGAAGTTAGAC
TACTGGGGTCAGGGAACCCTGGTCACCGTCTCGAGC

SEQ ID NO:14

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKSTGEVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPG
KGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLD
YWGQGTLVTVSS

SEQ ID NO:15

EVQLLESGGGLVQPGGSLRLSCAASGFTFGAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKFDYWGQGTLVTVSSASTKGPSE
VQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADS
VEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO:16

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEGRFTISR
DNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGKSTGDIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYH
TSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIKR

SEQ ID NO:17

EVQLLESGGGLVQPGGSLRLSCAASGFTFAHETMVWVRQAPGKGLEWVSHIPP**D**GQDPFYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY**H**CALLPKRGPWFDYWGQGTLVTVSS

SEQ ID NO:18

EVVAATPTSLLISWRHPHFPTRYYRITYGETGGNSPVQEFTVPLQPPTATISGLKPGVDYTI
TVYAVTDGRNGRLLSIPISINYRT

SEQ ID NO:19

DGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKA
VLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALE
DFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:20

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDYWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK

SEQ ID NO:21

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:22

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK

SEQ ID NO:23

EVQLLESGGGLVQPGGSLRLSCAASGFTFGAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKFDYWGQGTLVTVSS

SEQ ID NO:24

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTKGPSE
VQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADS
VEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO:25

STG

SEQ ID NO:26

ATGCATGTCGCCCAGCCAGCGGTGGTGCTGGCCAGCTCCCGCGGCATTGCCTCCTTCGTGTG
CGAGTACGCCAGCCCCGGCAAGGCCACCGAGGTGCGCGTCACGGTGCTCCGCCAGGCCGATA
GCCAGGTGACCGAAGTGTGTGCCGCTACGTACATGATGGGGAACGAGCTGACCTTCCTGGAC
GACTCTATCTGCACCGGGACCTCGAGCGGGAACCAGGTGAACCTGACCATCCAGGGCCTGCG
CGCGATGGACACGGGCCTGTACATCTGCAAGGTGGAGTTGATGTACCCCCCCCCGTACTACC
TGGGGATCGGCAACGGCACGCAGATCTACGTCATCGACCCCGAACCTTGCCCTGACAGCGAC
CAGGAGCCCAAGTCTAGTGACAAGACCCATACCTCTCCCCCCAGCCCCGCTCCAGAGCTGCT
GGGGGGCTCCAGCGTGTTCCTGTTTCCCCCCAAGCCTAAGGACACCCTGATGATCTCCAGAA
CCCCCGAGGTGACCTGCGTGGTCGTGGATGTGAGTCACGAGGACCCTGAGGTGAAGTTCAAC
TGGTACGTGGACGGGGTGGAGGTGCATAACGCCAAGACCAAGCCTCGCGAGGAGCAGTACAA
CAGTACCTACCGCGTGGTGTCCGTGCTCACTGTGCTGCATCAGGACTGGCTGAACGGCAAGG
AGTATAAGTGCAAGGTGTCTAACAAGGCCTTGCCCGCCCCCATCGAGAAAACAATCTCCAAG
GCCAAAGGGCAGCCCAGGGAACCTCAGGTGTACACCCTCCCTCCAAGCCGTGACGAGCTGAC
CAAGAACCAGGTCTCTCTGACCTGCTTGGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGG
AGTGGGAGTCCAACGGGCAGCCCGAGAACAACTACAAAACCACCCCGCCCGTGCTGGACTCT
GACGGCTCCTTCTTCCTGTACAGCAAACTGACCGTGGACAAGTCCAGGTGGCAGCAGGGAAA
CGTGTTCAGCTGCAGCGTCATGCATGAGGCCCTGCATAACCATTACACACAGAAGAGCCTGT
CCCTGAGCCCCGGCAAGGGATCCGAGGTGGTGGCCGCCACCCCCACCAGCCTGCTGATTTCC
TGGAGGCACCCCCACTTCCCCACACGCTACTACAGGATCACCTACGGCGAGACCGGCGGCAA
CAGCCCCGTGCAGGAGTTCACCGTGCCCCTGCAGCCTCCCACTGCCACCATCAGCGGCCTCA
AGCCCGGCGTGGACTACACCATCACCGTGTACGCCGTCACCGACGGAAGGAACGGCAGGCTG
CTGAGCATCCCCATCAGCATCAACTACAGGACC

SEQ ID NO:27

MHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLD
DSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
QEPKSSDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSEVVAATPTSLLIS
WRHPHFPTRYYRITYGETGGNSPVQEFTVPLQPPTATISGLKPGVDYTITVYAVTDGRNGRL
LSIPISINYRT

SEQ ID NO:28

ATGCATGTCGCCCAGCCAGCGGTGGTGCTGGCCAGCTCCCGCGGCATTGCCTCCTTCGTGTG
CGAGTACGCCAGCCCCGGCAAGGCCACCGAGGTGCGCGTCACGGTGCTCCGCCAGGCCGATA
GCCAGGTGACCGAAGTGTGTGCCGCTACGTACATGATGGGGAACGAGCTGACCTTCCTGGAC
GACTCTATCTGCACCGGGACCTCGAGCGGGAACCAGGTGAACCTGACCATCCAGGGCCTGCG
CGCGATGGACACGGGCCTGTACATCTGCAAGGTGGAGTTGATGTACCCCCCCCCGTACTACC
TGGGGATCGGCAACGGCACGCAGATCTACGTCATCGACCCCGAACCTTGCCCTGACAGCGAC
CAGGAGCCCAAGTCTAGTGACAAGACCCATACCTCTCCCCCCAGCCCCGCTCCAGAGCTGCT
GGGGGGCTCCAGCGTGTTCCTGTTTCCCCCCAAGCCTAAGGACACCCTGATGATCTCCAGAA
CCCCCGAGGTGACCTGCGTGGTCGTGGATGTGAGTCACGAGGACCCTGAGGTGAAGTTCAAC
TGGTACGTGGACGGGGTGGAGGTGCATAACGCCAAGACCAAGCCTCGCGAGGAGCAGTACAA
CAGTACCTACCGCGTGGTGTCCGTGCTCACTGTGCTGCATCAGGACTGGCTGAACGGCAAGG
AGTATAAGTGCAAGGTGTCTAACAAGGCCTTGCCCGCCCCCATCGAGAAAACAATCTCCAAG
GCCAAAGGGCAGCCCAGGGAACCTCAGGTGTACACCCTCCCTCCAAGCCGTGACGAGCTGAC
CAAGAACCAGGTCTCTCTGACCTGCTTGGTGAAGGGCTTCTACCCTAGCGACATCGCTGTGG
AGTGGGAGTCCAACGGGCAGCCCGAGAACAACTACAAAACCACCCCGCCCGTGCTGGACTCT
GACGGCTCCTTCTTCCTGTACAGCAAACTGACCGTGGACAAGTCCAGGTGGCAGCAGGGAAA
CGTGTTCAGCTGCAGCGTCATGCATGAGGCCCTGCATAACCATTACACACAGAAGAGCCTGT
CCCTGAGCCCCGGCAAGGGATCCGAGGTGCAGCTCCTGGTCAGCGGCGGCGGCCTGGTCCAG
CCCGGAGGCTCACTGAGGCTGAGCTGCGCCGCTAGCGGCTTCACCTTCAAGGCCTACCCCAT
GATGTGGGTCAGGCAGGCCCCCGGCAAAGGCCTGGAGTGGGTGTCTGAGATCAGCCCCAGCG
GCAGCTACACCTACTACGCCGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACAGC
AAGAACACCCTGTACCTGCAGATGAACTCTCTGAGGGCCGAGGACACCGCCGTGTACTACTG
CGCCAAGGACCCCAGGAAGCTGGACTATTGGGGCCAGGGCACTCTGGTGACCGTGAGCAGC

SEQ ID NO:29

MHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLD
DSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
QEPKSSDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**GS**EVQLLVSGGGLVQ
PGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNS
KNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:30

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SS

SEQ ID NO:31

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKR

SEQ ID NO:32

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

SEQ ID NO:33

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:34

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPG
FGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:35

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR

SEQ ID NO:36

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASNLETGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR

SEQ ID NO:37

DIQMTQSPSSLSASVGDRVTITCRASQAIDSYLHWYQQKPGKAPKLLIYSASNLETGVPSRF
SGSGSGTDFTLTISSLLIPEDFATYYCQQVVWRPFTFGQGTKVEIKR

SEQ ID NO:38

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSS

GGGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEP
TYADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSS

SEQ ID NO:39

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

SEQ ID NO:40

DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:41

QVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGFDPEDGETIYAQ
KFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGRSMFRGVIIPFNGMDVWGQGTTVTV
SS

SEQ ID NO:42

DIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKR

SEQ ID NO:43

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCA
ASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:44

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIRG

SEQ ID NO:45

EVVAATPTSLLISWRHPHFPTRYYRITYGETGGNSPVQEFTVPLQPPTATISGLKPGVDYTI
TVYAVTDGRNGRLLSIPISINYRT

SEQ ID NO:46

ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCCACCGCCACCGGCGTGCACAGCGAGGT
GCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCCGGCAGAAGCCTGAGACTGAGCTGTG

CCGCCAGCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTGAGGCAGGCCCCTGGCAAG
GGCCTGGAGTGGGTGTCCGCCATCACCTGGAATAGCGGCCACATCGACTACGCCGACAGCGT
GGAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGATGAACA
GCCTGAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGTGTCCTACCTGAGCACCGCC
AGCAGCCTGGACTACTGGGGCCAGGGCACCCTGGTGACAGTCTCGAGCGCTAGCACCAAGGG
CCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGCCCTGG
GCTGCCTGGTGAAGGACTACTTCCCCGAGCCTGTGACCGTGTCCTGGAATAGCGGAGCCCTG
ACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAG
CGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACA
AGCCCAGCAACACCAAAGTGGACAAGAAAGTGGAGCCCAAGAGCTGCGATAAGACCCACACC
TGCCCCCCCCTGCCCTGCCCCCGAGCTGGCCGGCGCCCCTAGCGTGTTCCTGTTCCCCCCCAA
GCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGATGTGA
GCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACAACGCC
AAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTGACCGT
GCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTGC
CTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGGTCTAC
ACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAA
GGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACT
ACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGCTGACC
GTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCT
GCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGTCGACCGGTGAGGTGC
AGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCC
GCCAGCGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGG
CCTGGAATGGGTGTCCGAGATCAGCCCCAGCGGCAGCTACACCTACTACGCCGACAGCGTGA
AGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGC
CTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTGGACTACTG
GGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO:47

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKSTGEVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPG
KGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLD
YWGQGTLVTVSS

SEQ ID NO:48

CTGCCCGCTCAGGTGGCCTTCACTCCCTACGCCCCAGAGCCCGGCTCTACCTGCAGGCTGAG
GGAGTACTACGACCAGACCGCCCAGATGTGCTGCAGCAAGTGCAGCCCCGGCCAGCACGCCA
AAGTGTTCTGCACCAAGACCAGCGACACCGTGTGCGATAGCTGCGAGGACAGCACCTACACC

CAGCTGTGGAACTGGGTCCCCGAGTGCCTGAGCTGCGGCTCTAGGTGTAGCAGCGACCAGGT
CGAGACCCAGGCCTGCACCAGGGAACAGAACCGGATCTGCACATGCAGGCCCGGCTGGTACT
GCGCCCTCAGCAAACAGGAGGGCTGCAGGCTGTGTGCCCCCCTCAGGAAGTGCAGGCCCGGG
TTTGGCGTGGCCAGGCCCGGAACCGAGACTAGCGACGTGGTGTGCAAACCCTGCGCCCCCGG
CACCTTCAGCAATACCACTAGCAGCACCGACATCTGCAGGCCTCACCAGATCTGCAACGTGG
TGGCCATTCCCGGCAACGCAAGCATGGACGCCGTGTGCACCAGCACCAGCCCCACCAGGTCA
ATGGCCCCTGGAGCCGTGCATCTGCCCCAGCCCGTGAGCACCAGAAGCCAGCACACCCAGCC
TACCCCCGAGCCCAGCACCGCCCCTAGCACCAGCTTCCTGCTGCCTATGGGCCCCTCCCCTC
CCGCCGAGGGCTCAACCGGCGACGAACCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCC
TGCCCCGCACCAGAACTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGA
CACCCTGATGATCAGCAGGACCCCCGAGGTGACCTGTGTGGTGGTGGACGTGAGCCACGAGG
ACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG
CCCAGGGAGGAGCAGTACAACAGCACCTACAGGGTGGTGAGCGTCCTGACCGTGCTGCACCA
GGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCCCA
TCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCAGGGAGCCACAGGTGTACACACTGCCC
CCCAGCAGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTA
TCCCAGCGATATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCA
CCCCCCCCGTCCTGGACTCCGACGGGAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAG
AGCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGTCGACCGGTGAGGTGCAGCTGCTGG
TGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGC
TTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATG
GGTGTCCGAGATCAGCCCCAGCGGCAGCTACACCTACTACGCCGACAGCGTGAAGGGCCGGT
TCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCC
GAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTGGACTACTGGGGCCAGGG
CACCCTGGTGACCGTGAGCAGC

SEQ ID NO:49

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPG
FGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSTGEVQLLVSGGGLVQPGGSLRLSCAASG
FTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRA
EDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:50

CTGCCCGCTCAGGTGGCCTTCACTCCCTACGCCCCAGAGCCCGGCTCTACCTGCAGGCTGAG
GGAGTACTACGACCAGACCGCCCAGATGTGCTGCAGCAAGTGCAGCCCCGGCCAGCACGCCA
AAGTGTTCTGCACCAAGACCAGCGACACCGTGTGCGATAGCTGCGAGGACAGCACCTACACC
CAGCTGTGGAACTGGGTCCCCGAGTGCCTGAGCTGCGGCTCTAGGTGTAGCAGCGACCAGGT

CGAGACCCAGGCCTGCACCAGGGAACAGAACCGGATCTGCACATGCAGGCCCGGCTGGTACT
GCGCCCTCAGCAAACAGGAGGGCTGCAGGCTGTGTGCCCCCCTCAGGAAGTGCAGGCCCGGG
TTTGGCGTGGCCAGGCCCGGAACCGAGACTAGCGACGTGGTGTGCAAACCCTGCGCCCCCGG
CACCTTCAGCAATACCACTAGCAGCACCGACATCTGCAGGCCTCACCAGATCTGCAACGTGG
TGGCCATTCCCGGCAACGCAAGCATGGACGCCGTGTGCACCAGCACCAGCCCCACCAGGTCA
ATGGCCCCTGGAGCCGTGCATCTGCCCCAGCCCGTGAGCACCAGAAGCCAGCACACCCAGCC
TACCCCCGAGCCCAGCACCGCCCCTAGCACCAGCTTCCTGCTGCCTATGGGCCCCTCCCCTC
CCGCCGAGGGCTCAACCGGCGACGAACCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCC
TGCCCCGCACCAGAACTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGA
CACCCTGATGATCAGCAGGACCCCCGAGGTGACCTGTGTGGTGGTGGACGTGAGCCACGAGG
ACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG
CCCAGGGAGGAGCAGTACAACAGCACCTACAGGGTGGTGAGCGTCCTGACCGTGCTGCACCA
GGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCCCA
TCGAGAAGACCATCAGCAAGGCCAAAGGCCAGCCCAGGGAGCCACAGGTGTACACACTGCCC
CCCAGCAGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTA
TCCCAGCGATATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCA
CCCCCCCCCGTCCTGGACTCCGACGGGAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAG
AGCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGACCGTGGCGGCGCCCAGCACGGTGG
CCGCCCCCTCCACCGTCGCCGCGCCAAGCACCGTGGCTGCTCCGTCGACCGGTGAGGTGCAG
CTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGC
CAGCGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCC
TGGAATGGGTGTCCGAGATCAGCCCCAGCGGCAGCTACACCTACTACGCCGACAGCGTGAAG
GGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCT
GCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTGGACTACTGGG
GCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO:51

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPG
FGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTVAAPSTVAAPSTVAAPSTVAAPSTGEVQ
LLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:52

ATTATGGGATCCACCGGCGAGGTGCAGCTGTTGGTGT

SEQ ID NO:53

GCTGGGGCCCTTGGTGCTAGCGCTCGAGACGGTGACCAGG

SEQ ID NO:54
CTCGAGCGCTAGCACCAAGGGCCCCAGCGACATCCAGATGACCC

SEQ ID NO:55
TTATGTCAAGCTTTTACCGTTTGATTTCCACCTTGGT

SEQ ID NO:56
ATTATGGGATCCACCGGCGACATCCAGATGACCCAGTCTCC

SEQ ID NO:57
GCGCCGCCACCGTACGTTTGATTTCCACCTTGGTCCC

SEQ ID NO:58
CAAACGTACGGTGGCGGCGCCGAGCGAGGTGCAGCTGTTGGTGTC

SEQ ID NO:59
TTATGTCAAGCTTTTAGCTCGAGACGGTGACCAG

SEQ ID NO:60
GGTGGAAATCAAACGTACGGTGGCGGCGCCGAGCGA

SEQ ID NO:61
GAGGTGCAGCTGTTGGTGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGTCTCTC
CTGTGCAGCCTCCGGATTCACCTTTAAGGCTTATCCGATGATGTGGGTCCGCCAGGCTCCAG
GGAAGGGTCTAGAGTGGGTTTCAGAGATTTCGCCTTCGGGTTCTTATACATACTACGCAGAC
TCCGTGAAGGGCCGGTTCACCATCTCCCGCGACAATTCCAAGAACACGCTGTATCTGCAAAT
GAACAGCCTGCGTGCCGAGGACACCGCGGTATATTACTGTGCGAAAGATCCTCGGAAGTTAG
ACTACTGGGGTCAGGGAACCCTGGTCACCGTCTCGAGCGCTAGCACCAAGGGCCCCAGCGAC
ATCCAGATGACCCAGTCTCCATCCTCTCTGTCTGCATCTGTAGGAGACCGTGTCACCATCAC
TTGCCGGGCAAGTCAGAGCATTGATAGTTATTTACATTGGTACCAGCAGAAACCAGGGAAAG
CCCCTAAGCTCCTGATCTATAGTGCATCCGAGTTGCAAAGTGGGGTCCCATCACGTTTCAGT
GGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGC
TACGTACTACTGTCAACAGGTTGTGTGGCGTCCTTTTACGTTCGGCCAAGGGACCAAGGTGG
AAATCAAACGG

SEQ ID NO:62
EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTKGPSD
IQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFS
GSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR

SEQ ID NO:63
GACATCCAGATGACCCAGTCTCCATCCTCTCTGTCTGCATCTGTAGGAGACCGTGTCACCAT
CACTTGCCGGGCAAGTCAGAGCATTGATAGTTATTTACATTGGTACCAGCAGAAACCAGGGA
AAGCCCCTAAGCTCCTGATCTATAGTGCATCCGAGTTGCAAAGTGGGGTCCCATCACGTTTC

AGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTT
TGCTACGTACTACTGTCAACAGGTTGTGTGGCGTCCTTTTACGTTCGGCCAAGGGACCAAGG
TGGAAATCAAACGTACGGTGGCGGCGCCGAGCGAGGTGCAGCTGTTGGTGTCTGGGGGAGGC
TTGGTACAGCCTGGGGGGTCCCTGCGTCTCTCCTGTGCAGCCTCCGGATTCACCTTTAAGGC
TTATCCGATGATGTGGGTCCGCCAGGCTCCAGGGAAGGGTCTAGAGTGGGTTTCAGAGATTT
CGCCTTCGGGTTCTTATACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCCGC
GACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGCGTGCCGAGGACACCGCGGT
ATATTACTGTGCGAAAGATCCTCGGAAGTTAGACTACTGGGGTCAGGGAACCCTGGTCACCG
TCTCGAGC

SEQ ID NO:64

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKRTVAAPSEVQLLVSGGG
LVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:65

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSEQKLISEEDLN

SEQ ID NO:66

GSTVAAPSGSTVAAPSGS

SEQ ID NO:67

GSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:68

GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:69

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGSTVAAPSGSEVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMM
WVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA
KDPRKLDYWGQGTLVTVSS

SEQ ID NO:70

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSEVQLLVSGGGLVQPGGSLRLSCAASGF
TFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAE
DTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:71

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSEVQLLVSGGGLVQPGGSLR
LSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYL
QMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:72

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSEVQLLVSGGGL
VQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:73

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPG
FGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTVAAPSEVQLLVSGGGLVQPGGSLRLSCA
ASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNS
LRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:74

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPG
FGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTVAAPSDIQMTQSPSSLSASVGDRVTITC
RASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFAT
YYCQQYMFQPMTFGQGTKVEIKR

SEQ ID NO:75

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTYT
QLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPG
FGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRS
MAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTVAAPSDGGGIRRSMSGTWYLKAMTVDRE
FPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYII
PSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSE
TCSPG

SEQ ID NO:76

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK
GPSEVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPT
YAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SV

SEQ ID NO:77

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSDIQMTQSPSS

LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:78

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK
GPSQVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGFDPEDGETI
YAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGRSMFRGVIIPFNGMDVWGQGTT
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:79

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSDIRMTQSPSS
LSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:80

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK
GPSEVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPT
YAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

SEQ ID NO:81

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSDIQLTQSPSS
LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:82

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGKTVAAPSEVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQA
PGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRK
LDYWGQGTLVTVSS

SEQ ID NO:83

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGKTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKP
GKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGT
KVEIKR

SEQ ID NO:84

EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAEIRSKSINSATHY
AESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGKTVAAPSDGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTLL
KGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQLH
GKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:85

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSEVQLLVSGGGLVQPGGSLRLSCAASGFT
FKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED
TAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:86

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQ

LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQW
IGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
YMFQPMTFGQGTKVEIKR

SEQ ID NO:87

DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKYASESMSGIPSRF
SGSGSGTDFTLSINTVESEDIADYYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDGGGIRRSMSGTWYLKAMTVDREFPEMN
LESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIPSAVR
DHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:88

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSEVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEP
TYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:89

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSDIQMTQSPSS
LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:90

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSQVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGFDPEDGET
IYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGRSMFRGVIIPFNGMDVWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:91

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSDIRMTQSPSS
LSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:92

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSEVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEP
TYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

SEQ ID NO:93

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSDIQLTQSPSS
LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:94

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYAD
SVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKTVAAPSDGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTL
LKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQL
HGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:95

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSEVQLLVSGGGLVQPGGSLRLSCAASGFT
FKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED
TAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:96

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQW
IGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
YMFQPMTFGQGTKVEIKR

SEQ ID NO:97

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSRF
SGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDGGGIRRSMSGTWYLKAMTVDREFPEMN
LESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIPSAVR
DHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:98

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SSASTKGPSEVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINT
YTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:99

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSDIQMTQSPS
SLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

SEQ ID NO:100

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SSASTKGPSQVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGFDP
EDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGRSMFRGVIIPFNGMDV
WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR

EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:101

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSDIRMTQSPS
SLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

SEQ ID NO:102

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SSASTKGPSEVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINT
YTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

SEQ ID NO:103

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSDIQLTQSPS
SLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC

SEQ ID NO:104

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGKTVAAPSEVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPM
MWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC
AKDPRKLDYWGQGTLVTVSS

SEQ ID NO:105

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV

SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGKTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELR
WYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPM
TFGQGTKVEIKR

SEQ ID NO:106

QVQLVESGGGVVQPGRSLRLSCAASGFIFSSYAMHWVRQAPGNGLEWVAFMSYDGSNKKYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRGIAAGGNYYYYGMDVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGKTVAAPSDGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTP
MTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFY
SEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:107

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSEVQLLVSGGGLVQPGGSLRLSCAASGF
TFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAE
DTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:108

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQ
WIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QYMFQPMTFGQGTKVEIKR

SEQ ID NO:109

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDGGGIRRSMSGTWYLKAMTVDREFPEM
NLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIPSAV
RDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSETCSP
G

SEQ ID NO:110

QVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPTYAD

KFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSSASTKGPS
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK

SEQ ID NO:111

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRTVAAPSDIQMTQSPSS
LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:112

QVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPTYAD
KFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSSASTKGPS
QVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGFDPEDGETIYAQ
KFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATGRSMFRGVIIPFNGMDVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:113

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRTVAAPSEIVLTQSPAT
LSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT
LTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIKRTVAAPSDIRMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQSYSTPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV
TKSFNRGEC

SEQ ID NO:114

QVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPTYAD
KFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSSASTKGPS
EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA

DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

SEQ ID NO:115

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRTVAAPSDIQLTQSPSS
LSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

SEQ ID NO:116

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSS
GGGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEP
TYADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSSAST
KGPSEVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYT
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:117

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSS
GGGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEP
TYADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSSAST
KGPSDIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIKR

SEQ ID NO:118

DIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRF
SGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSS
GGGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEP
TYADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSSAST
KGPSDGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQ
EVKAVLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNL
EALEDFEKAAGARGLSTESILIPRQSETCSPG

SEQ ID NO:119

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKRTVAAPSDIQMTQSPSS
LSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIKR

SEQ ID NO:120

DIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRF

SGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKRTVAAPSDGGGIRRSMS
GTWYLKAMTVDREFPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKK
YTADGGKHVAYIIPSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARG
LSTESILIPRQSETCSPG

SEQ ID NO:121

VSDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTATISGL
KPGVDDTITVYAVTNHHMPLRIFGPISINHRTTVAAPSEVQLLVSGGGLVQPGGSLRLSCAA
SGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYADSVKGRFTISRDNSKNTLYLQMNSL
RAEDTAVYYCAKDPRKLDYWGQGTLVTVSS

SEQ ID NO:122

VSDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTATISGL
KPGVDDTITVYAVTNHHMPLRIFGPISINHRTTVAAPSDIQMTQSPSSLSASVGDRVTITCR
ASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQYMFQPMTFGQGTKVEIKR

SEQ ID NO:123

VSDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTATISGL
KPGVDDTITVYAVTNHHMPLRIFGPISINHRTTVAAPSDGGGIRRSMSGTWYLKAMTVDREF
PEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKAVLGRTKERKKYTADGGKHVAYIIP
SAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALEDFEKAAGARGLSTESILIPRQSET
CSPG

SEQ ID NO:124

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGKTVAAPSDIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQ
QRPGKAPKLLIYSAFNRYTGVPSRFSGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFG
QGTKLEVKRGGGGSGGGGSGGGGSSGGGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYG
MNWVRQAPGKGLEWMGWINTYTGEPTYADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYY
CARERGDAMDYWGQGTLVTVSS

SEQ ID NO:125

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDIVMTQSPSSLSASVGDRVTLTCTASQS
VSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRFSGRGYGTDFTLTISSLQPEDVAVYYCQQ

DYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSSGGGSQVQLVQSGAEVKKPGASVKVSCTA
SGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPTYADKFKDRFTFSLETSASTVYMELTSL
TSDDTAVYYCARERGDAMDYWGQGTLVTVSS

SEQ ID NO:126

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGKTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQ
QKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFG
QGTKVEIKR

SEQ ID NO:127

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQS
IDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
VVWRPFTFGQGTKVEIKR

SEQ ID NO:128

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAA
DFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGKTVAAPSVSDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYG
ETGGNSPVREFTVPHPEVTATISGLKPGVDDTITVYAVTNHHMPLRIFGPISINHRT

SEQ ID NO:129

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSVSDVPRDLEVVAATPTSLLISWDTHNAY
NGYYRITYGETGGNSPVREFTVPHPEVTATISGLKPGVDDTITVYAVTNHHMPLRIFGPISI
NHRT

SEQ ID NO:130

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCA
ASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTV
AAPSDIVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGV
PSRFSGRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGG
GGSSGGGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTY
TGEPTYADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVS
S

SEQ ID NO:131

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCA
ASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTV
AAPSDIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR

SEQ ID NO:132

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNC
TARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCA
ASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTV
AAPSVSDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTAT
ISGLKPGVDDTITVYAVTNHHMPLRIFGPISINHRT

SEQ ID NO:133

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTKGPSD
IVMTQSPSSLSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRFS
GRGYGTDFTLTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSSG
GGSQVQLVQSGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPT
YADKFKDRFTFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSS

SEQ ID NO:134

EVQLLVSGGGLVQPGGSLRLSCAASGFTFKAYPMMWVRQAPGKGLEWVSEISPSGSYTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPRKLDYWGQGTLVTVSSASTKGSPV
SDVPRDLEVVAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTATISGLK
PGVDDTITVYAVTNHHMPLRIFGPISINHRT

SEQ ID NO:135

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIKRTVAAPSDIVMTQSPSS
LSASVGDRVTLTCTASQSVSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRFSGRGYGTDFT
LTISSLQPEDVAVYYCQQDYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSSGGGSQVQLVQ
SGAEVKKPGASVKVSCTASGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPTYADKFKDRF
TFSLETSASTVYMELTSLTSDDTAVYYCARERGDAMDYWGQGTLVTVSS

SEQ ID NO:136

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIKRTVAAPSDIQMTQSPSS
LSASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQVVWRPFTFGQGTKVEIKR

SEQ ID NO:137

DIQMTQSPSSLSASVGDRVTITCRASQWIGPELRWYQQKPGKAPKLLIYHTSILQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQYMFQPMTFGQGTKVEIKRTVAAPSVSDVPRDLEV
VAATPTSLLISWDTHNAYNGYYRITYGETGGNSPVREFTVPHPEVTATISGLKPGVDDTITV
YAVTNHHMPLRIFGPISINHRT

SEQ ID NO:138

DGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKA
VLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALE
DFEKAAGARGLSTESILIPRQSETCSPGTVAAPSDIVMTQSPSSLSASVGDRVTLTCTASQS
VSNDVVWYQQRPGKAPKLLIYSAFNRYTGVPSRFSGRGYGTDFTLTISSLQPEDVAVYYCQQ
DYNSPRTFGQGTKLEVKRGGGGSGGGGSGGGGSSGGGSQVQLVQSGAEVKKPGASVKVSCTA
SGYTFTHYGMNWVRQAPGKGLEWMGWINTYTGEPTYADKFKDRFTFSLETSASTVYMELTSL
TSDDTAVYYCARERGDAMDYWGQGTLVTVSS

SEQ ID NO:139

DGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKA
VLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALE
DFEKAAGARGLSTESILIPRQSETCSPGTVAAPSDIQMTQSPSSLSASVGDRVTITCRASQS
IDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQ
VVWRPFTFGQGTKVEIKR

SEQ ID NO:140

DGGGIRRSMSGTWYLKAMTVDREFPEMNLESVTPMTLTLLKGHNLEAKVTMLISGRCQEVKA
VLGRTKERKKYTADGGKHVAYIIPSAVRDHVIFYSEGQLHGKPVRGVKLVGRDPKNNLEALE

DFEKAAGARGLSTESILIPRQSETCSPGTVAAPSVSDVPRDLEVVAATPTSLLISWDTHNAY
NGYYRITYGETGGNSPVREFTVPHPEVTATISGLKPGVDDTITVYAVTNHHMPLRIFGPISI
NHRT

SEQ ID NO:141

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCCGGCAGAAGCCTGAGACTGAG
CTGTGCCGCCAGCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTGAGGCAGGCCCCTG
GCAAGGGCCTGGAGTGGGTGTCCGCCATCACCTGGAATAGCGGCCACATCGACTACGCCGAC
AGCGTGGAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGAT
GAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGTGTCCTACCTGAGCA
CCGCCAGCAGCCTGGACTACTGGGGCCAGGGCACCCTGGTGACAGTCTCGAGCGCTAGCACC
AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCTGTGACCGTGTCCTGGAATAGCGGAG
CCCTGACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTG
AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA
CCACAAGCCCAGCAACACCAAAGTGGACAAGAAAGTGGAGCCCAAGAGCTGCGATAAGACCC
ACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGGCCGGCGCCCCTAGCGTGTTCCTGTTCCCC
CCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGA
TGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACA
ACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGC
CCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGG
TCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
CAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGC
TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG
GCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGGGATCCACCGT
GGCCGCTCCCAGCGGATCAGAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTG
GCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAAGGCCTACCCCATGATG
TGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCAGCCCCAGCGGCAG
CTACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGA
ACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCC
AAGGACCCCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO:142

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCCGGCAGAAGCCTGAGACTGAG
CTGTGCCGCCAGCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTGAGGCAGGCCCCTG
GCAAGGGCCTGGAGTGGGTGTCCGCCATCACCTGGAATAGCGGCCACATCGACTACGCCGAC
AGCGTGGAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGAT
GAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGTGTCCTACCTGAGCA
CCGCCAGCAGCCTGGACTACTGGGGCCAGGGCACCCTGGTGACAGTCTCGAGCGCTAGCACC
AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCTGTGACCGTGTCCTGGAATAGCGGAG
CCCTGACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTG

AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA
CCACAAGCCCAGCAACACCAAAGTGGACAAGAAAGTGGAGCCCAAGAGCTGCGATAAGACCC
ACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGGCCGGCGCCCCTAGCGTGTTCCTGTTCCCC
CCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGA
TGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACA
ACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGC
CCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGG
TCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
CAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGC
TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG
GCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGGGATCCACAGT
GGCTGCACCTTCCGGGTCAACCGTCGCCGCCCCCAGCGGAAGCGAGGTGCAGCTGCTGGTGT
CTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTC
ACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGT
GTCCGAGATCAGCCCCAGCGGCAGCTACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCA
CCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAG
GACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAAGCTGGACTACTGGGGCCAGGGCAC
CCTGGTGACCGTGAGCAGC

SEQ ID NO:143

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCCGGCAGAAGCCTGAGACTGAG
CTGTGCCGCCAGCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTGAGGCAGGCCCCTG
GCAAGGGCCTGGAGTGGGTGTCCGCCATCACCTGGAATAGCGGCCACATCGACTACGCCGAC
AGCGTGGAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGAT
GAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGTGTCCTACCTGAGCA
CCGCCAGCAGCCTGGACTACTGGGGCCAGGGCACCCTGGTGACAGTCTCGAGCGCTAGCACC
AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCTGTGACCGTGTCCTGGAATAGCGGAG
CCCTGACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTG
AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA
CCACAAGCCCAGCAACACCAAAGTGGACAAGAAAGTGGAGCCCAAGAGCTGCGATAAGACCC
ACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGGCCGGCGCCCCTAGCGTGTTCCTGTTCCCC
CCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGA
TGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACA
ACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGC
CCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGG
TCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
CAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGC
TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG

GCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGGGATCCACCGT
CGCCGCACCAAGCGGGTCAACAGTGGCCGCTCCCTCCGGCAGCACTGTGGCTGCCCCCAGCG
GAAGCGAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGA
CTGAGCTGCGCCGCCAGCGGCTTCACCTTCAAGGCCTACCCCATGATGTGGGTGCGGCAGGC
CCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCAGCCCCAGCGGCAGCTACACCTACTACG
CCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTG
CAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGACCCCCGGAA
GCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO:144

GAGGTGCAGCTGGTGGAGTCTGGCGGCGGACTGGTGCAGCCCGGCAGAAGCCTGAGACTGAG
CTGTGCCGCCAGCGGCTTCACCTTCGACGACTACGCCATGCACTGGGTGAGGCAGGCCCCTG
GCAAGGGCCTGGAGTGGGTGTCCGCCATCACCTGGAATAGCGGCCACATCGACTACGCCGAC
AGCGTGGAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACAGCCTGTACCTGCAGAT
GAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGTGTCCTACCTGAGCA
CCGCCAGCAGCCTGGACTACTGGGGCCAGGGCACCCTGGTGACAGTCTCGAGCGCTAGCACC
AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCTGTGACCGTGTCCTGGAATAGCGGAG
CCCTGACCTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTG
AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA
CCACAAGCCCAGCAACACCAAAGTGGACAAGAAAGTGGAGCCCAAGAGCTGCGATAAGACCC
ACACCTGCCCCCCCTGCCCTGCCCCCGAGCTGGCCGGCGCCCCTAGCGTGTTCCTGTTCCCC
CCCAAGCCTAAGGACACCCTGATGATCAGCAGGACCCCCGAAGTGACCTGCGTGGTGGTGGA
TGTGAGCCACGAGGACCCTGAAGTGAAGTTCAACTGGTACGTGGACGGCGTGGAAGTGCACA
ACGCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACCGCGTGGTGTCTGTGCTG
ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGC
CCTGCCTGCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGAGAGCCCCAGG
TCTACACCCTGCCTCCCTCCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
CAACTACAAGACCACCCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACTCCAAGC
TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAG
GCCCTGCACAATCACTACACCCAGAAGAGTCTGAGCCTGTCCCCTGGCAAGGGATCCACCGT
CGCCGCACCAAGCGGATCTACCGTCGCAGCCCCTTCCGGGTCAACAGTGGCCGCTCCCTCCG
GCAGCACTGTGGCTGCCCCCAGCGGAAGCGAGGTGCAGCTGCTGGTGTCTGGCGGCGGACTG
GTGCAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAAGGCCTA
CCCCATGATGTGGGTGCGGCAGGCCCCTGGCAAGGGCCTGGAATGGGTGTCCGAGATCAGCC
CCAGCGGCAGCTACACCTACTACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGAC
AACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTA
CTACTGCGCCAAGGACCCCCGGAAGCTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGA
GCAGC

SEQ ID NO:145

PASGS

SEQ ID NO:146
PASPASGS

SEQ ID NO:147
PASPASPASGS

SEQ ID NO:148
GGGGSGGGGS

SEQ ID NO:149
GGGGSGGGGSGGGGS

SEQ ID NO:150
TVAAPSTVAAPSGS

SEQ ID NO:151
TVAAPSTVAAPSTVAAPSGS

SEQ ID NO:152
GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:153
GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:154
PAVPPPGS

SEQ ID NO:155
PAVPPPPAVPPPGS

SEQ ID NO:156
PAVPPPPAVPPPPAVPPPGS

SEQ ID NO:157
TVSDVPGS

SEQ ID NO:158
TVSDVPTVSDVPGS

SEQ ID NO:159
TVSDVPTVSDVPTVSDVPGS

SEQ ID NO:160
TGLDSPGS

SEQ ID NO:161
TGLDSPTGLDSPGS

SEQ ID NO:162
TGLDSPTGLDSPTGLDSPGS

SEQ ID NO:163
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGNINPNNGGTNYNQ
KFKDRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARWILYYGRSKWYFDVWGRGTLVTVSSA

STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGKGSEVVAATPTSLLISWRHPHFPTRYYRITYGETGGNSPVQEFT
VPLQPPTATISGLKPGVDYTITVYAVTDGRNGRLLSIPISINYRT

SEQ ID NO:164

DIVMTQSPLSLPVTPGEPASISCRSSQSIVQSNGDTYLEWYLQKPGQSPQLLIYRVSNRFSG
VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:165

MHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLD
DSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
QEPKSSDKTHTSPPSPAPELLGGSSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGVQLLESGGGLVQ
PGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDNS
KNTLYLQMNSLRAEDTAVYYCATAEDEPGYDYWGQGTLVTVSS

Claims

1. A composition comprising a TNFα antagonist and a VEGF antagonist for use in preventing or treating a disease of the eye.

2. The composition of claim 1, wherein the TNFα antagonist and the VEGF antagonist are antigen binding proteins.

3. The composition of claim 1 or claim 2, wherein the TNFα antagonist and the VEGF antagonist are present in the form of a dual targeting protein.

4. The composition of claim 3, wherein the dual targeting protein comprises at least one paired VH/VL domain which binds TNFα or a TNFα receptor, and at least one paired VH/VL domain which binds VEGF or a VEGF receptor.

5. The composition of claim 4, wherein the dual targeting molecule is a DVD-Ig.

6. The composition of claim 3 or 4, wherein the dual targeting protein is a bispecific antibody.

7. The composition of claim 3, wherein the dual targeting protein is a dAb-dAb in-line fusion.

8. The composition of claim 3, wherein the dual targeting protein is a receptor-Fc fusion which is linked to one or more epitope binding domains.

9. The composition of claim 2, wherein the TNFα antagonist is an anti-TNFα antibody.

10. The composition of claim 2, wherein the VEGF antagonist is an anti-VEGF antibody.

11. The composition of claim 3, wherein the TNFα antagonist portion of the dual targeting protein is an anti-TNF antibody and wherein the VEGF antagonist portion of the dual targeting protein is an anti-VEGF epitope binding domain.

12. The composition of claim 3, wherein the VEGF antagonist portion of the dual targeting protein is an anti-VEGF antibody and the TNFα antagonist portion of the dual targeting protein is an anti-TNF epitope binding domain.

13. The composition of claim 8, 11 or 12, wherein the epitope binding domain is a dAb.

14. The composition of claim 13, wherein the dAb is a human dAb.

15. The composition of claim 8, 11 or 12, wherein the epitope binding domain is derived from a non-Ig scaffold.

16. The composition of claim 15 wherein the epitope binding domain is selected from CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ-crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin).

17. The composition according to claim 8 or any one of claims 11 to 16, wherein the epitope binding domain is directly attached to the antigen binding protein with a linker consisting of from 1 to 30 amino acids.

18. The composition according to claim 17, wherein the linker is selected from those set out in SEQ ID NO: 3-8 and 25, or any combination or multiple thereof.

19. The composition according to any one of claims 11 to 18, wherein the epitope binding domain is linked to the N-terminus of the antigen binding protein heavy chain.

20. The composition according to any one of claims 11 to 18, wherein the epitope binding domain is linked to the N-terminus of the antigen binding protein light chain.

21. The composition according to any one of claims 11 to 18, wherein the epitope binding domain is linked to the C-terminus of the antigen binding protein heavy chain.

22. The composition according to any one of claims 11 to 18, wherein the epitope binding domain is linked to the C-terminus of the antigen binding protein light chain.

23. A composition according to any one of claims 2 to 7, or 8 to 22, wherein the antigen binding protein comprises the CDRH1, CDRH2 and CDRH3 contained in the heavy chain set out in SEQ ID NO:10 and the CDRL1, CDRL2 and CDRL3 contained in the light chain set out in SEQ ID NO:12.

24. The composition according to claim 23 which comprises the heavy chain sequence of SEQ ID NO:14, 15, 47, 69, 70, 71 or 72 and the light chain sequence of SEQ ID NO:12.

25. The composition as claimed in any one of claims 3-8 or 11-24, wherein the composition is to be administered intravitreally every 4-6 weeks.

26. The composition as claimed in any one of claims 1-25, wherein the composition comprises a further active agent, optionally an anti-inflammatory agent.

27. Use of a composition as defined in any one of claims 1-26 for the manufacture of a medicament for use in preventing or treating a disease of the eye.

28. A TNFα antagonist selected from the group consisting of adalimumab, infliximab, etanercept, ESBA105, PEP1-5-19, PEP1-5-490, PEP1-5-493, an adnectin of SEQ ID NO:2, golimumab, certolizumab, ALK-6931, and an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31, for use in preventing or treating an eye disease, wherein the TNFα antagonist is to be administered in combination with a VEGF antagonist selected from the group consisting of bevacizumab, ranibizumab, r84, aflibercept, CT01, DOM15-10-11, DOM15-26-593, PRS-050, PRS-051, MP0012, CT-322, ESBA903, EPI-0030, EPI-0010, and DMS1571.

29. A VEGF antagonist selected from the group consisting of bevacizumab, ranibizumab, r84, aflibercept, CT01, DOM15-10-11, DOM15-26-593, PRS-050, PRS-051, MP0012, CT-322, ESBA903, EPI-0030, EPI-0010 and DMS1571, for use in preventing or treating an eye disease, wherein the VEGF antagonist is to be administered in combination with a TNFα antagonist selected from the group consisting of adalimumab, infliximab, etanercept, ESBA105, PEP1-5-19, PEP1-5-

490, PEP1-5-493, an adnectin of SEQ ID NO:2, golimumab, certolizumab, ALK-6931, and an antibody comprising a heavy chain of SEQ ID NO:30 and a light chain of SEQ ID NO:31

30. A TNFα antagonist as claimed in claim 28 or the VEGF antagonist as claimed in claim 29, wherein the TNFα antagonist is adalimumab and the VEGF antagonist is ranibizumab.

31. A pharmaceutical composition comprising a composition as claimed in any one of claims 1 to 24 and a pharmaceutically acceptable carrier.

32. A pharmaceutical compositions as claimed in claim 31, wherein the composition comprises a further active agent, optionally an anti-inflammatory agent.

33. A polynucleotide sequence encoding an antigen binding protein as claimed in any one of claims 2 to 24.

34. A polynucleotide sequence encoding a heavy chain or light chain of a composition according to any one of claims 5, 6 or 9 to 24.

35. A polynucleotide sequence as claimed in claim 34, wherein the sequence is as set forth in SEQ ID NO: 11, 13, or 46.

36. A recombinant transformed or transfected host cell comprising one or more polynucleotide sequences as claimed in any one of claims 33-35.

37. A method for the production of a composition according to any one of claims 2 to 24 which method comprises the step of culturing a host cell of claim 36 and isolating the antigen binding protein.

38. A composition as claimed in any one of claims 1 to 24, which is for delivery via the intravitreal route.

39. A composition as claimed in any one of claims 1 to 24, which is for delivery via the periocular route.

40. A composition according to claim 39 which is for delivery via trans-scleral, subconjunctival, sub-tenon, peribulbar, topical, retrobulbar route or which is for delivery to the inferior, superior or lateral rectus muscle.

41. A composition according to any one of claims 1 to 24 wherein the disease of the eye is diabetic macula edema, cystoid macula edema, uveitis, AMD (Age related macular degeneration), choroidal neovascular AMD, diabetic retinopathy, retinal vein occlusion and other maculopathies and ocular vasculopathies.

42. A method of preventing or treating a patient afflicted with an eye disease comprising administering a prophylactically or therapeutically effective amount of a composition or dual targeting protein according to any one of claims 1 to 24 systemically or topically to the eye of the patient.

43. The method of claim 42, wherein said patient is suffering from at least one of the following diseases or disorders: diabetic macula edema, cystoid macula edema, uveitis, AMD (Age related macular degeneration), choroidal neovascular AMD, diabetic retinopathy, retinal vein occlusion and other maculopathies and ocular vasculopathies.

44. A dual targeting antigen binding molecule comprising a TNFα antagonist portion, a VEGF antagonist portion and a linker connecting said TNFα antagonist portion to said VEGF antagonist portion, wherein:

the TNFα antagonist portion comprises an amino acid sequence of any one of the TNFα antagonists listed in table 1;

the VEGF antagonist portion comprises an amino acid sequence of any one of the VEGF antagonists listed in table 2;

the linker is an amino acid sequence from 1 – 150 amino acids in length; and

the dual targeting molecule is not DMS4000 or DMS4031.

45. A dual targeting antigen binding molecule comprising a TNFα antagonist portion, a VEGF antagonist portion and a linker connecting said TNFα antagonist portion to said VEGF antagonist portion, wherein:

the TNFα antagonist portion comprises an amino acid sequence of any one of the TNFα antagonists listed in table 1;

the VEGF antagonist portion comprises an amino acid sequence of any one of the VEGF antagonists listed in table 2;

the linker is an amino acid sequence from 1 – 150 amino acids in length; and wherein the dual targeting antigen binding molecule is for use in preventing or treating a disease of the eye and is to be administered intravitreally every 4-6 weeks.

46. A dual targeting molecule as claimed in claim 44 or 45, wherein the linker is selected from those set out in SEQ ID NO: 3-8, 25, 66-68, and 145-162 or any combination or multiple thereof.

47. A dual targeting antigen binding molecule as claimed in any one of claims 44-46, consisting of an amino acid sequence of SEQ ID NO:62 or SEQ ID NO 64.

48. An antigen binding protein comprising the heavy chain sequence of SEQ ID NO:69, 70, 71 or 72 and the light chain sequence of SEQ ID NO:12.

49. A pharmaceutical composition comprising an antigen binding protein as claimed in claim 48 and a further active agent, optionally an anti-inflammatory agent

50. A polynucleotide sequence encoding the antigen binding protein of claim 48.

51. A polynucleotide sequence as claimed in claim 50, wherein the polynucleotide comprises SEQ ID NO:141, 142, 143 or 144 and SEQ ID NO:11.

Figures

Figure 1.

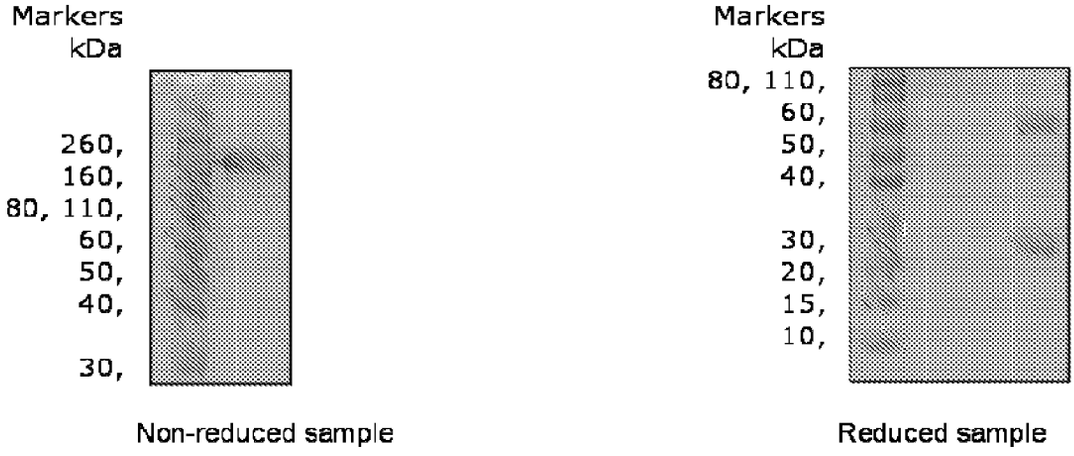


Figure 2.

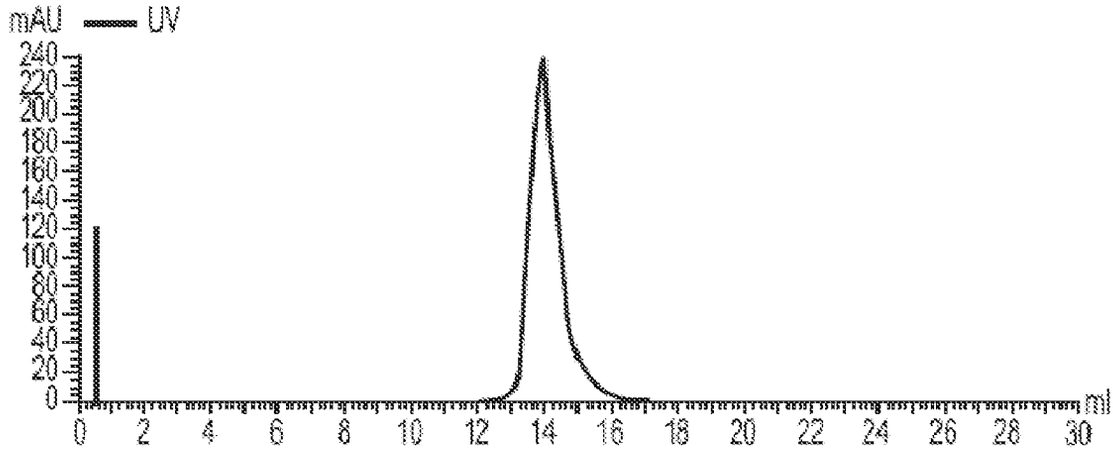


Figure 3.

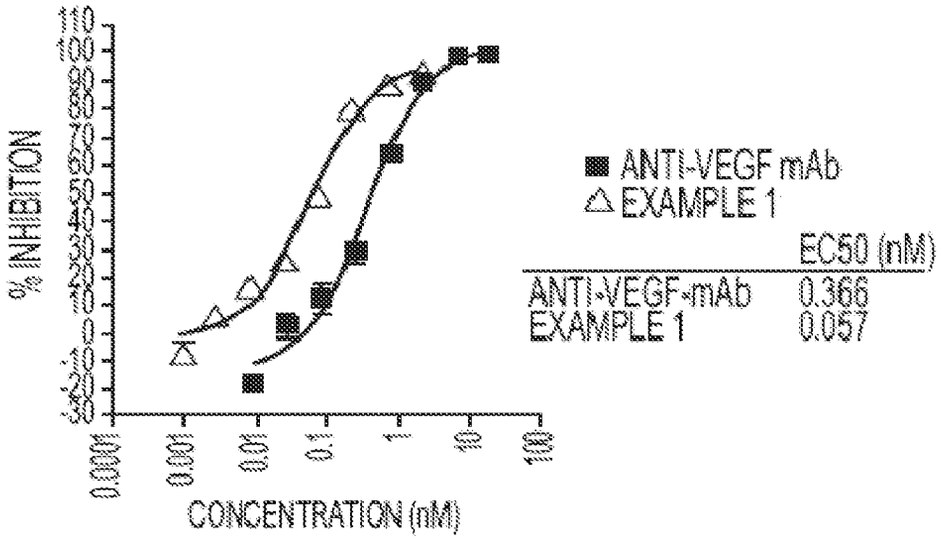


Figure 4.

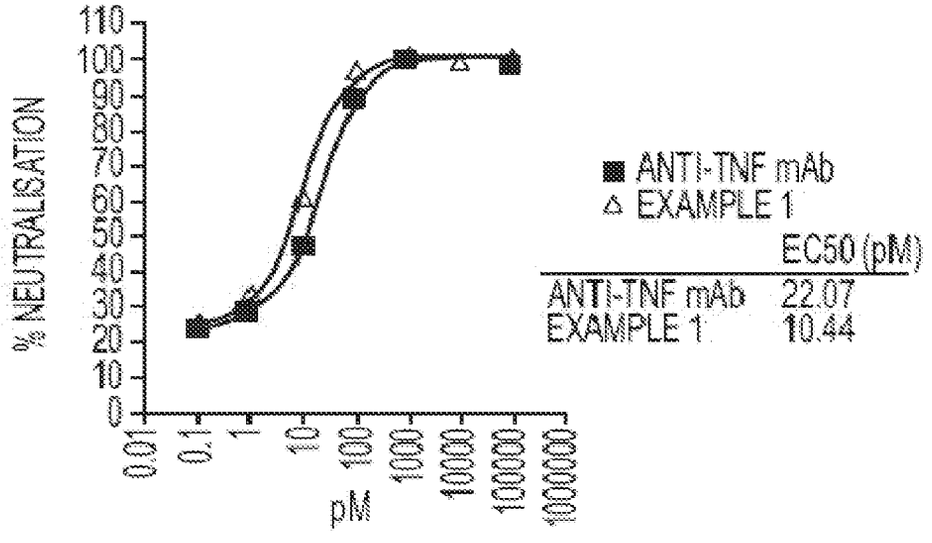


Figure 5

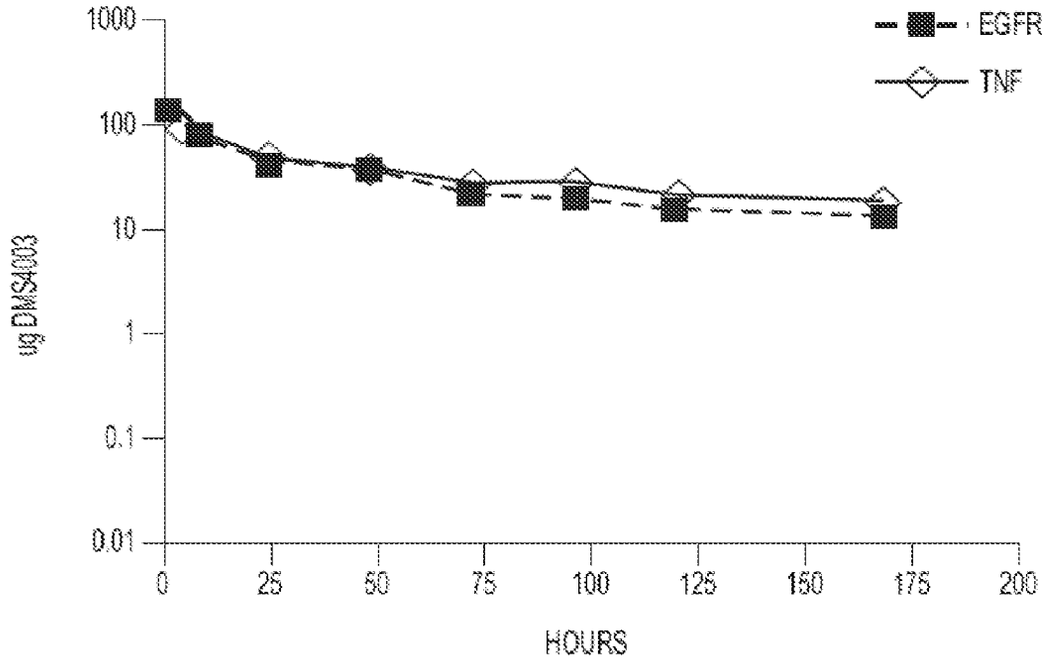


Figure 6

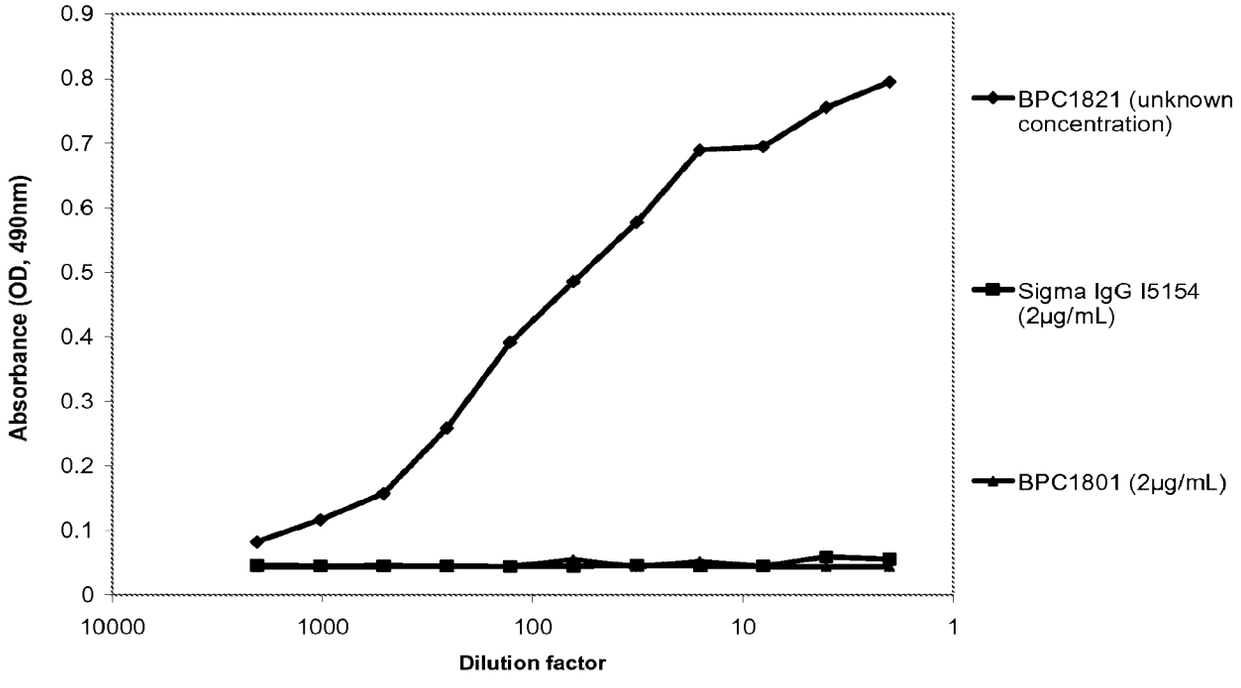


Figure 7

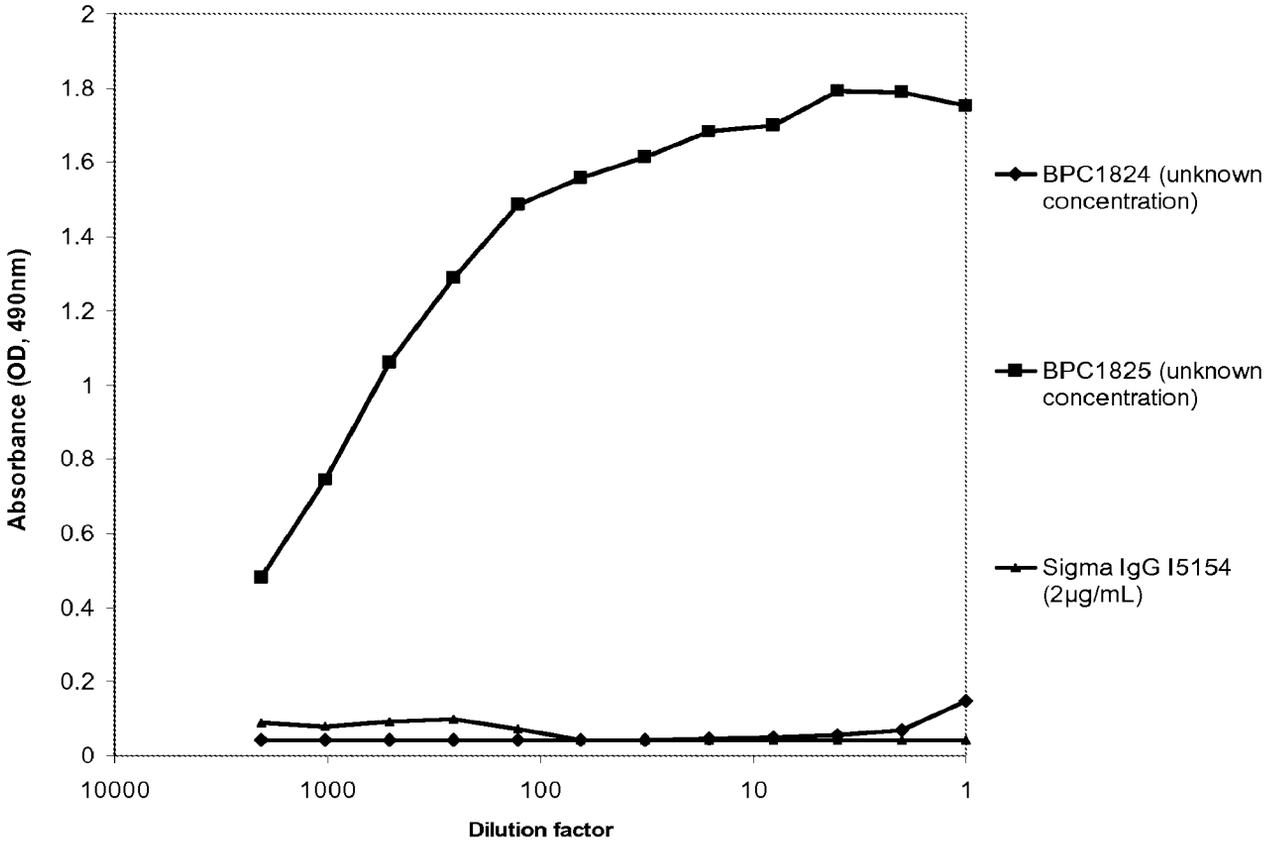


Figure 8 (a)

		Secondary Agents-->	Bevacizumab	r84 (Affitech)	Aflibercept	Ranibizumab	ESBA	DOM15-26-593	DOM15-10-11	PRS-50 / 55	MP0112
		Anti-VEGF Agents	Avastin		VEGF-Trap	Lucentis					
			Humanized mAb	mAb (Peregrine)	Receptor-Fc fusion	Humanized Fab	Humanized ScFv	Human VH dAb	Human Vk dAb	Anticalin (A/C)	Darpin (Dpn)
	Primary Agent Anti-TNFa Agents										
Etanercept	Enbrel	Receptor-Fc fusion					Rec-Fc-ScFv	Rec-Fc-dAb	Rec-Fc-dAb	Rec-Fc-A/C	Rec-Fc-Dpn
Infliximab	Remicade	Chimaeric mAb	DVD-Ig	DVD-Ig		DVD-Ig	mAb-ScFv	mAb-dAb	mAb-dAb	mAb-A/C	mAb-Dpn
Adalimumab	Humira	Human mAb	DVD-Ig	DVD-Ig		DVD-Ig	mAb-ScFv	mAb-dAb	mAb-dAb	mAb-A/C	mAb-Dpn
Golimumab	Simponi	Human mAb	DVD-Ig	DVD-Ig		DVD-Ig	mAb-ScFv	mAb-dAb	mAb-dAb	mAb-A/C	mAb-Dpn
Certolizumab	Cimzia	Humanized Fab (PEGylated)	DVD-Ig	DVD-Ig	Rec-Fc-Fab	Fab-Fab	Fab-ScFv	Fab-dAb	Fab-dAb	Fab-A/C	Fab-Dpn
ESBA105		Humanized ScFv	ScFv-	ScFv-	ScFv-Rec-Fc	ScFv-Fab	ScFv-ScFv	ScFv-dAb	ScFv-dAb	ScFv-A/C	ScFv-Dpn
PEP1-5-19 etc		Human Vk dAb	dAb-mAb?	dAb-mAb?	dAb-Rec-Fc	dAb-Fab?	dAb-ScFv	dAb-dAb	dAb-dAb	dAb-A/C	dAb-Dpn
ALX-0401		Humanized camelid VHH	dAb-mAb?	dAb-mAb?	VHH-Rec-Fc	VHH-Fab	VHH-ScFv	VHH-dAb	VHH-dAb	VHH-A/C	VHH-Dpn
CT-???		Adnectin	dAb-mAb?	dAb-mAb?	Axn-Rec-Fc	Axn-Fab	Axn-ScFv	Axn-dAb	Axn-dAb	Axn-A/C	Axn-Dpn

Figure 8 (b)

		Secondary Agents →	Etanercept	Infliximab	Adalimumab	Golimumab	Certolizumab	ESBA105	PEP1-5-19	ALX-0401	CT-???
			Enbrel	Remicade	Humira	Simponi	Cimzia		etc		
		Anti-TNFa Agents	Receptor-Fc fusion	Chimaeric mAb	Human mAb	Human mAb	Humanized Fab (PEGylated)	Humanized ScFv	Human Vk dAb	Humanized camelid VHH	Adnectin (Axn)
	Primary Agent										
	Anti-VEGF Agents										
Bevacizumab	Avastin	Humanized mAb		DVD-Ig	DVD-Ig	DVD-Ig	DVD-Ig	mAb-ScFv	mAb-dAb	mAb-VHH	mAb-Axn
r84 (Affitech)		Humanized mAb (Peregrine)		DVD-Ig	DVD-Ig	DVD-Ig	DVD-Ig	mAb-ScFv	mAb-dAb	mAb-VHH	mAb-Axn
Aflibercept	VEGF-Trap	Receptor-Fc fusion					Rec-Fc-Fab	Rec-Fc-ScFv	Rec-Fc-dAb	Rec-Fc-VHH	Rec-Fc-Axn
Ranibizumab	Lucentis	Humanized Fab		DVD-Ig	DVD-Ig	DVD-Ig	Fab-Fab	Fab-ScFv	Fab-dAb	Fab-VHH	Fab-Axn
ESBA		Humanized ScFv	ScFv-Rec-Fc	ScFv-mAb/DVD-Ig?	ScFv-mAb/DVD-Ig?	ScFv-mAb/DVD-Ig?	ScFv-Fab	ScFv-ScFv	ScFv-dAb	ScFv-VHH	ScFv-Axn
DOM15-26-593		Human VH dAb	dAb-Rec-Fc	dAb-mAb	dAb-mAb	dAb-mAb	dAb-Fab	ScFv-dAb	dAb-dAb	dAb-VHH	dAb-Axn
DOM15-10-11		Human Vk dAb	dAb-Rec-Fc	dAb-mAb	dAb-mAb	dAb-mAb	dAb-Fab	ScFv-dAb	dAb-dAb	dAb-VHH	dAb-Axn
PRS-50 / 55		Anticalin	A/C-Rec-Fc	A/C-mAb	A/C-mAb	A/C-mAb	A/C-Fab	A/C-ScFv	A/C-dAb	A/C-VHH	A/C-Axn
MP0112		Darpin	Dpn-Rec-Fc	Dpn-mAb	Dpn-mAb	Dpn-mAb	Dpn-Fab	Dpn-ScFv	Dpn-dAb	Dpn-VHH	Dpn-Axn

Figure 9

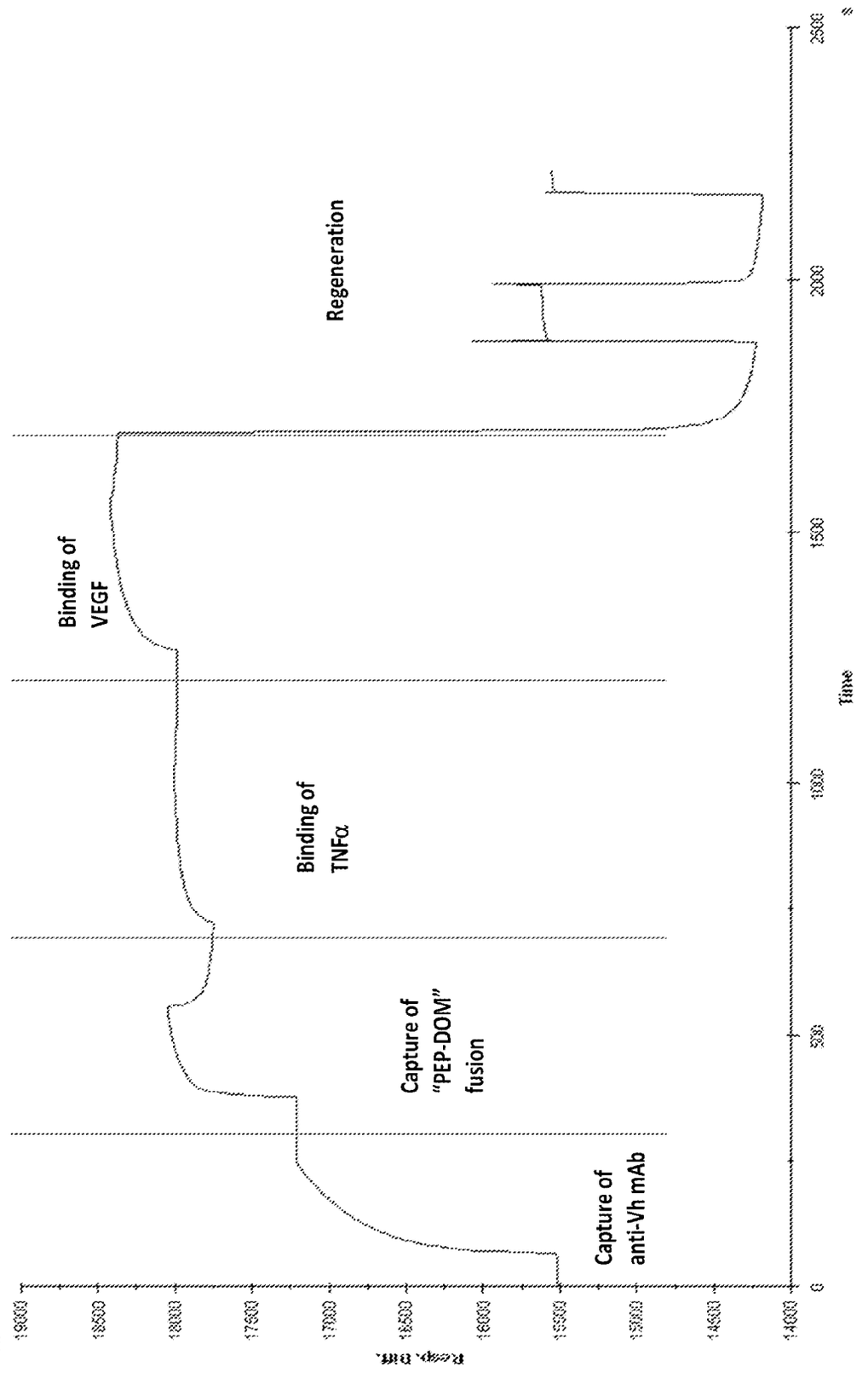


Figure 10

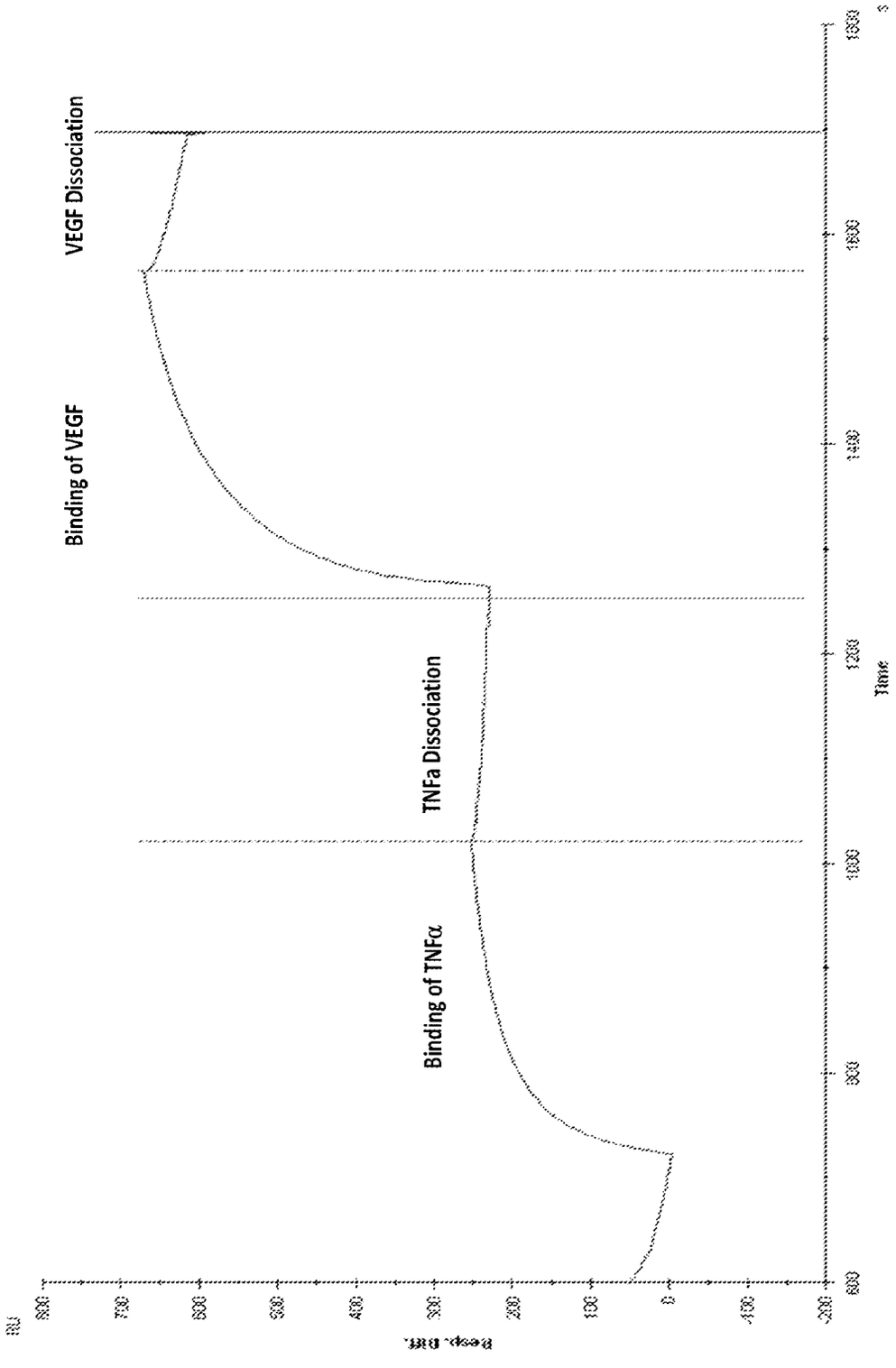
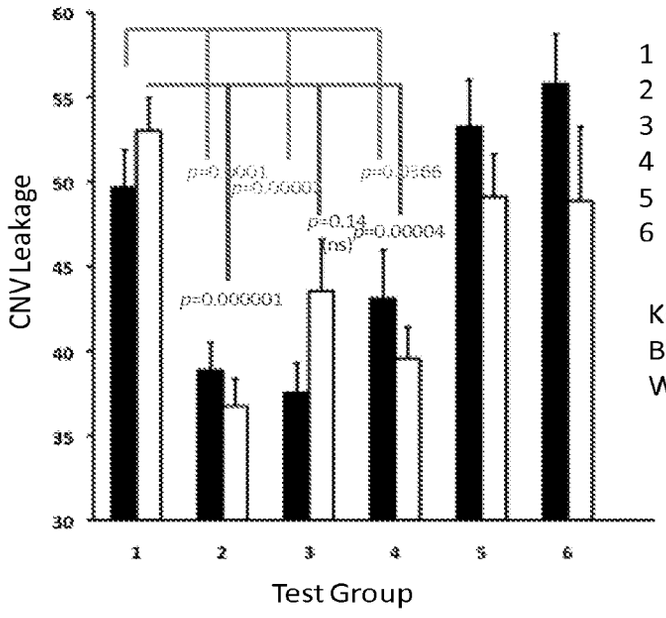


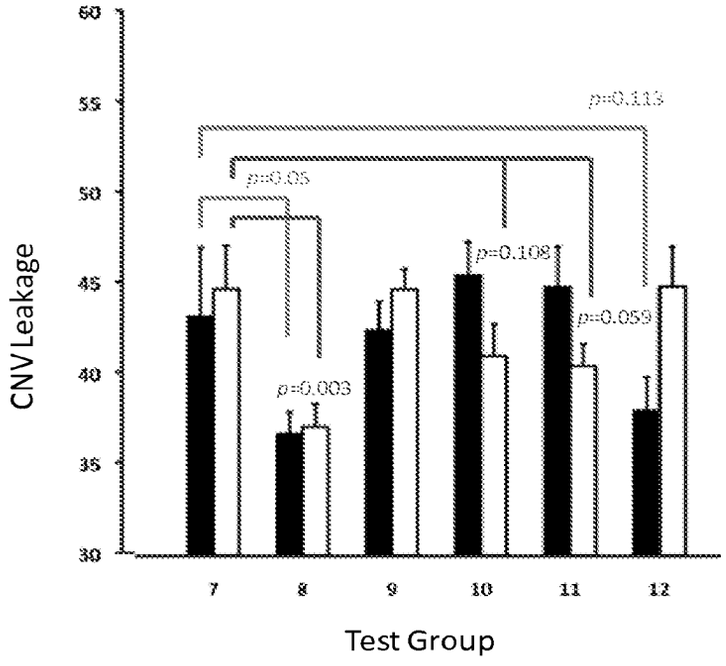
Figure 11



1 Vehicle
2 1mg/ml anti-VEGF Dab
3 0.5mg/ml anti-VEGF Dab
4 0.25mg/ml anti-VEGF Dab
5 0.1mg/ml anti-VEGF Dab
6 0.05mg/ml anti-VEGF Dab

Key
Black bars = day 7 results
White bars = day 14 results

Figure 12



7 Enbrel **(Vehicle only)**
8 Enbrel 15mg/ml (30ug)
9 Enbrel 5mg/ml (10ug)
10 Enbrel 1.5mg/ml (3ug)
11 Enbrel 0.5mg/ml (1ug)
12 Enbrel 0.15mg/ml (0.3ug)

Key
Black bars = day 7 results
White bars = day 14 results

Figure 13

Figure 14

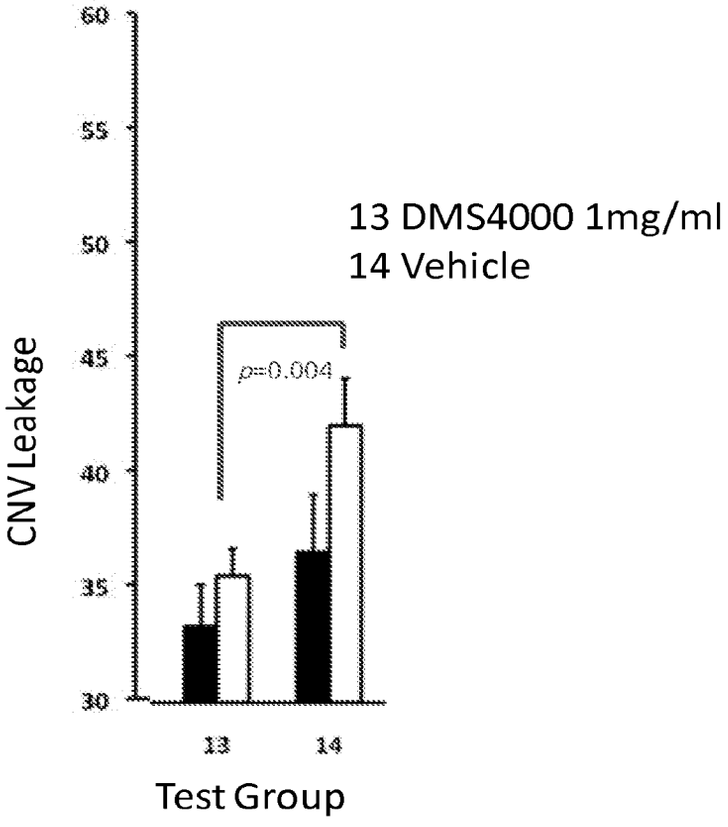
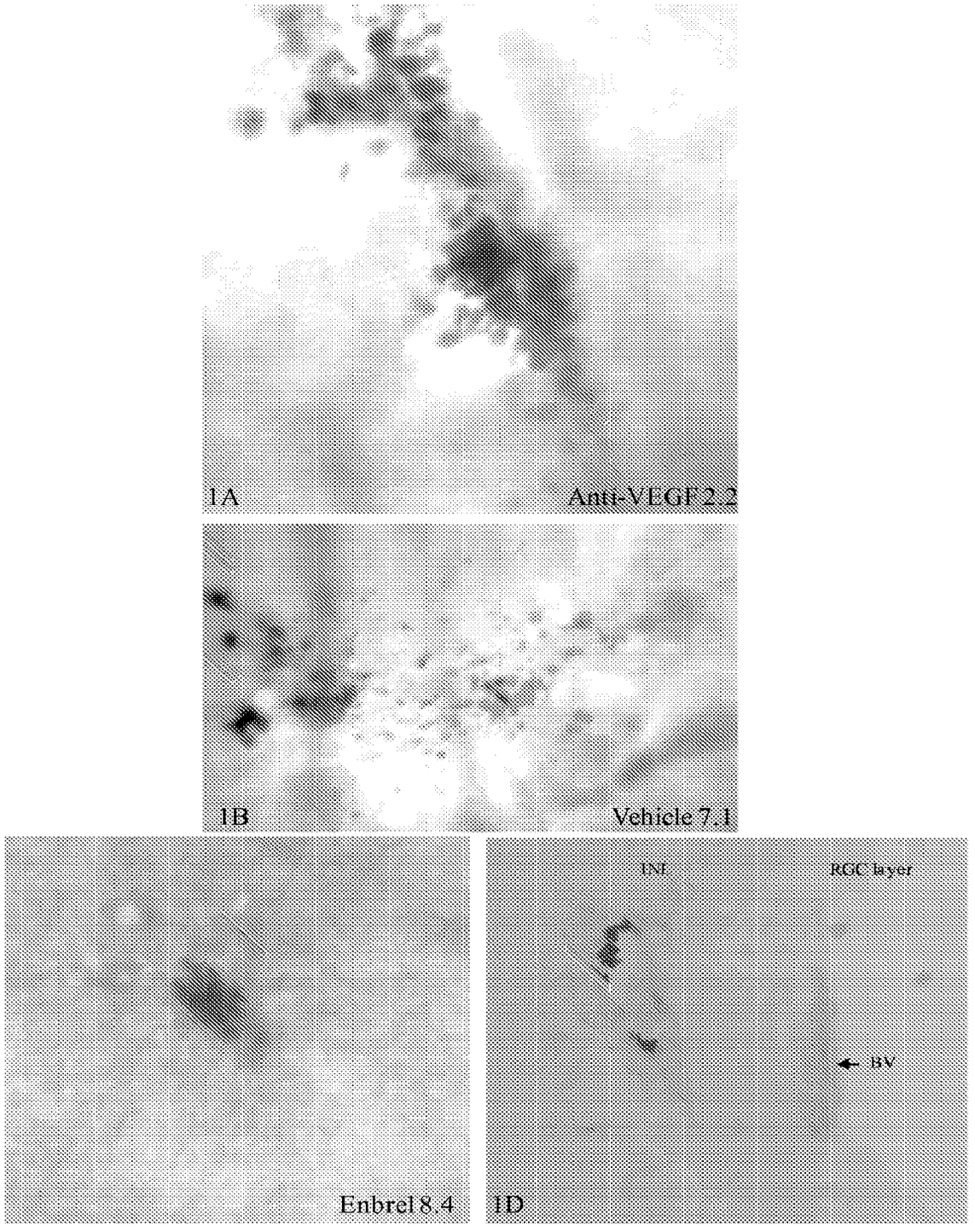


Figure 15



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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 December 2010 (02.12.2010)

PCT

(10) International Publication Number
WO 2010/136492 A3

(51) **International Patent Classification**:
A61K 39/395 (2006.01) ***A61P 27/02*** (2006.01)
C07K 16/22 (2006.01) *C07K 14/715* (2006.01)
C07K 16/24 (2006.01)

(21) **International Application Number**:
PCT/EP2010/057246

(22) **International Filing Date**:
26 May 2010 (26.05.2010)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/181,887 28 May 2009 (28.05.2009) US

(71) **Applicant** *(for all designated States except US)*: **GLAXO GROUP LIMITED** [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford Middlesex UB6 0NN (GB).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **ADAMSON, Peter** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **ERTL, Peter, Franz** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **GERMASCHEWSKI, Volker** [DE/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **GOUGH, Gerald, Wayne** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB). **STEWARD, Michael** [GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Stevenage Hertfordshire SG1 2NY (GB).

(74) **Agents**: **REDDISH, Anna** et al.; GlaxoSmithKline, Global Patents CN925.1, 980 Great West Road, Brentford Middlesex TW8 9GS (GB).

(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, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

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))*

(88) **Date of publication of the international search report**:
24 February 2011

(54) **Title**: COMBINATION OF A TNF-ALPHA ANTAGONIST AND A VEGF ANTAGONIST FOR USE IN THE TREATMENT OR PREVENTION OF DISEASES OF THE EYE.

(57) **Abstract**: Combinations of TNF&alpha,- antagonists with VEGF antagonists for use in treating diseases of the eye, and provides antigen-binding proteins which bind to TNFα or a TNFα receptor and/or VEGF or a VEGF receptor.

WO 2010/136492 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/057246

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/22 C07K16/24 A61P27/02
ADD. C07K14/715

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 A61K A61P

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, BIOSIS, EMBASE, WPI Data, 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	A. ADAMIS ET AL.: "Immunological mechanisms in the pathogenesis of diabetic retinopathy.", SEMINARS IN IMMUNOPATHOLOGY, vol. 30, no. 2, 14 March 2008 (2008-03-14), pages 65-84, XP019625605, Germany page 76, left-hand column, line 21 - line 26 page 79, left-hand column, paragraph 3 - page 80, left-hand column, last paragraph ----- -/--	1-51

[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
6 December 2010	14/12/2010

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	Nooij, Frans

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/057246

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	L. MAINES ET AL.: "Pharmacologic manipulation of sphingosine kinase in retinal endothelial cells: Implications for angiogenic ocular diseases.", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 47, no. 11, November 2006 (2006-11), pages 5022-5031, XP002592513, USA page 5022, left-hand column, paragraphs 1,3 page 5025, left-hand column, paragraph 1 - page 5026, right-hand column, paragraph 1	1-51
Y	WO 2006/003388 A2 (DOMANTIS LIMITED) 12 January 2006 (2006-01-12) examples 23-25 page 162, last paragraph - page 163, paragraph 1	1-51
A	US 2004/091455 A1 (ZELDIS) 13 May 2004 (2004-05-13) paragraph [0018] - paragraph [0020] paragraph [0030] - paragraph [0031] paragraph [0040] - paragraph [0041]	1-51
A	A. ROTHOVA: "Inflammatory cystoid macular edema.", CURRENT OPINION IN OPHTHALMOLOGY, vol. 18, no. 6, November 2007 (2007-11), pages 487-492, XP008124386, USA page 490, left-hand column, line 7 - right-hand column, line 1	1-51
A	E. RODRIGUES ET AL.: "Therapeutic monoclonal antibodies in ophthalmology.", PROGRESS IN RETINAL AND EYE RESEARCH, vol. 28, no. 2, March 2009 (2009-03), pages 117-144, XP025948069, England page 139, left-hand column, line 37 - line 41	1-51
Y	L. HOLT ET AL.: "Domain antibodies: proteins for therapy.", TRENDS IN BIOTECHNOLOGY, vol. 21, no. 11, November 2003 (2003-11), pages 484-490, XP004467495, page 489, left-hand column, paragraph 2	1-51
A,P	WO 2009/068649 A2 (GLAXO GROUP LIMITED) 4 June 2009 (2009-06-04) example 11 sequence 75	44,46-51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/057246

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

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

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

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-43, 45(completely); 46, 47(partially)

A (pharmaceutical) composition comprising a TNF-alpha antagonist and a VEGF antagonist for use in preventing or treating a disease of the eye. Polynucleotide sequences. Recombinant host cell. Method of production. Method of preventing or treating a patient afflicted with an eye disease. Dual targeting antigen binding molecule comprising a specified TNF-alpha antagonist portion, a specified VEGF antagonist portion and a linker, for use in preventing or treating a disease of the eye and is to be administered intravitreally every 4-6 weeks.

---

2. claims: 44(completely); 46, 47(partially)

A dual targeting antigen binding molecule comprising a specified TNF-alpha antagonist portion, a specified anti-VEGF antagonist portion and a linker, wherein the dual targeting molecule is not DMS4000 or DMS4031.

---

3. claims: 48-51

An antigen binding protein comprising the heavy chain sequence of SEQ ID NO:69, 70, 71 or 72 and the light chain sequence of SEQ ID NO:12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/057246

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006003388 A2	12-01-2006	AU 2005259006 A1	12-01-2006
		AU 2010201856 A1	27-05-2010
		CA 2571536 A1	12-01-2006
		EA 200700175 A1	31-08-2007
		EP 1761565 A2	14-03-2007
		KR 20070050912 A	16-05-2007
		KR 20100049700 A	12-05-2010
US 2004091455 A1	13-05-2004	US 2008213219 A1	04-09-2008
WO 2009068649 A2	04-06-2009	AR 069495 A1	27-01-2010
		AU 2008328726 A1	04-06-2009
		CA 2706419 A1	04-06-2009
		EP 2222709 A2	01-09-2010
		GB 2468232 A	01-09-2010
		KR 20100097720 A	03-09-2010
		PE 12342009 A1	07-09-2009
		US 2009148905 A1	11-06-2009
		UY 31504 A1	17-07-2009

Electronic Acknowledgement Receipt

EFS ID:	15939984
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:	PAT055157-US-NP
Receipt Date:	04-JUN-2013
Filing Date:	25-JAN-2013
Time Stamp:	10:47:56
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	Information Disclosure Statement (IDS) Form (SB08)	_PAT055157-US-NP_IDS_SB08_2013Jun4.pdf	673133 ee95b75ab1d938626e9bb1a7216083de5e96a718	no	5

Warnings:	
Information:	

2	Foreign Reference	_26_WO07084765.pdf	6008664 42267ea82d2325f5cee498f410283f0bc9f18ed3	no	97
Warnings:					
Information:					
3	Foreign Reference	_23_WO2006128564.pdf	908186 6951f1f8082b6341bf240281bd2898e607f69887	no	26
Warnings:					
Information:					
4	Foreign Reference	_21_WO2006047325.pdf	2351274 44192fbc0a02711292876ce37e61add30f103125	no	46
Warnings:					
Information:					
5	Foreign Reference	_27_CN201578690_U_EN_Abstract.pdf	74956 87f66871da08ac0c276321c08468f91244d739e8	no	1
Warnings:					
Information:					
6	Foreign Reference	_28_EP2371406.pdf	294175 548482c45d2edfc8102121205d2c1cc3fa462e2f	no	23
Warnings:					
Information:					
7	Foreign Reference	_29_JP_Pat_Appl_2001_EN_ABSTRACT_.pdf	81328 948d633815d853e03c787daa7926b939157d31ad	no	1
Warnings:					
Information:					
8	Foreign Reference	_30_WO2010136492.pdf	1039402 5050c24d0a93712b6275dd0e185aaeff6a6d7f68	no	163
Warnings:					
Information:					
			Total Files Size (in bytes):	11431118	

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 Confirm No. 5306

Sigg, Juergen et al.

APPLICATION NO: 13/750,352

FILED: January 25, 2013

FOR: SYRINGE

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above-referenced patent application, please enter the following preliminary amendments.

Amendments to the Claims are reflected in the listing of the claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

CASE PAT055157-US-NP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Sigg, Juergen et al.
APPLICATION NO: 13/750,352
FILED: January 25, 2013
FOR: SYRINGE

Confirm No. 5306

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above-referenced patent application, please enter the following preliminary amendments.

Amendments to the Claims are reflected in the listing of the claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1.(currently amended) A pre-filled syringe for intravitreal injection, the syringe comprising a glass body forming a barrel, 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~~ and containing 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 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.(original) 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.(original) A pre-filled syringe according to claim 1, wherein the syringe is filled with about 0.165ml of said VEGF antagonist solution.

4.(original) 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.(original) 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.(currently amended) 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~~.

7.(currently amended) A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating 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.(currently amended) A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of ~~more than~~ from about 1µg to less than about 500µg~~, more than about 3µg, more than about 5µg, more than about 7µg or more than about 10µg~~ silicone oil.

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

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

11. (original) A pre-filled syringe according to claim~~s~~ 1, wherein the syringe is silicone oil free.

12. (original) 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 ≥25µm in diameter per ml, and (ii) no more than 50 particles ≥10µm in diameter per ml.

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

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

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

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

17. (original) A pre-filled syringe according to claim~~s~~ 1 wherein the VEGF antagonist is a non-antibody VEGF antagonist.

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

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

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

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

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

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

24. (original) A pre-filled syringe according to claims 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.(original) 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. (original) 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. (original) 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. (original) A blister pack comprising a pre-filled syringe according to claims 25, wherein $\leq$5% of the VEGF antagonist is alkylated.

29. (original) 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. (original) 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 claim 1.

31. (original) 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. (original) 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.

REMARKS/ARGUMENTS

The foregoing amendments to the specification places the claims in better form and remove multiple dependencies.

Claim 1 has further been amended to specify that the glass body of the claimed syringe forms a barrel – this amendment was performed to provide antecedent basis for the term "barrel" in feature (c) of the same claim. Claim 1 has also been amended to specify that the claimed syringe is used to facilitate intravitreal injection. Support for these amendments may be found on p. 2, lines 7-8 of the original specification. Finally, claim 1 has been amended to delete elements that relate to the arrangement and functionality of the body, the stopper and the plunger, because these elements represent typical features of conventional syringes. Thus, no new matter has been added. Applicants reserve the right to prosecute the subject matter of the cancelled claims in subsequent divisional applications.

Should the Examiner have any questions, please contact the undersigned attorney.

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: August 16, 2013

CASE PAT055157-US-NP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF Confirm No. 5306

Sigg, Juergen et al.

APPLICATION NO: 13/750,352

FILED: January 25, 2013

FOR: SYRINGE

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above-referenced patent application, please enter the following preliminary amendments.

Amendments to the Claims are reflected in the listing of the claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1.(currently amended) A pre-filled syringe for intravitreal injection, the syringe comprising a glass body forming a barrel, 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~~ and containing 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 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.(original) 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.(original) A pre-filled syringe according to claim 1, wherein the syringe is filled with about 0.165ml of said VEGF antagonist solution.

4.(original) 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.(original) 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.(currently amended) 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~~.

7.(currently amended) A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating 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.(currently amended) A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of ~~more than~~ from about 1μg to less than about 500μg~~, more than about 3μg, more than about 5μg, more than about 7μg or more than about 10μg~~ silicone oil.

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

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

11. (original) A pre-filled syringe according to claim~~s~~ 1, wherein the syringe is silicone oil free.

12. (original) 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 ≥25μm in diameter per ml, and (ii) no more than 50 particles ≥10μm in diameter per ml.

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

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

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

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

17. (original) A pre-filled syringe according to claim~~s~~ 1 wherein the VEGF antagonist is a non-antibody VEGF antagonist.

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

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

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

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

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

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

24. (original) A pre-filled syringe according to claims 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.(original) 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. (original) 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. (original) 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. (original) A blister pack comprising a pre-filled syringe according to claims 25, wherein $\leq$5% of the VEGF antagonist is alkylated.

29. (original) 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. (original) 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 claim 1.

31. (original) 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. (original) 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.

REMARKS/ARGUMENTS

The foregoing amendments to the specification places the claims in better form and remove multiple dependencies.

Claim 1 has further been amended to specify that the glass body of the claimed syringe forms a barrel – this amendment was performed to provide antecedent basis for the term "barrel" in feature (c) of the same claim. Claim 1 has also been amended to specify that the claimed syringe is used to facilitate intravitreal injection. Support for these amendments may be found on p. 2, lines 7-8 of the original specification. Finally, claim 1 has been amended to delete elements that relate to the arrangement and functionality of the body, the stopper and the plunger, because these elements represent typical features of conventional syringes. Thus, no new matter has been added. Applicants reserve the right to prosecute the subject matter of the cancelled claims in subsequent divisional applications.

Should the Examiner have any questions, please contact the undersigned attorney.

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: August 16, 2013

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1.(currently amended) A pre-filled syringe for intravitreal injection, the syringe comprising a glass body forming a barrel, 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~~ and containing 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 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.(original) 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.(original) A pre-filled syringe according to claim 1, wherein the syringe is filled with about 0.165ml of said VEGF antagonist solution.

4.(original) 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.(original) 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.(currently amended) 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~~.

7.(currently amended) A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating 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.(currently amended) A pre-filled syringe according to claim 1, wherein the syringe barrel has an internal coating of ~~more than~~ from about 1µg to less than about 500µg~~, more than about 3µg, more than about 5µg, more than about 7µg or more than about 10µg~~ silicone oil.

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

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

11. (original) A pre-filled syringe according to claim~~s~~ 1, wherein the syringe is silicone oil free.

12. (original) 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 ≥25µm in diameter per ml, and (ii) no more than 50 particles ≥10µm in diameter per ml.

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

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

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

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

17. (original) A pre-filled syringe according to claim~~s~~ 1 wherein the VEGF antagonist is a non-antibody VEGF antagonist.

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

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

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

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

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

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

24. (original) A pre-filled syringe according to claims 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.(original) 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. (original) 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. (original) 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. (original) A blister pack comprising a pre-filled syringe according to claims 25, wherein $\leq$5% of the VEGF antagonist is alkylated.

29. (original) 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. (original) 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 claim 1.

31. (original) 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. (original) 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.

REMARKS/ARGUMENTS

The foregoing amendments to the specification places the claims in better form and remove multiple dependencies.

Claim 1 has further been amended to specify that the glass body of the claimed syringe forms a barrel – this amendment was performed to provide antecedent basis for the term "barrel" in feature (c) of the same claim. Claim 1 has also been amended to specify that the claimed syringe is used to facilitate intravitreal injection. Support for these amendments may be found on p. 2, lines 7-8 of the original specification. Finally, claim 1 has been amended to delete elements that relate to the arrangement and functionality of the body, the stopper and the plunger, because these elements represent typical features of conventional syringes. Thus, no new matter has been added. Applicants reserve the right to prosecute the subject matter of the cancelled claims in subsequent divisional applications.

Should the Examiner have any questions, please contact the undersigned attorney.

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: August 16, 2013

Electronic Acknowledgement Receipt

EFS ID:	16612031
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:	PAT055157-US-NP
Receipt Date:	16-AUG-2013
Filing Date:	25-JAN-2013
Time Stamp:	14:42:29
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_PPHPaperwork_2013Aug16.pdf	12941309 5405ce29e0cb58632564be6f7cc30c6e1843ea1a	yes	32

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Petition to make special under Patent Prosecution Hwy	1	27
	Preliminary Amendment	28	32
Warnings:			
Information:			
	Total Files Size (in bytes):	12941309	

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.

PTO/SB/20UK (02-12)
Approved for use through 01/31/2015. OMB 0651-0058
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

REQUEST FOR PARTICIPATION IN THE PATENT PROSECUTION HIGHWAY (PPH) PILOT PROGRAM BETWEEN THE UNITED KINGDOM INTELLECTUAL PROPERTY OFFICE (UKIPO) AND THE USPTO

Application No.:	13/750,352	First Named Inventor:	Juergen Sigg
Filing Date:	January 25, 2013	Attorney Docket No.:	55157-US-NP
Title of the Invention:	SYRINGE		

THIS REQUEST FOR PARTICIPATION IN THE PPH PILOT PROGRAM ALONG WITH THE REQUIRED DOCUMENTS MUST BE SUBMITTED VIA EFS-WEB. INFORMATION REGARDING EFS-WEB IS AVAILABLE AT HTTP://WWW.USPTO.GOV/EBC/EFS_HELP.HTML.

APPLICANT HEREBY REQUESTS PARTICIPATION IN THE PATENT PROSECUTION HIGHWAY (PPH) PILOT PROGRAM AND PETITIONS TO MAKE THE ABOVE-IDENTIFIED APPLICATION SPECIAL UNDER THE PPH PILOT PROGRAM.

The above-identified application and the corresponding UK application(s) have the same priority/filing date. If UKIPO is not the office of first filing (OFF), identify the OFF and the OFF application no.

The UK application number(s) is/are: GB1301368.5

The filing date of the UK application(s) is/are: GB1301368.5 was filed January 25, 2013, and claims priority to EP12174680, which was filed July 3, 2012, and is the same priority application as that for 13/750,352

I. List of Required Documents:

a. **A copy of the latest UK office action prior to the "Decision to Grant a Patent"* in the above-identified UK application(s)**

☑ is attached.

☐ is not attached because the UK application was allowed in a first office action.

* It is not necessary to submit a copy of the "Decision to Grant a Patent."

b. **(1) An information disclosure statement listing the documents cited in the UK office action**

☐ is attached.

☑ has already been filed in the above-identified U.S. application on March 19, 2013 and June 4, 2013.

(2) Copies of all documents (except for U.S. patents or U.S. patent application publications)

☐ are attached.

☑ have already been filed in the above-identified U.S. application on March 19, 2013 and June 4, 2013.

PTO/SB/20UK (02-12)
Approved for use through 01/31/2015. OMB 0651-0058
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

REQUEST FOR PARTICIPATION IN THE PPH PILOT PROGRAM BETWEEN UKIPO AND THE USPTO
(continued)

Application No.:	13/750,352	First Named Inventor:	Juergen Sigg

II. Claims Correspondence Table:

Claims in US Application	Patentable Claims in UK Application	Explanation regarding the correspondence
1, 2, 15, 16, 18, 19, 21, 23, 26, 27	1, 2, 15, 16, 18, 19, 21, 23, 26, 27	The US and UK claims are the same.
3	3	US claim 3 depends from claim 1. UK claim 3 is multiply dependent on claims 1 and 2.
4	4	US claim 3 depends from claim 1. UK claim 3 is multiply dependent on claims 1-3.
5	5	US claim 3 depends from claim 1. UK claim 3 is multiply dependent on claims 1-4.
6	6	The US and UK claims both specify a syringe barrel thickness of about 450 nm or less. The UK claim recites other upper limits less than 450 nm.
7-9	7-9	The US and UK claims both specify between about 1 and less than about 500 µg silicone oil as the syringe barrel coating. I
10, 12-14, 20, 22, 25	10, 12-14, 20, 22, 25	The US claims depend from claim 1. The UK claims are multiply dependent on all previous claims.
11	11	US claim 11 depends from claim 1. UK claim 11 is multiply dependent on claims 1-5.
17	17	US claim 17 depends from claim 1. UK claim 17 is multiply dependent on claims 1-13.
24	24	US claim 24 depends from claim 20. UK claim 24 is multiply dependent on claims 20-23.
28	28	US claim 28 depends from claim 25. UK claim 28 is multiply dependent on claims 25-27.
29	29	US claim 29 depends from claim 25. UK claim 29 is multiply dependent on claims 25-28.
30	34, 35	US method claim 30 depends from claim 1 and converts from multiple dependent UK use claim 34 and combines the features of UK claims 34 & 35.
31	36	US claim 31 depends from claim 30. UK claim 36 depends on claim 35, which depends on claim 34, which multiply depends on all previous claims.
32	37	US claim 32 depends from claim 30. UK claim 37 depends on claims 35 & 36.

III. All the claims in the US application sufficiently correspond to the patentable/allowable claims in the UK application.

Signature / Andrew Holmes /	Date August 16, 2013
Name (Print/Typed) Andrew Holmes	Registration Number 51,813

CARPMAELS & RANSFORD

David Spinner
Novartis Pharma AG
Patent and Trademark Department
Postfach
4002 Basel
Switzerland

Chartered Patent Attorneys
European Patent Attorneys
Trade Mark Attorneys
Regulated by IPReg

Carpmaels & Ransford
One Southampton Row
London WC1B 5HA
United Kingdom
T +44 20 7242 8692
F +44 20 7405 4166
E email@carpmaels.com
www.carpmaels.com

By email only

Your Ref PAT55157-GB-NP
Our Ref P061025GB:CJM/JPG
Date 10th April 2013

Dear David,

Re: United Kingdom Patent Application No. 1301368.5
NOVARTIS AG

I have now received the combined search and examination report from the UKIPO. The due date for responding to the report is **3rd July 2014**. A copy of the report is enclosed for your records.

The Examiner implicitly acknowledged that the claimed syringe is novel over the cited prior art. However, the Examiner objected to the claims for lack of inventive step. In particular, the Examiner has alleged that the claims merely define a collocation of features well-known in the prior art which in combination do not introduce an inventive step.

The Examiner has argued that typical syringe volumes used in ophthalmic applications include 1 ml pointing to various patent applications that relate to the intravitreal administration of a VEGF-antagonist molecule. For example, the Examiner cited Genentech's WO 2006/047325, which discloses the use of a low-volume syringe such as a tuberculin syringe containing 50µl of a ranibizumab solution.

In addition, the Examiner pointed to OcuJect's US 2012/0078224 which discloses that "*the drug reservoir is silicone oil-free (lacks silicone oil or one of its derivatives) and is not internally covered or lubricated with silicone oil, […] which ensures that silicone oil does not get inside the eye causing floaters or intraocular pressure elevation*" (see paragraph [0145)]. However, this application appears to relate to an intraocular injection device that comprises other parts in addition to a syringe.

The Examiner further cites Baxter's WO 2006/128564, which relates to a silicone-free syringe. However, the disclosed syringe comprises a polypropylene body with a laminated piston in order to avoid the use of silicone oil.

The Examiner further objected to the claims for failing to make clear which part of the claim is the inventive concept. The Examiner appears to be concerned that claim 1 is not sufficiently concise and that mainly features that distinguish the claimed subject-matter from the prior art should be recited in the claim.

I have not reviewed the application in greater detail, but it appears that the Examiner's concerns regarding inventive step could be addressed by defining the amount of silicone oil in the barrel more precisely, *e.g.* by specifying that the amount of silicone oil is in the range of about 3µg-about 200µg (see page 5, line 28 of the description).

Please let me know if you would like us to prepare a draft response to the outstanding examination report for your review. I look forward to receiving your further instructions.

Should you have any questions regarding the above, please do not hesitate to contact me.

Yours sincerely,

Cameron Marshall

Encl. Combined search and examination report

DUE: 3.07.14 CJM.

Page by Page Check Done by Records	AM
Copied for Drop Dead	AM
Confirmed All Required Checks Done	AM

VJPG GDB → JPM.D

INTELLECTUAL PROPERTY OFFICE

Novartis AG
c/o Carpmaels & Ransford
One Southampton Row
LONDON
WC1B 5HA

Patents Directorate

Concept House
Cardiff Road, Newport
South Wales, NP10 8QQ

Direct Line: 01633 814706
†**E-Mail:** matthew.parker@ipo.gov.uk
Switchboard: 0300 300 2000
Fax: 01633 817777
Minicom: 0300 0200 015

Your Reference: P061025GB:CJM/JPG
Application No: GB1301368.5

22 March 2013

Dear Sirs

Patents Act 1977:
Combined Search and Examination Report under Sections 17 and 18(3)

Latest date for reply: **3 July 2014**

I enclose two copies of my search and examination report and a copy of the citations.

By the above date you should either file amendments to meet the objections in the report or make observations on them. If you do not, the application may be refused.

Online e-filing

You may file such amendments or observations electronically if you wish, using the online patent filing services detailed in www.ipo.gov.uk/p-apply-online.

Other search results

If you have applied to another patent office for a patent for this invention you will be receiving from them the results of their search. If you decide to proceed with the present application you are asked to provide a copy of any such official search report or details of any documents cited and category assigned in the report. You may file such information electronically using the online patent filing services detailed in www.ipo.gov.uk/p-apply-online.

Cut-off date This request applies to search reports that you have received before the date when you send a response to our first examination report under section 18(3) or section 18(4);

if you make no response to an initial section 18(4) report the cut-off date is two months after the date of that report. Tell us about a search report sooner rather than later if that would allow it to be considered during our first examination.

Exceptions You do not have to supply details of a search report that (1) shows a nil response, or (2) has been published by WIPO or EPO, or (3) you have already supplied to us on a previous GB application.

Publication

I estimate that, provided you have met all the formal requirements, preparations for publication of your application will be completed soon after **26 November 2013**. At this time you will receive a letter confirming the exact date when the preparations for publication will be completed. This letter will also tell you the publication number and date of publication of your application. However, it should not be relied upon as a reminder if you are intending to withdraw your application before publication.

On the date of publication details of your application, including your name and address, will be entered in the Register of Patents and will become publicly available, including on our website. Some documents and correspondence from your application file will also be made publicly available on our website at www.ipo.gov.uk/p-ipsum.

Withdrawal

If you wish to withdraw your application to prevent publication you must withdraw it before the preparations for publication are complete. One way that you can withdraw your application is by emailing withdraw@ipo.gov.uk. Further details on withdrawal are available from www.ipo.gov.uk/p-withdraw. **WARNING** – once preparations for publication are complete it will NOT be possible to prevent publication.

Amendment

If you wish to file amended claims for inclusion with the published application you must do so before the preparations for publication are completed.

Correspondence

If you write to the Office less than 3 weeks before 26 November 2013 please mark your letter prominently: **"URGENT - PUBLICATION IMMINENT"**.

Yours faithfully

MP

Dr Matthew Parker
Examiner

Important information about combined search and examination

I also ask that you take note of the following points. These might have a bearing on the future stages of your application because the examination report has been sent to you before your application has been published.

(a) You may file voluntary amendments before making a full response to my examination report. We will publish with your application any new or amended claims you file voluntarily or as a full response, provided that they are received before preparations for publication are completed. It would help us when you file amendments before publication if you could **prominently indicate** in a covering letter whether or not the amendments are intended as a full response to the examination report.

(b) If you file a full response to the examination report before your application is published I will consider it as soon as possible. However, if this would disrupt the publication of your application, I would have to delay taking any action until the application had been published. This delay could be up to 3 months, depending upon when we receive your response.

(c) There is another situation when there might be a delay between you filing a full response and the Office responding to it. This would arise if you met all my objections but your application had not or had only recently been published. I could not report the outcome of my re-examination until I was satisfied that the search was complete for documents published before the priority date of your invention and that anybody interested in the application has had three months following publication of the application to make observations on the patentability of your invention.

(d) Provided that the requirements of the Act have been met, I can send your application to grant as early as three months after publication. Before doing so I will bring the original search up to date and raise with you any further objection that might result from this top-up search. However, there is a possibility that at that time I may not have access to all the patent applications published after the priority date of your invention and of possible relevance to your application. If this is the case I would have to complete the search after grant and if necessary raise any new found novelty objection then.

INTELLECTUAL PROPERTY OFFICE

Your ref :	P061025GB:CJM/JPG	**Examiner :**	Dr Matthew Parker
Application No:	GB1301368.5	**Tel :**	01633 814706
Applicant :	Novartis AG	**Date of report :**	22 March 2013
Latest date for reply:	3 July 2014	Page 1/2	

Patents Act 1977
Combined Search and Examination Report under Sections 17 & 18(3)

Patentability

1. Section 4A(1)(a) states that 'a patent shall not be granted for the invention of – (a) a method of treatment of the human or animal body by surgery or therapy'. Claims 33-37 clearly define a method of therapy and therefore these are not patentable.

Collocation and clarity

2. The combination of a series of known features, each playing its usual part in the final entity, is a matter of design or mere collocation, and not of invention. See Manual of Patent Practice section 3.17.

3. Claim1 of your application seems to contain a number of features which are well known in the art and it is unclear where you believe your invention lies. The first part of the claim describes a standard variable volume syringe with a plunger, body, stopper, outlet, wherein the plunger forces the stopper towards the outlet reducing the volume of the variable chamber. This is entirely conventional in the syringe art. The claim then goes onto describe an ophthalmic solution which comprises a VEGF-antagonist. As described in your description such a fluid is well known in the art and is produced commercially as ranibizumab (Lucentis ^{RTM}), bevacizumab (Avastin ^{RTM}), and aflibercept (Eylea ^{RTM}). The claim defines a syringe with a nominal maximum fill volume of between 0.5ml and about 1ml. As described in your description on page 1, lines 13-15, low volume syringes are common in the art. The claim defines the syringe barrel as comprising less the 500µg silicon oil. Low silicon oil and silicon oil free syringes are also well known in the art. Therefore, it seems that the claim is merely a combination of well known features and it is unclear which part of your claim is the inventive concept.

4. If you believe that your inventive concept is a novel VEGF antagonist solution then your main claim should be directed to this and the details about the syringe construction should be omitted from the claim. If you believe that the syringe and plunger has some sort of novel inventive concept then your main claim should be directed to this and the details about the VEGF antagonist solution should be omitted from the claim.

Inventive step

5. The invention as defined in claims 1-32 is obvious in view of what has already been disclosed in the following documents:

[Examination Report contd.]

US2013/012918 A1 (FOSTER), see paragraph [0050]
US2012/078224 A1 (OCUJECT), see paragraphs [0145], [0147], [0148]
US2006/172944 A1 (REGENERON), see abstract
WO2007/149334 A2 (REGENERON), see abstract
WO2007/084765 A1 (POTENTIA), see Figure 9, paragraph [0218]
WO2007/035621 A1 (OSI EYETECH), see abstract and paragraph [0106]
WO2006/128564 A1 (BAXTER), see abstract
WO2006/047325 A1 (GENENTECH), see abstract and paragraph [0130]
CN201578690 U (JIANYOU), see abstract

6. As discussed above your claims define a collocation of well known features which in combination do not introduce an inventive step. All the above documents show some of the features of claim 1 at least.

7. The FOSTER document shows a typical ophthalmic syringe which can have a typical syringe volume of 1ml. The OCUJECT document a syringe for injection of Lucentis ^{RTM} (ranibizumab), or Avastin ^{RTM} (bevacizumab), the syringe is additionally silicon oil free. The two REGENERON documents show that ophthalmic solution using VEGF antagonist are well known. The POTENTIA shows the use of such an ophthalmic solution such as Avastin ^{RTM} with a small volume syringe. The OSI EYETECH shows an ophthalmic syringe for use with VEGF antagonist. The BAXTER and JIANYOU documents show that syringes which are produced without silicon oil are well known. The GENENTECH disclose the injection of 0.05 mL ranibizumab solution into the eye.

Clarity

8. Your statement of invention on page 1 lines 28-35 is not consistent with claim 1.

9. The description on page 2, line 21 states that 'the body may be made from a plastic material' and this is inconsistent with claim which defines the syringe comprising a glass body.

Further search

10. On amendment a further search form and fee may be requested.

INTELLECTUAL PROPERTY OFFICE

Application No:	GB1301368.5	**Examiner:**	Dr Matthew Parker
Claims searched:	1-32	**Date of search:**	21 March 2013

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
Y	1-32	US2013/012918 A1 (FOSTER), see paragraph [0050]
Y	1-32	US2012/078224 A1 (OCUJECT), see paragraphs [0145], [0147], [0148]
Y	1-32	US2006/172944 A1 (REGENERON), see abstract
Y	1-32	WO2007/149334 A2 (REGENERON), see abstract
Y	1-32	WO2007/084765 A1 (POTENTIA), see Figure 9, paragraph [0218]
Y	1-32	WO2007/035621 A1 (OSI EYETECH), see abstract and paragraph [0106]
Y	1-32	WO2006/128564 A1 (BAXTER), see abstract
Y	1-32	WO2006/047325 A1 (GENENTECH), see abstract and paragraph [0130]
Y	1-32	CN201578690 U (JIANYOU), see abstract

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[X] :

Worldwide search of patent documents classified in the following areas of the IPC

A61K; A61M

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI

International Classification:

Subclass	Subgroup	Valid From
A61M	0005/31	01/01/2006
A61M	0005/178	01/01/2006

INTELLECTUAL PROPERTY OFFICE

Novartis AG
c/o Carpmaels & Ransford LLP
One Southampton Row
London
WC1B 5HA

Patents Directorate

Concept House
Cardiff Road, Newport
South Wales, NP10 8QQ

Direct Line: 01633 814706
E-Mail: matthew.parker@ipo.gov.uk
Switchboard: 0300 300 2000
Fax: 01633 817777
Minicom: 0300 0200 015

Your Reference: P061025GB:CJM/JPG
Application No: GB1301368.5

28 June 2013

Dear Sirs

Patents Act 1977: Examination Report under Section 18(3)

Latest date for reply: **3 July 2014**

I have re-examined your application in response to your agent's letter of 3 May 2013 and enclose two copies of my further examination report.

By the above date you should either file amendments to meet the objections in the enclosed report or make observations on them. If you do not, the application may be refused.

Online e-filing

You may file such amendments or observations electronically if you wish, using the online patent filing services detailed in www.ipo.gov.uk/p-apply-online.

Yours faithfully

Dr Matthew Parker
Examiner

INTELLECTUAL PROPERTY OFFICE

Your ref :	P061025GB:CJM/JPG	**Examiner :**	Dr Matthew Parker
Application No:	GB1301368.5	**Tel :**	01633 814706
Applicant :	Novartis AG	**Date of report :**	28 June 2013
Latest date for reply:	3 July 2014	Page 1/1	

Patents Act 1977
Examination Report under Section 18(3)

Clarity

1. Your claims are now allowable. You are respectfully reminded that your description should be brought in line with your claims. You should particularly note the objections in paragraphs 8 and 9 in the examination report of 22dn March 2013.

INTELLECTUAL PROPERTY OFFICE

Your ref :	P061025GB:CJM/JPG	**Examiner :**	Dr Matthew Parker
Application No:	GB1301368.5	**Tel :**	01633 814706
Applicant :	Novartis AG	**Date of report :**	28 June 2013
Latest date for reply:	3 July 2014	Page 1/1	

Patents Act 1977
Examination Report under Section 18(3)

Clarity

1. Your claims are now allowable. You are respectfully reminded that your description should be brought in line with your claims. You should particularly note the objections in paragraphs 8 and 9 in the examination report of 22dn March 2013.

CARPMAELS & RANSFORD

Chartered Patent Attorneys
European Patent Attorneys
Trade Mark Attorneys
Regulated by IPReg

Carpmaels & Ransford
One Southampton Row
London WC1B 5HA
United Kingdom
T +44 20 7242 8692
F +44 20 7405 4166
E email@carpmaels.com
www.carpmaels.com

UK Intellectual Property Office
Concept House
Cardiff Rd
Newport
NP10 8QQ

Electronically submitted

Your Ref GB1301368.5
Our Ref P061025GB:CJM/JPG
Date 3rd May 2013

Dear Sirs,

Re: United Kingdom Patent Application No. 1301368.5
NOVARTIS AG

I am responding to the combined search and examination report dated 22nd March 2013.

Introduction

The present invention relates to a pre-filled syringe with a reduced silicone content containing an ophthalmic solution which comprises a VEGF antagonist for intravitreal administration.

With "standard" siliconised syringes known in the art, small amounts of silicone can detach from the syringe barrel during storage and use, and this material is injected into the eye with each administration. Over time, droplets of silicone in the eye can build up and aggregate, causing "floaters" in the vision. In addition, silicone oil can cause aggregation of proteinaceous products such as the VEGF antagonists referred to in the claims. By reducing the silicone levels as much as possible, the amount of silicone that detaches from the syringe barrel wall is minimised.

Silicone-free syringes are known in the art; however, such syringes are made of plastic. Silicone-free plastic syringes cannot be used as pre-filled syringes for a number of reasons.

Typically health authorities require that the outer surface of pre-filled syringes is sterile. Although they may be filled under aseptic conditions, only the contents would therefore be sterile. Thus, they are typically packaged in blister packs and then subjected to sterilisation. The sterilising process has to be aggressive enough to enter the blister pack to sterilise the outer surface of the syringe, and the syringe sealing has to be tight enough to prevent ingress of the sterilising agent into the syringe.

The seal provided by plastic silicone-free syringes is not tight enough, meaning that terminal sterilisation of the syringes cannot be carried out without the sterilising agent entering the syringe. Biologic products such as the VEGF antagonists referred to in the claims are particularly sensitive to

such sterilising agents, which can oxidise/denature the proteins.

Nor is it possible to use silicone-free glass-barrelled syringes. In glass-barrelled syringes, rubber stoppers are used to ensure suitable tightness of the seal. Therefore terminal sterilisation is possible without the sterilising agent entering the syringe. However, there is a high friction level between the rubber and glass meaning that the break-loose and slide forces for moving the stopper would be so high as to render the syringe unusable in the absence of silicone.

The inventors show for the first time that silicone levels can be reduced far below prior art levels while still obtaining a usable syringe (see page 6, line 5 to page 7, line 16). This syringe can be terminally sterilised after filling without damaging the VEGF antagonist contained in the barrel of the syringe.

Amendments

An amended set of claims 1-37 is submitted herewith, which shall replace the claims currently on file.

Claim 1 has been amended by specifying that the glass body forms a barrel in order to provide proper antecedent basis for the term "barrel" in feature (c). In addition, it has been specified that the syringe is for intravitreal injection. Basis for this amendment is found in the description as originally filed at page 2, lines 1 and 2.

Claim 1 has further been amended by deleting features that relate to the arrangement and functionality of the body, the stopper and the plunger as far as these are typical for conventional syringes.

The above amendments do not constitute an abandonment of subject-matter, and the applicant reserves the right to reintroduce any deleted subject-matter into the present application and/or any divisional applications.

Collocation and Clarity

The Examiner has alleged that claim 1 is merely a combination of well-known features and that it is unclear which part of the claim relates to the inventive concept. In particular, the Examiner has alleged that both low-volume syringes and low-silicone syringes were known in the art. The applicant disagrees with the Examiner's assessment since none of the references cited in the combined search and examination report appear to disclose a low-volume, low-silicone pre-filled syringe with a glass body (see discussion of the individual references under inventive step).

The Examiner further requested that the claim be re-drafted such that features that do not relate to the inventive concept are omitted. More specifically, the Examiner suggested omitting either details relating to the construction of the syringes or details regarding the VEGF antagonist solution. Without prejudice, claim 1 has been amended by deleting any features describing the syringe that appear not to be relevant for the inventive concept.

However, as highlighted in the introductory remarks, the problems associated with siliconised syringes are of particular concern with the VEGF antagonists referred to in the claims and the description. As already discussed, droplets of silicone from the syringe can form aggregates in the eye, causing "floaters" in the vision.

With intravenous or intramuscular injections, which are used in administering other protein-based products such as antibodies and vaccines, this is less of a problem as the silicone droplets are less likely to localise and aggregate. In addition, the volumes in which the VEGF antagonists are administered are by an order of magnitude smaller than those used for *e.g.* the administration of vaccine, which may also be provided in pre-filled syringes. The exact administration of small volumes is technically more challenging than the administration of larger volumes which allow more room for error.

The problem underlying the invention directly relates to the administration of VEGF antagonists to the eye by means of a pre-filled syringe. Hence a relationship between syringe construction and details of the VEGF antagonist solution exists that is properly reflected in the claims.

In order to further clarify wherein the invention lies, claim 1 has been amended to specify that the syringe is for intravitreal injection.

The Examiner has objected to the passage at page 1, lines 28-35, and page 2, line 21, as being inconsistent with the claims. For reason of procedural economy, it is requested that amendments to the description are postponed until the Examiner has agreed to an allowable wording of the claims.

Inventive Step

The Examiner has objected to claim 1 for lack of inventive step. In particular, the Examiner has alleged that the invention merely relates to a collocation of well-known features. Therefore the Examiner has cited several documents that disclose at least some of the features of claim 1.

US2013/012918 relates to a disposable hypodermic syringe for stable, one-handed dispensing of fluids and precision positioning (see abstract). A pre-filled syringe, much less a pre-filled syringe with a glass body, wherein the syringe barrel comprises less than about 500µg silicone oil, is not disclosed anywhere in US2013/012918. The only statement that appears to be remotely relate to the present application is found in paragraph [0050], which states that typical syringe volumes used in ophthalmic surgery may include 1 ml, 3 ml, or 5 ml. Thus, US2013/012918 does not appear to be of any relevance with regard to the present invention.

WO2006/047325 relates to a dosing regimen administering a therapeutically effective amount of VEGF antagonist to a mammal suffering from, or at risk for, an intraocular neovascular disorder (abstract). This application further discloses the use of a 30 gauge, 1/2-inch needle attached to a low volume (*e.g.* tuberculin) syringe for administering 50 µl of the study drug solution (see page 32, lines 10 and 11). No further details regarding the syringe are disclosed. In particular, no pre-filled syringe with a glass body having a barrel comprising less than about 500µg silicone oil is disclosed. Therefore WO2006/047325 does not appear to be relevant for considering the inventive concept of the present invention.

US2006/172944 relates to methods of reducing or treating angiogenesis and/or inflammation associated with eye injury in a subject in need thereof, comprising administering an agent capable of blocking or inhibiting VEGF (abstract). There is no mention of administration of the disclosed VEGF antagonist by means of a syringe, much less by a pre-filled syringe with low-silicone content. Therefore US2006/172944 also does not appear to be of any relevance with regard to the present invention.

WO2007/149334 is directed to pharmaceutical formulations suitable for intravitreal administration comprising agents capable of inhibiting VEGF (abstract). Ophthalmic formulations can be provided in a pre-filled syringe suitable for intravitreal administration (see page 5, paragraph [0036]). A pre-filled glass syringe containing a 40 mg/ml VEGF Trap liquid formulation is also disclosed (see *e.g.* example 4). No disclosure regarding the silicone content of the syringe is made. Therefore WO2007/149334 also does not appear to be relevant with respect to the present invention.

WO2007/084765 provides composition, methods, and articles of manufacture for treating an eye disorder such as macular degeneration, choroidal neovascularization, or retinal neovascularization (see abstract). This application further discloses a syringe containing a first therapeutic agent dissolved in a liquid medium located in the syringe and a needle that contains an ocular implant with a sustained-release formulation of a second therapeutic agent (see *e.g.* paragraph [0010]). No disclosure regarding the silicone content in a glass syringe of the volume specified in claim 1 is found in WO2007/084765. Therefore this application is also of no relevance with regard to the present invention.

CN201578690 relates to a clinical syringe (*i.e.* not a pre-filled syringe) that does not need to be coated with silicone oil (*i.e.* is silicone-free). Therefore this application also does not appear to be relevant to the present invention.

WO2007/035621 relates to an ophthalmic syringe for performing intravitreous injections (see page 1, lines 8 and 9). The application discloses that particulate contaminants present in a drug, in a syringe, or in or on materials used at the time of injection may have the potential to induce detrimental effects when injected into the vitreous (page 2, lines 29-31). Furthermore, a syringe with a 1 ml glass barrel sealed with a rubber stopper is disclosed (page 9, lines 13-15). A pre-filled syringe is also contemplated (page 9, lines 16 and 17). While a silicone coating is considered for the needle (see *e.g.* page 7, lines 20-26), silicone coating of the syringe barrel is not addressed anywhere in the application. Therefore WO2007/035621 appears to be of no relevance with regard to the present invention.

US2012/078224 discloses a device for delivering pharmaceutical formulations into the eye. The application specifically discloses that the drug reservoir of the device *"is silicone oil-free (lacks silicone oil or one of its derivatives) and is not internally covered or lubricated with silicone oil, [...] which ensures that silicone oil does not get inside the eye causing floaters or intraocular pressure elevation"* (see paragraph [0145]). In this respect, the application appears to address a similar problem as the present invention. However, the solution provided to this problem is different from the present invention in as far as US2012/078224 teaches the avoidance of silicone rather than the reduction of the silicone content in the syringe barrel to less than about 500µg and consequently proposes that the reservoir is made of a material that contains a cyclic olefin series resin (see *e.g.* paragraph [0146]). The use of a plastic resin in place of glass as the reservoir material of choice makes sense because, as stated above, glass would be incompatible with a silicone-free reservoir: it would be impossible to move the plunger to expel the drug from the reservoir (cf. paragraph [0110] of US2012/078224).

WO2006/128584 relates to a syringe composed of a polypropylene hollow barrel and a sliding coated rubber piston (cf. title). This application states that one disadvantage of the use of silicone-coated pistons in commercial syringes is that that the silicone oils contaminate the content of the syringe body (see page 1, paragraph [002], last sentence). Therefore this application relates to the

same problem that is addressed by the present invention. WO2006/128564 further discloses that, in order to avoid the above mentioned disadvantage, laminated pistons were developed which are silicone-free (page 1, paragraph [003]). The application is based on the discovery that conventional hollow-barrel bodies made of polypropylene can be combined with certain pistons with certain coatings (page 1, paragraph [006]). Thus, while WO2006/128564 addresses the same problem as the present application, a completely different solution is provided.

Hence, while US2012/078224 and WO2006/128564 both appear to address the problem underlying the invention, neither of the two applications provides any pointers that would point the skilled person the solution provided in the instant application. In particular, neither of the two applications addresses the specific problem associated with pre-filled syringes containing one of the VEGF antagonists referred to in the claims. As discussed in the introduction, silicone-free syringes are not suitable for use with these VEGF antagonists because they typically are made of materials other than glass and therefore do not provide a tight enough seal to prevent sterilising agents from entering the syringe barrel and oxidising or denaturing the VEGF antagonist. The use of a glass body is therefore essential to provide a tight enough seal to prevent the sterilising agent from coming into contact with the VEGF antagonist contained in the syringe. However, the use of a glass body requires the use of silicone as a lubricant to reduce the friction between the barrel and the plunger.

The inventors showed for the first time that the amount of silicone used to coat the barrel of the syringe can be reduced dramatically without affecting the functionality of the syringe (cf. page 13, lines 15 and 16 the description). This was unexpected. By reducing the amount of silicone, the likelihood of introducing silicone into the eye in an amount that could lead to the formation of floaters is also dramatically reduced. In addition, the amount of silicone is low enough to avoid aggregate formation of the VEGF antagonist during storage of the syringe.

It follows from the above that the claimed subject-matter has an inventive step in view of the cited prior art.

Requests

The Examiner is encouraged to contact the applicant's agent, should further clarifications or amendments be required. In particular, a description which has been amended to match the claims will be submitted, should the Examiner agree that the amended set of claims appears allowable.

An interview with the Examiner is requested, if this is deemed to be expedient for accelerating examination of the present application.

Yours truly,

// ELECTRONICALLY SIGNED AND SUBMITTED //

Carpmaels & Ransford

Encl. Amended set of claims 1-37 (clean and marked-up versions)

CLAIMS

1. A pre-filled syringe for intravitreal injection, ~~the syringe~~ comprising a glass body forming a barrel, 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~~ and containing 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 or claim 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 is filled with dosage volume of about 0.05ml of a VEGF antagonist solution.

5. 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.

6. 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, 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.

7. 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, preferably less than about 10µg silicone oil.

8. A pre-filled syringe according to any previous claim, 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 any previous claim, 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 any previous claim, wherein the silicone oil is DC365 emulsion.

11. A pre-filled syringe according to any one of claims 1-5, wherein the syringe is silicone oil free.

12. 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.

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 claim 15, wherein the ranibizumab is at a concentration of 10mg/ml.

17. A pre-filled syringe according to any one of claims 1-13 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.

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 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.

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 pre-filled syringe according to any of claims 20-23, 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 any previous claim, 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 ≤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 ≤0.1mg.

28. A blister pack comprising a pre-filled syringe according to any one of claims 25-27, wherein ≤5% of the VEGF antagonist is alkylated.

29. A blister pack comprising a pre-filled syringe according to any of claims 25-28, wherein the syringe has been sterilised using EtO or H_2O_2 with a Sterility Assurance Level of at least 10^{-6}.

30. A blister pack according to any of claims 25-29, 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.

31. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-24, or a blister pack comprising a pre-filled syringe according to any one of claims 25-30, (ii) a needle, and optionally (iii) instructions for administration.

32. A kit according to claim 31, wherein the needle is a 30-gauge x ½ inch needle.

33. A pre-filled syringe according to any one of claims 1-24 for use in therapy.

34. A pre-filled syringe according to any one of claims 1-24 for use in a method of treating a patient suffering from 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.

35. The pre-filled syringe for the use according to claim 34, wherein the method comprises the step of administering an ophthalmic solution to the patient using the pre-filled syringe.

36. The pre-filled syringe for the use according to claim 35, wherein the method 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.

37. The pre-filled syringe for the use according to claim 35 or 36, wherein the VEGF antagonist administered is a non-antibody VEGF antagonist and wherein the patient has previously received treatment with an antibody VEGF antagonist.

CLAIMS

1. A pre-filled syringe for intravitreal injection comprising a glass body forming a barrel, a stopper and a plunger and containing 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 or claim 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 is filled with dosage volume of about 0.05ml of a VEGF antagonist solution.

5. 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.

6. 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, 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.

7. 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, preferably less than about 10µg silicone oil.

8. A pre-filled syringe according to any previous claim, 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 any previous claim, 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 any previous claim, wherein the silicone oil is DC365 emulsion.

11. A pre-filled syringe according to any one of claims 1-5, wherein the syringe is silicone oil free.

12. 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.

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 claim 15, wherein the ranibizumab is at a concentration of 10mg/ml.

17. A pre-filled syringe according to any one of claims 1-13 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.

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 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.

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 pre-filled syringe according to any of claims 20-23, 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 any previous claim, 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 any one of claims 25-27, wherein $\leq$5% of the VEGF antagonist is alkylated.

29. A blister pack comprising a pre-filled syringe according to any of claims 25-28, wherein the syringe has been sterilised using EtO or H_2O_2 with a Sterility Assurance Level of at least 10^{-6}.

30. A blister pack according to any of claims 25-29, 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.

31. A kit comprising: (i) a pre-filled syringe according to any one of claims 1-24, or a blister pack comprising a pre-filled syringe according to any one of claims 25-30, (ii) a needle, and optionally (iii) instructions for administration.

32. A kit according to claim 31, wherein the needle is a 30-gauge x ½ inch needle.

33. A pre-filled syringe according to any one of claims 1-24 for use in therapy.

34. A pre-filled syringe according to any one of claims 1-24 for use in a method of treating a patient suffering from 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.

35. The pre-filled syringe for the use according to claim 34, wherein the method comprises the step of administering an ophthalmic solution to the patient using the pre-filled syringe.

36. The pre-filled syringe for the use according to claim 35, wherein the method 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.

37. The pre-filled syringe for the use according to claim 35 or 36, wherein the VEGF antagonist administered is a non-antibody VEGF antagonist and wherein the patient has previously received treatment with an antibody VEGF antagonist.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13750352
	Filing Date	2013-01-25
	First Named Inventor	Juergen Sigg
	Art Unit	3763
	Examiner Name	N. D. Shah
	Attorney Docket Number	PAT055157-US-NP

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					
If you wish to add additional U.S. Patent citation information please click the Add button.						

U.S.PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					
If you wish to add additional U.S. Published Application citation information please click the Add button.						

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2002241264	JP	A2	2002-08-28	HITOMI KOJI	Abstract	☐
	2	0264273	EP	A2	1988-04-20	OKUDA TAMOTSU	eq SHO63-97173	☐
	3	0879611	EP	A2	1998-11-25	SUDO MASAMICHI	eq HEI10-314305	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13750352
	Filing Date	2013-01-25
	First Named Inventor	Juergen Sigg
	Art Unit	3763
	Examiner Name	N. D. Shah
	Attorney Docket Number	PAT055157-US-NP

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	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, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1		☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13750352
	Filing Date	2013-01-25
	First Named Inventor	Juergen Sigg
	Art Unit	3763
	Examiner Name	N. D. Shah
	Attorney Docket Number	PAT055157-US-NP

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature		Date (YYYY-MM-DD)	2013-10-28
Name/Print	Jim Lynch	Registration Number	54,363

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 1 hour 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, U.S. Department of Commerce, 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.**

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

(11) Publication number: **0 264 273**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **87309088.0**

(22) Date of filing: **14.10.87**

(51) Int. Cl.⁴: **A 61 M 5/315**

(30) Priority: **15.10.86 JP 243122/86**

(43) Date of publication of application:
20.04.88 Bulletin 88/16

(84) Designated Contracting States: **DE FR GB IT**

(71) Applicant: **DAIKYO GOMU SEIKO LTD.**
No. 38-2, Sumida 3-chome Sumida-ku
Tokyo (JP)

(72) Inventor: **Okuda, Tamotsu**
c/o Daikyo Gomu Seiko Ltd. 38-2 Sumida 3-chome
Abiko-shi Chiba-ken (JP)

Muraki, Tomoyasu
1-4 Aoyamadai 1-chome
Abiko-shi Chiba-ken (JP)

(74) Representative: **Horton, Andrew Robert Grant et al**
BOULT, WADE & TENNANT 27 Furnival Street
London, EC4A 1PQ (GB)

(54) **A laminated sliding stopper for a syringe.**

(57) A hypodermic syringe includes a barrel (1) containing liquid medicament (8) and a stopper (3) which has a surface in contact with the medicament and is slidably moved within the barrel when the syringe is operated. The stopper (3) comprises a rubber body (5) which is completely covered on the surfaces which contact the liquid in the barrel and slidably contact the barrel respectively with a coating (7) of tetrafluoroethylene resin.

EP 0 264 273 A2

Bundesdruckerei Berlin

Description

"A LAMINATED SLIDING STOPPER FOR A SYRINGE"

BACKGROUND OF THE INVENTION

1. Field of the invention.

This invention relates to a sliding or movable stopper or a piston of a syringe used for the hypodermic injection of liquid medicaments.

2. Description of the Prior Art.

A syringe generally comprises a main barrel shaped body and a plunger which may be a rod carrying or adapted to engage a stopper which is slidingly pushed into the main barrel. Alternatively the plunger may itself be a barrel which has a stopper adapted for engagement by a spigot within the main barrel, and the stopper being thereby pierced and being slidingly pushed back into the barrel shaped plunger when the syringe is operated. The main barrel and plunger were also formerly made of glass but other materials such as polyethylene, polypropylene and polycarbonates have been used. The feature of a stopper which slides within a barrel (whether formed in the main body or the plunger) containing the medicament is well-known, and is in common use where the barrel serves as a storage container for the medicament.

For the purpose of preventing deterioration of medicament due to a slidable stopper, it is known from Japanese Utility Model Publication No. 19435/1977 to provide a slidable stopper with a part, in contact with a liquid medicament, coated with a thin film of a fluoro-resin. A sliding stopper consisting of a rubber laminated with polyethylene (hereinafter referred to as PE) or polypropylene (hereinafter referred to as PP) is known from Japanese Utility Model Publication No. 32602/1980. A slidable stopper partly laminated with a fluoro-resin film is known from DE-3346351-Al. In any case, these proposals are directed to improvement of the chemical resistance of the stopper of a rubber elastic body and do not take the slidability of the stopper into consideration.

Therefore, the slidability of a rubber-like elastic body stopper is maintained by coating the inner wall of a barrel of a syringe and the stopper with a suitable quantity of silicone oil. Syringes free from silicone-based oils and intended for medicaments have not been commercially available.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an improved stopper for a syringe used for the injection of a liquid medicament.

It is a further object of the present invention to provide a sliding stopper whose position in a barrel can readily be visually confirmed.

A preferred form of the invention comprises a sliding stopper for a syringe, consisting of a rubber elastic body whose part to be contacted with a liquid medicament and sliding part on the inner wall of the barrel are fully laminated with a film of tetrafluoroethylene resin.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a cross-sectional view of one embodiment of the laminated rubber stopper of the present invention when applied to a syringe of disposable type.

Fig. 2 is a cross-sectional view of another embodiment of the laminated rubber stopper of the present invention when applied to a syringe additionally serving as a container.

Fig. 3 is a cross-sectional view of a further embodiment of the laminated rubber stopper of the present invention when applied to a syringe additionally serving as a container for a liquid medicament.

Fig. 4 and Fig. 5 are cross-sectional views of preferred embodiments of the sliding rubber stopper of the present invention.

Fig. 6 is a cross-sectional view of one example of a sliding stopper of the prior art, whose only part to be contacted with a liquid medicament is laminated with a fluoro-resin.

DETAILED DESCRIPTION OF THE INVENTION

Of late, bad influences upon a human body due to foreign matters of fine particles in an injection medicament have been considered as a serious question in the field of medicines or medicaments and in USA and other countries, the legislations have been made for the purpose of regulation of the influences (see USP XX[1], British Standards Institution 3236). Since the foreign matters or fine particles are mainly caused by silicone oils used as a lubricant for syringes, it has eagerly been desired to develop a syringe and medical instrument without using such a silicone oil.

In order to answer the above described question, the inventors have proposed sliding stoppers for syringes or syringes serving additionally as containers for liquid medicaments, laminated with tetrafluoroethylene-ethylene copolymer resins films (Japanese Patent Application Nos. 281083/1985 and 293070/1985). The present invention has been made to further improve these earlier inventions.

Accordingly, the present invention provides a sliding stopper or piston for a syringe, consisting of a rubber

elastic body whose part to be contacted with a liquid medicament and sliding part on the inner wall of the barrel are completely laminated with a film of tetrafluoroethylene resin. In this specification, "stopper" is hereinafter used including the meaning of "piston".

In the preferred embodiments of the present inven tion, the film of tetrafluoroethylene resin (hereinafter referred to as TFE) has a thickness of 0.010 to 0.2 mm, the syringe additionally serves as a container for a medicament and the TEF film is colored, for example, in black.

As shown in Fig. 1, the feature of the present invention consists in a sliding stopper 3 consisting of a rubber base material 5 and a laminated part 7 with a TFE film on the surface thereof, in which the TFE laminated part 7 is provided on not only a part 11 to be contacted with a liquid medicament 8 but also a sliding part 10 on the inner wall of a barrel 1 of a syringe, whereby the coating of a silicone oil, as commonly effected in the prior art, is not necessary and deterioration of a medicament can be prevented.

When a colored TFE film is laminated in the sliding rubber stopper of the present invention, the position of the end of the sliding rubber stopper in a syringe can readily be seen with the naked eye and a material with a lower percent photo-transmission can favourably be used for the barrel of the syringe.

Up to the present time, liquid lubricants such as silicone oils have frequently been used to improve the sliding property of a sliding stopper of a syringe, but the liquid lubricant tends to contaminate a liquid medicament used in the syringe and accordingly, it is required to apply to the surface of a rubber base material a solid and lubricating material in such a manner that it is hardly stripped therefrom.

Among known materials capable of satisfying the above described requirement, fluoro-resins have the lowest friction resistance, namely, a friction coefficient of 0.02 to 0.07, whereas PP and PE each has a friction coefficient of 1 to 4. Above all, TFE has the lowest friction coefficient, namely 0.02 to 0.03.

Thus, a TFE film is chosed for the laminating material of a sliding stopper of a syringe, which needs a high sliding property, and it is found that the sliding stopper consisting of a rubber body the surface of which is laminated with a TFE film can give best results and exhibit an excellent initial sliding value without using any silicone oil.

Furthermore, it is found that when the sliding property of the sliding stopper of the present invention is examined by applying it to injection barrels or cylinders of various materials such as glass, PP, PE and polycarbonate, there is also such a problem that it is difficult to visualy confirm the position of the end of the sliding stopper in the barrel of the material with a lower transparency such as plastics, although there is no such problem in the case of using a transparent material such as glass as the material of the barrel. PP and PE each has a lower transparency, namely a percent photo-transmission of 50 to 90 % and 10 to 80 % respectively. This is due to that since TFE is intrinsically milk white, the laminated part becomes milk white and there is not contrast difference between the laminated part and barrel.

The inventors have succeeded in solving this problem by laminating a sliding stopper with a colored TFE film to give a contrast difference between the TFE film and barrel. As a coloring agent for coloring TFE, there can be used any material which is capable of giving a strong contrast by its color without having any injurious action on a human body and without elution in liquid medicaments, for example, ultramarine blue, carbon black, red oxide and the like. On the other hand, the use of a colored rubber material laminated with a milk white TFE film is not preferable because the black of rubber is not clear and a color unevenness appear on the shaped article.

The base of the TFE laminated sliding stopper according to the present invention is made of a rubber or elastomeric material selected from synthetic rubbers or natural rubbers, for example, isoprene, butadiene, styrene-butadiene, ethylene-propylene, isopreneisobutylene and nitrile rubbers and the like. These rubbers are generally blended with fillers and bridging agents in conventional manner.

Lamination of the TFE film according to present invention is generally carried out by the method described in Japanese Patent Publication No. 53184/1982, incorporated herein by reference, which the inventors have proposed. The thickness of the laminated TFE film is generally in the range of 0.010 to 0.2 mm, preferably 0.010 to 0.1 mm, since if it exceeds 0.2 mm, the laminating operation is easy, but the sealing property is lowered because of the increased hardness.

Embodiments of the sliding stopper for a syringe according to the present invention will now be illustrated with reference to the accompanying drawings:

Fig. 1 is a cross-sectional view of one embodiment of the present invention, in which a sliding stopper 3 of the present invention consisting of a rubber base material 5 laminated with a TFE film 7 as a surface 11 to be contacted with a liquid medicament 8 and a sliding surface 10 on the inner wall of a barrel 1 is used in a syringe of disposal type, comprising a barrel 1, plunger rod 2, needle 4 and support recess 6.

Fig. 2 is a cross-sectional view of another embodiment of the present invention, in which a syringe additionally serving as a container 1 is filled with a liquid medicament 8 and sealed by a sliding stopper 3 of the present invention, which are just ready to be engaged with an auxiliary means 9 of the syringe. When using this syringe, the auxiliary means 9 is screwed in a threaded part 6′ of the sliding stopper 3 to allow a duplex head needle 4′ to penetrate into the sliding stopper and further thrust to move the stopper 3 to the right in Fig. 2, whereby the corresponding quantity of the liquid medicament 8 is flowed out into the duplex head needle 4′, reaching the needle 4.

Fig. 3 is a cross-sectional view of a further embodiment of the present invention, in which a syringe barrel 1 is filled with a liquid medicament 8 and sealed by a sliding stopper 3 of the present invention. When using this syringe, a plunger rod 2 is screwed in a threaded part 6′ of the sliding stopper 3.

Fig. 4 and Fig. 5 are cross-sectional views of sliding stoppers of the present invention, respectively used in the embodiments of Fig. 1 and Fig. 2, in each of which the rubber base material 5 is fully laminated with the TFE film 7 at the part 11 to be contacted with a liquid medicament and the sliding part 10 on the inner wall of the barrel 1.

In the sliding stopper 3 of the present invention as shown in Fig. 1 to Fig. 5, a colored TFE film can of course be used as the laminated part 7 depending on the material of the syringe barrel 1.

Fig. 6 is a cross-sectional view of a sliding stopper 3 of the prior art, in which a TFE film 7 is laminated on only a part 11 to be contacted with a liquid medicament 8 to retain the rubber surface exposed at a sliding part 10 on the inner wall of the barrel 1.

The sliding stopper of the present invention has the following advantages:

1) In all of commercially available syringes, silicone oils are used to improve the sliding property. According to the present invention, however, the use of the silicon oil is not required and contamination of a liquid medicament with foreign matters during injecting can be prevented to a greater extent.

2) Since the quantity of materials dissolved out of the sliding stopper is largely reduced, the sliding stopper can be applied to a syringe additionally serving as a container for a liquid medicament with such an advantage that the quality of the liquid medicament can be maintained for a long time as it is, when the liquid medicament was prepared.

3) Since knocking due to the coating unevenness of a silicone oil in a syringe can be prevented, it is made possible to dose precisely a medicament.

4) Deairing can favorably be carried out and the deairing operation time from aspiration of a liquid medicament in a syringe to dosing of the medicament can be shortened. Thus, there is little danger of misinjection of air in a human body.

5) Even if a barrel is of a semitransparent material, the movement and position of the sliding stopper can readily be confirmed by coloring the TFE film, in particular, in black.

The following examples are given in order to illustrate the present invention in greater detail without limiting the same.

Preliminary Test:

Comparison of the sliding resistances of TFE-, PE- and PP- laminated sliding stoppers

Rubber Composition

Polybutadiene Rubber (JSR BR 01 -commercial name- made by Japan Synthetic Rubber Co.)	80 parts
Polyisoprene Rubber (Nipol 2200 -commercial name- made by Nippon Zeon Co.)	20 parts
Wet Process Silica (Carplex 1120 -commercial name- made by Shionogi Seiyaku Co.)	15 parts
Calcined Clay (Burgess Iceberg -commercial name- made by Burgess Pigment Co.)	20 parts
Carbon Black (Asahi Carbon No. 35 -commercial name- made by Asahi Carbon Co.)	5 parts
Low Molecular Weight Polyethylene (HI-WAX No. 110 P -commercial name- made by Mitsui Sekiyu Kagaku Co.)	2 parts
α,α'-bis(t-butylperoxy-m-isopropyl)benzene (Perbutyl P -commercial name- Nippon Yushi Co.)	1.5 parts

Material of Laminating Film

TFE Film: Neoflon TFE® - commercial name- made by Daikin Kogyo Co., 75 um thickness, one side etched by sputtering

Polyethylene Film: Sholex® - commercial name- made by Showa Denko Co. 75 um thickness

Polypropylene Film: Mitsui Polypro ® - commercial name- made by Mitsui Sekiyu Kagaku CO., 75 um thickness

The rubber composition with the above described formulation was kneaded using two rolls for rubber according to the "Rubber Test Method" by The Society of Rubber Industry, Japan. The resulting nonvulcanized rubber was shaped into a sheet on which each of the above described laminating films was superimposed in

such a manner that the sputtered surface of the film was contacted with the rubber, placed on a lower metal mold having a recess corresponding to the shape of the sliding stopper shown in Fig. 4 in such a manner that the film surface was contacted with the mold and then molded by heating at a temperature of 165 to 185 °C and a pressure of 50 to 100 kg/cm^2 for 7 hours by the use of an upper metal mold having a projection corresponding to the recess 6 of the sliding stopper 3. The thus molded article was then subjected to punching to remove the burr formed round it and washed with warm water, thus obtaining a laminated sliding stopper. The whole surface of a part of the stopper to be contacted with a liquid medicament and another part sliding on the inner surface of a barrel was fully laminated with the film.

Sliding Test:

The resulting sliding stopper was inserted into a barrel of a syringe made of PP at the maximum scale mark (6 ml) and then subjected to measurement of a drawing resistance using Shimadzu Autograph DCS-100 (commercial name, made by Shimadzu Seisakujo Co.), thus attaining an initial value as a statical friction force and a sliding value as a kinetic friction force, as shown in Table 1:

Table 1

Kind of Laminating Film	Initial Value (g)	Sliding Value(g)
TFE	230 - 240	120 - 140
PE	12000 - 14000	11000 - 12000
PP	9000 - 10000	7000 - 8000

As is evident from these results, the TFE film laminated sliding stopper gives a very small initial value and sliding value in the absence of a silicone oil.

Example 1

Rubber Composition	
Polybutadiene Rubber (Nipol BR -commercial name-, made by Nippon Zeon Co.)	68 wt %
Polyisoprene Rubber (Nipol IR -commercial name-, made by Nippon Zeon Co.)	2.7 wt %
Reinforcing Agent	24.1 wt %
Organic Crosslinking	1.8 wt %

The procedures of the Preliminary Test were repeated except using the above described rubber composition to obtain a TFE laminated sliding stopper, as shown in Fig. 4, which was then subjected to various tests described below, thus obtaining results as shown in Table 2.

Example 2

Example 1 was repeated except using a black-colored TFE film as the TFE laminating film to obtain a TFE laminated sliding stopper with the same shape as that of Example 1. In this case, the coloring was carried out by mixing a finely powdered TFE with 5 % by weight of carbon black, sintering the mixture and then subjecting to a skiving treatment to obtain a colored TFE film. The resulting sliding stopper was then subjected to the

same tests, thus obtaining results shown in Table 2.

Comparative Examples 1

Example 1 was repeated except laminating the part to be calculated with a liquid medicament and only a part of the sliding part as shown in Fig. 6 with a TFE film (not colored) to obtain a sliding stopper for comparison, which was then subjected to the same tests, thus obtaining results shown in Table 2.

Comparative Example 2

Example 1 was repeated except laminating the whole surface of the part to be contacted with a liquid medicament and the sliding part with a PP film (not colored) to obtain a sliding stopper for comparison, which was then subjected to the same tests, thus obtaining results shown in Table 2.

Comparative Examples 3 to 6

Commercially available stoppers A, B, C and D, not laminated, were subjected to the same tests, thus obtaining results shown in Table 2.

In Table 2, "ND" means an amount of lower the limit which can be detected.

Test Methods

(1) Legal Tests I to IV

Tests I to III: Notification No. 442 of the Welfare Ministry, Standard for Syringe Barrel of Disposal Type
Test IV: Test Method of Rubber Stopper for Liquid Transfusion according to 11th Revision, Japanese Pharmacopoeia

Test I

Elution test with water (70 °C x 30 min.) to determine (i) the outward appearance, (ii) pH, and the amounts of (iii) heavy metals, (iv) potassium permanganate reducing materials and (v) evaporation residues

Test II

Elution test with a solvent such as trifluorotrichloroethane to determine the amount of silicone oil dissolved out (n = 20)
In each Example, 21, samples were subjected to this test to determine quantitatively silicone oil used in a syringe using trifluorotrichloroethane.

Test III

Physical tests including (i) pressure test, (ii) aspiration test and (iii) movement test

Test IV

Elution or Extraction test to determine (i) pH, (ii) percent transmission of visible rays, (iii) ultraviolet absorption spectrum and (iv) potassium permanganate reducing materials

(2) Independent Tests V and VI

Test V

Physical tests including (i) sliding test, (ii) knocking test and (iii) deairing test

(i) Sliding Test

When a syringe barrel to which an injection needle is not attached is fixed and a plunger rod is thrust to move a sliding stopper, the load (initial value and sliding value) is measured in an analogous manner to the Preliminary test.

(ii) Knocking Test (Fluctuation of Sliding Value)

"Good" means such a state that when a plunger rod is thrust in a syringe barrel, it is moved in smooth and continuous manner, while "knocking" means such a state that the plunger rod is intermittently moved. The knocking property is an important property to judge whether an injection medicament is precisely dosed as predetermined or not.

(iii) Deairing Test

After purified water is aspirated in a syringe to the maximum scale mark and then discharged by 1 ml by thrusting the plunger under such a state that the needle is directed upward, the presence or absence of bubbles is visually examined. Mark O shows bubble-free state and mark x shows a bubbled state.

Test VI

Test VI is a fine particle test effected by aspirating 5 ml of purified water by a syringe, discharging the water by thrusting and collecting, repeating this procedure three times to obtain a test liquid and after allowing to

stand for 30 minutes, subjecting 12 ml of the test liquid to measurement using an optical fine particle tester (RION). In each Example, 20 samples were used and subjected to this test and the results are shown in Table 2 as mean values per one sample.

The general assessment of the results is represented by "Very Good: ◎, "Normal" Δ and "Unsuitable" X.

Table 2

Test Items			Example 1	Example 2	Comparative Example 1	Comparative Example 2	Comparative Example 3	Comparative Example 4	Comparative Example 5	Comparative Example 6	Standard
		Sample	Present Invention	Present Invention (colored)	Comparison	Comparison	Commercially Available Article A	Commercially Available Article B	Commercially Available Article C	Commercially Available Article D	
		Laminating	TFE whole	Colored TFE whole	TFE Only End	PP whole	No	No	No	No	
Legal Test I to III	I	(i) Appearance	○	○	○	○	○	○	○	○	Colorless clear no foreign matters
		(ii) pH	+0.02	+0.02	+0.04	0.1	+0.30	+0.25	+0.30	+0.04	<2.0
		(iii) Heavy Metals	ND	ND	ND	ND	ND	ND	ND	ND	<2 ppm
		(iv) $KMnO_4$ Reducing Materials	0.05 ml	0.06 ml	0.08 ml	0.1 ml	0.23 ml	0.31 ml	0.25 ml	0.12 ml	<2 ml
		(v) Evaporation Residues	ND	ND	ND	ND	0.7 mg	2.2 mg	0.8 mg	0.8 mg	<1.0 mg
	II	Elution with Trifluorotrichloroethane (Silicone Oil)	ND	ND	ND	ND	$\bar{x}$=4985μg max=5380μg min=3730	$\bar{x}$=3823μg max=4800μg min=3430	$\bar{x}$=3903μg max=5100μg min=3180	$\bar{x}$=3212μg max=4320μg min=2900	5 mg
	III	(i) Pressure Test	suitable	suitable	suitable	unsuitable	suitable	suitable	suitable	suitable	
		(ii) Aspiration Test	-do-	-do-	-do-	-do-	-do-	-do-	-do-	-do-	
		(iii) Movement Test	-do-	-do-	unsuitable	-do-	-do-	-do-	-do-	-do-	

Table 2 (continued)

Legal Test IV	IV	(i) pH		-0,25	-0,28	-0,45	-0,35	+1,12	+2,05	+2,73	+0.59	
		(ii) Percent Transmission of Visible Rays										
		VR	430Nm	99,9%	99,9%	99,8%	99,8%	99,4%	88,0%	80,8%	99,8%	>99,0%
			650Nm	100,0%	100,0%	100,0%	99,9%	99,7%	93,1%	89,9%	100,0%	-do-
		(iii) UV Absorption Spectrum 220～350Nm		0,012	0,014	0,025	0,032	0,105	0,760	1,256	0,036	less than 0,2 absorbance
		(iv) KMnO4 Reducing Materials (OXD)		0,41ml	0,45ml	0,90 ml	0,85ml	1,80ml	26,10ml	31,20ml	1,91ml	<2,0ml
Independent Tests V and VI	V	Sliding Test Initial Value		230g	240g	10kg	9,5kg	780g	930g	850g	600g	
		Sliding Test Sliding Value		120g	140g		7,7kg	410g	390g	420g	330g	
		Knocking Property		good	good	knocking	knocking	some knocking	some knocking	some knocking	some knocking	
		Deairing Property		○	○	○	○	x	x	x	x	
	VI	Fine Particles	2 μ	183	230			6581	5195	5036	3510	
			5 μ	49	58			2200	1729	1669	1230	
			10 μ	5	15			430	382	402	165	
			20 μ	0	0			150	103	162	30	
			30 μ	0	0			25	15	48	4	
General Assessment				◎	◎	x	x	△	△	△	△	

0 264 273

Test Results

Test results shown in Table 2 are summarized below:

I Elution Test with Water

There is a great difference between the laminated samples of Examples 1 and 2, and Comparative Examples 1 and 2, and the non-laminated samples of Comparative Examples 3 to 6. As to the Evaporation Residues, in particular, Comparative Examples 3 to 6 give values near the standard or exceeding it (Comparative Example 4).

II Elution Test with Solvent

Silicone oil cannot be found in the laminated samples, but a considerable amount of silicone oil exceeding the standard value is determined in the non-laminated samples.

III Physical Test

All the samples except those of Comparative Examples 1 and 2 satisfy the standard. In Comparative Examples 1 and 2, silicone oil was not used and accordingly, the sliding property of the stopper was worse. In Examples 1 and 2 according to the present invention, on the other hand, silicone oil was not used, but good results were given.

IV Elution or Extraction Test

Since this elution test is carried out under severer conditions than the Elution Tests I and II, there is a clearer difference between the stoppers of Examples 1 and 2 according to the present invention and Comparative Examples.

V Physical Test (Independent Test)

The movement load test is a test assuming the condition that a syringe is really used. Examples 1 and 2 show more excellent results concerning the sliding property as compared with Comparative Examples. The stoppers of Comparative Examples 1 and 2 are not suitable for practical use, since the sliding property is worse unless silicone oil is used. In the knocking test, the stoppers of Examples 1 and 2 do not meet with knocking, but those of Comparative Examples meet with knocking, more or less. This is probably due to partial coating unevenness of silicone oil on the inner wall of a syringe barrel and the plunger part. In the dealring test, more bubbles are found in the case of using a large quantity of silicone oil.

VI Fine Particle Test

This test teaches that the stoppers of the present invention give much less fine particles of foreign matters and in particular, those with a particle size of about 2 μm can be decreased to 1/35 of the prior art according to the present invention. Furthermore, fine particle foreign matters with a particle size of at least 20 μm cannot be found in the present invention.

As a general estimation, the sliding stoppers of the present invention can favorably be compared with the comparative samples in all the Tests I to VI.

Claims

1. A sliding stopper for a syringe, consisting of a rubber elastic body (5) whose part to be contacted with a liquid medicament (8) and sliding part on the inner wall of a barrel (1) are fully laminated with a film (7) of tetrafluoroethylene.

2. A sliding stopper according to claim 1, wherein the film (7) of tetrafluoroethylene has a thickness of 0.01 to 0.2 mm.

3. A sliding stopper according to claim 1, wherein the film (7) of tetrafluoroethylene is colored.

4. A sliding stopper according to claim 3, wherein the coloring is imparted by a coloring agent selected from the group consisting of ultramarine blue, carbon black and red oxide.

5. A sliding stopper according to claim 1, wherein the rubber elastic body is made of a rubber or elastic material selected from the group consisting of isoprene, butadiene, styrene-butadiene, ethylene-propylene, isoprene-isobutylene and nitrile rubbers.

6. A syringe including a main body and a plunger of which either the main body or the plunger serves as a barrel (1) for containing liquid medicament and is closed by an elastic stopper (3) which in use of the syringe moves slidingly in the barrel as liquid is forced out of the barrel, characterised in that those surfaces of the stopper which are in contact with either the medicament or the barrel are entirely coated with a film (7) of tetrafluoroethylene resin.

7. A syringe according to claim 6 in which the film (7) is distinctively colored.

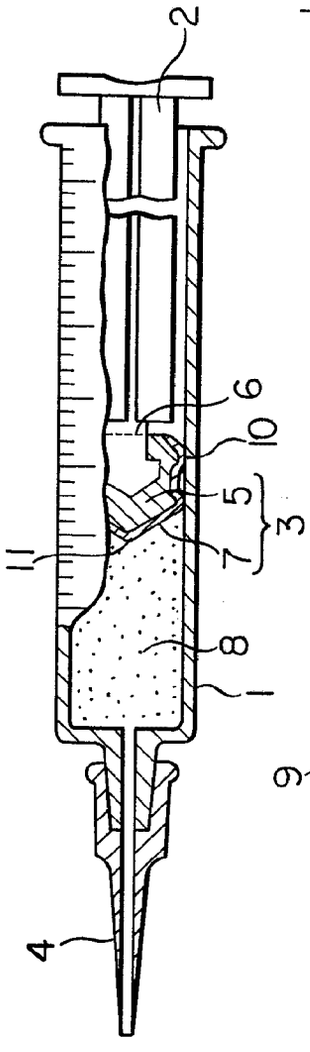


FIG. 1

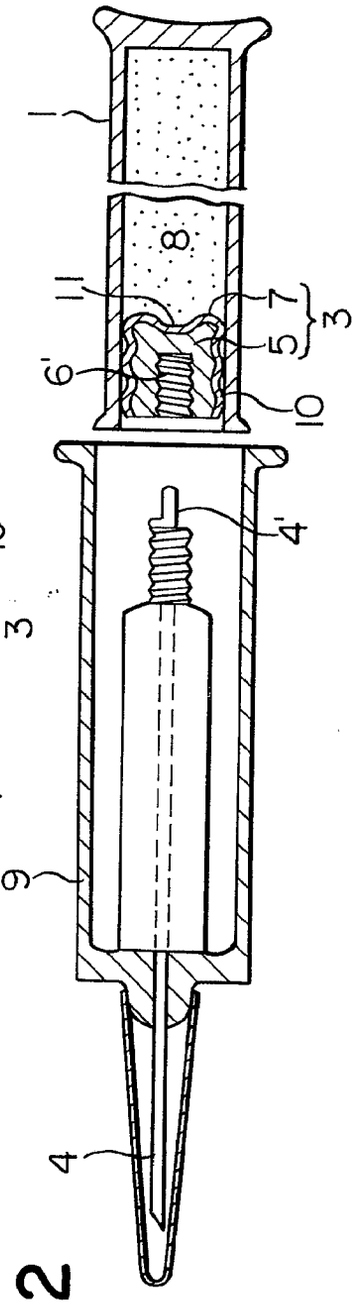


FIG. 2

FIG. 3

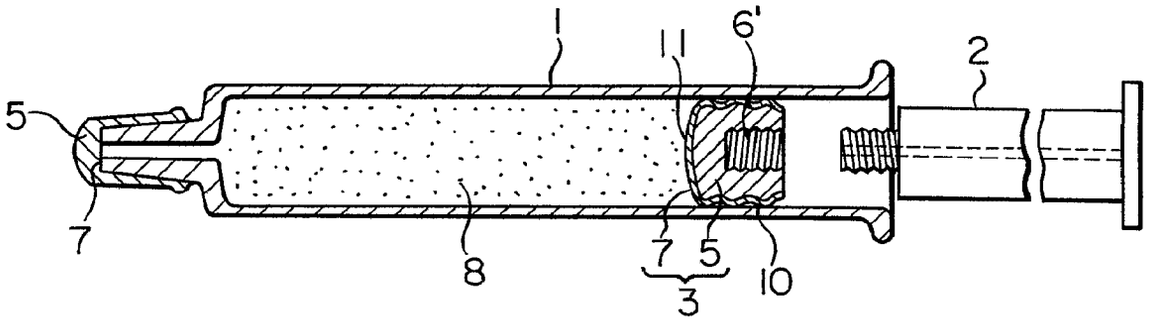


FIG. 4

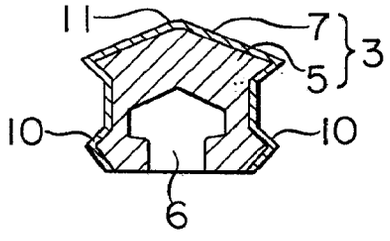


FIG. 5

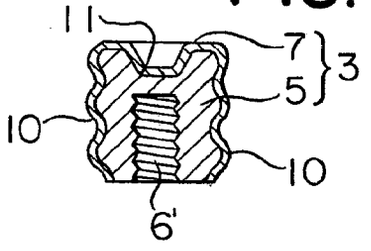
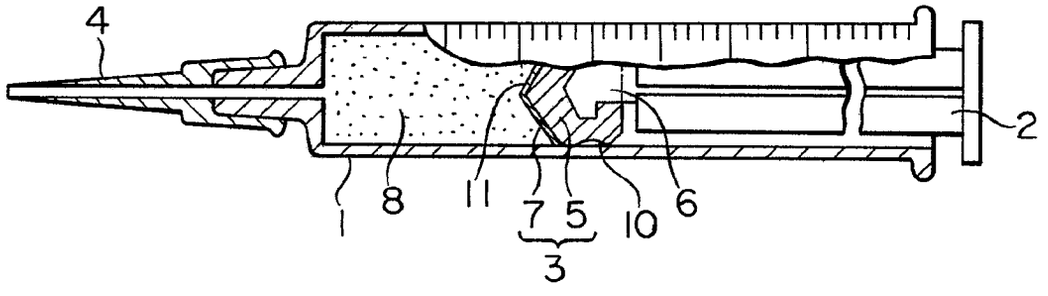


FIG. 6



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

(11) EP 0 879 611 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
25.11.1998 Bulletin 1998/48

(51) Int Cl.[6]: **A61M 5/315**

(21) Application number: **98303964.5**

(22) Date of filing: **19.05.1998**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **22.05.1997 JP 132297/97**

(71) Applicant: **DAIKYO SEIKO, LTD.**
Tokyo (JP)

(72) Inventors:
- **Sudo, Masamichi**
  Sumida-ku, Tokyo (JP)
- **Muraki, Tomoyasu**
  Sumida-ku, Tokyo (JP)

(74) Representative:
Baverstock, Michael George Douglas et al
BOULT WADE TENNANT,
27 Furnival Street
London EC4A 1PQ (GB)

(54) A sealing stopper for a syringe and a prefilled syringe

(57) There can be provided a sealing stopper for a syringe, having very high sealing property and sliding property, and a prefilled syringe using this sealing stopper and capable of preserving a medicament for a long time and operating in easy and precise manner during injecting. This syringe is also excellent in sanitary and operating property during a step of formulation or preservation of a medicament. In this sealing stopper for a syringe, a surface of the rubber body is laminated with a tetrafluoroethylene resin film or ultrahigh molecular weight polyethylene film having an average roughness Ra on the central line of the surface in a range of at most 0.05 μm and a kinematic friction coefficient of at most 0.2.

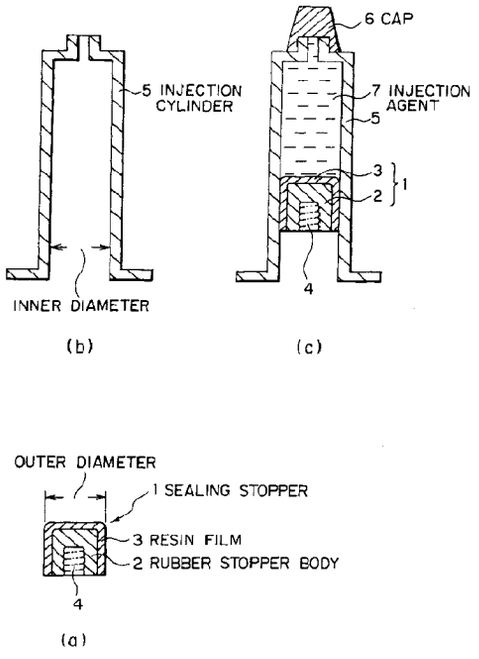


FIG. 1

EP 0 879 611 A2

Printed by Jouve, 75001 PARIS (FR)

Description

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a sealing stopper for a syringe and a prefilled syringe consisting of an injection cylinder or two-component cylinder in which a medicament is sealed by the use of the sealing stopper for a syringe.

2. Description of the Prior Art

An injection agent as one agent form of a medicament includes a solid formulation to be dissolved in administering and a liquid formulation prepared in the form of a solution. As a means for administering an injection agent in the body, there are a method comprising directly administering a medicamnet liquid in the body from a syringe and a method comprising mixing an injection agent with another medicament liquid held by another container just before administering and then introducing the mixture into the body through an administering system, for example, another medical instrument than syringes, such as drip injection set.

In the so-called prefilled syringe, an injection agent is previously filled in an injection cylinder-cum-container, transported or kept in custody, while sealing the end thereof by a sealing stopper, and for the adminisration of the injection agent, an injection needle or administration device is fitted to the pointed end, after which the sealing stopper is thrusted toward the pointed end and slidably moved to allow the injection agent to flow out of the injection needle side and administer it. This syringe of prefilled type has various advantages that①operation thereof is very simple,②administration of a medicament is feasible with a correct administration quantity without misuse of a medicament even in case of emergency and③removal of a medicament is not required to prevent the medicament from contamination with microorganisms and to maintain highly sanitary. Thus, the syringe of prefilled type has lately been used often so as to improve the efficiency of medical treatment in the actual medical scene and to prevent contamination with microorganisms. Further, it has been recommended to use a so-called kit article consisting of a system of a solid agent, water for dissoving the solid agent and a medicament liquid, in combination, because of the same reason.

Such a prefilled syringe is convenient as described above, but when a medicament is kept in custody, high sealing property is required and simultaneously, slidable movement of a sealing stopper is required in administering. Namely, the prefilled syringe must have a function of opposite properties, that is, sealing property and slidable property.

In syringes of the prior art, silicone oils have been coated onto a piston to unite both the sealing property and slidable property. Of late, however, there arise problems, for example, lowering of the potency due to adsorption of effective components of a medicament on the silicone oil, contamination of a medicament with fine grains as a stripped product of a silicone oil and bad influences upon the human body thereby (poisonous character of silicone oil). Accordingly, there is a late tendency of avoiding use of silicone oils.

On the other hand, a movable sealing rubber stopper (which will hereinafter be referred to as "sealing stopper" in some cases) whose main body consists of a rubber has hitherto been known, for example, one having a fluoro resin film such as tetrafluoroethylene laminated on the surface to be contacted with a medicament liquid (Japanese Utility Model Publication No. 8990/1973), a sealing rubber stopper for a prefilled syringe having a polypropylene resin film laminated on all sites to be contacted with an inner surface of a syringe (US Patent No. 4,554,125), etc.

Under the situation, the inventors have developed and proposed syringes or two-component syringes capable of satisfying both the sealing property and slidable property without using silicone oils and having high sanitary and safety property, for example, a sealing stopper whose surface is coated with a tetrafluoroethylene-ethylene copolymer resin (which will hereinafter be referred to as "ETFE" in some cases), as disclosed in Japanese Patent Laid-Open Publication No. 139668/1987, a sealing stopper whose surface is coated with a polytetrafluoroethylene resin film (which will hereinafter be referred to as "PTFE" in some cases), as disclosed in Japanese Patent Laid-Open Publication No. 97173/1988 and a sealing stopper laminated with PTFE, ETFE or ultrahigh molecular polyethylene resin film having a shape suitable for a prefilled syringe, as disclosed in Japanese Utility Model Laid-Open Publication No. 138454/1989 or 138455/1989. Furthermore, there has been proposed a syringe consisting of a cyclic olefin plastic capable of satisfying both the sealing property and slidable property in combination with the sealing stopper as described above, as disclosed in Japanese Patent Laid-Open Publication No. 181164/1991.

In the general formulation provisions of the Japanese Pharmacopoeia of 13th Revision, it is provided that a container for an injection agent must be a hermetic container and the hermetic container is defined as a container capable of daily handling and preventing a medicament from contamination with gases or microorganisms during ordinary storage. Considering the prior art in view of this official provision, the resin film-laminated sealing stopper has a large effect on inhibition of dissolving-out of a rubber component of the stopper body, but the sealing property tends to be lowered because of not using silicone oil.

In the above described sealing stopper the inventors have developed, it is necessary in order to maintain sufficient the sealing property to design so that a difference between the outer diameter of the sealing stopper and the inner diameter of the syringe is somewhat larger and consequently, there arises a problem that the sliding resistance during administering a medicament is somewhat increased.

On the other hand, the inventors have made various studies about resins to be laminated on surfaces of sealing stoppers and consequently, have reached a conclusion that PTFE is most suitable and high moecular weight polyethylene (which will hereinafter be referred to as "UHMWPE" some times) is preferably used in addition to fluoro resins, as compared with other fluoro resins, for example, tetrafluoroethylene-perfluoroethylene copolymer (PFA), tetrafluoroethylene-hexafluoropropylene copolymer (FEP), tetrafluoroethylene-ethylene copolymer (ETFE), trichlorotrifluoroethylene (PCTFE), polyvinylidene fluoride (PVDF), polyvinyl fluoride (PVF), etc. The reasons therefor will be illustrated below.

The above described other fluoro resins can be subjected to thermal melt molding, for example, injection molding or extrusion molding, but PTFE having a melt flow rate (MFR) of substantially zero at its melting point of 327 °C and being non-sticky cannot be subjected to thermal melt molding [Cf. "Plastic No Jiten (Plastic Dictionary)", page 836-838, published by Asakura Shoten, March 1, 1992]. Accordingly, a film of PTFE is obtained by compression molding to give a sheet, by shaping in a block and cutting or slicing the block to give a relatively thick sheet or by skiving working to give a thinner film.

The skiving method will further be illustrated in detail. A suitable amount of a powdered resin raw material for shaping working, obtained by suspension polymerization to give a grain diameter of ~ 10 μm, is charged in a metallic mold for sintering shaping, previously shaped at room temperature and at a pressure of 100 to 1000 kg/cm^2 in a compression press and then sintered at 360 to 380°C for several hours ordinarily but depending on the size of a shaped product. Then, the metallic mold is cooled at normal pressure or at some pressure, thus obtaining a primary shaped product in the form of a sheet, block or cylinder. The shaped product of PTFE in the form of a cylinder, obtained in the above described compression shaping, is fitted to a lathe and revolved, during which an edged tool is pressed against the shaped product at a constant pressure and a specified angle to obtain a PTFE film with a thickness of 40 to 50 μm and at most 200 μm.

The film prepared by this skiving method has a disadvantage that there remain pinholes or skiving scratches on the surface thereof and accordingly, the film is not suitable for laminating a sealing stopper for preventing it from leaching of rubber components in a medicament and contaminating the medicament.

On the other hand, a casting method comprising adding a latex emulsion to a suspension of fine grains of a fluoro resin, thinnly spreading the mixture on a metallic surface and then burning to obtain a film has been known as disclosed in US Patent No. 5,194,335. According to this method, a film with a thickness of up to about 3 μm can be produced.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a sealing stopper for a syringe and a prefilled syringe, whereby the above described problems can be resolved.

It is another object of the present invention to provide a sealing rubber stopper for a syringe, in which a surface of the rubber body is laminated with a PTFE film or UHMWPE film, whereby more sufficient and excellent sealing property and slidable property as compared with those of the prior art can be given without using silicone oil.

It is a further object of the present invention to provide a sealing rubber stopper for a syringe, in which a surface of the rubber body is laminated with a PTFE film or UHMWPE film, having no pinholes nor scratches and having high sanitary property.

It is a still further object of the present invention to provide a prefilled syringe, in which a medicament is enclosed and sealed in an injection cylinder or two-component cylinder by the use of the sealing stopper for a syringe.

These objects can be attained by a sealing stopper for a syringe, in which a surface of the rubber body is laminated with a tetrafluoroethylene resin film or ultra-high molecular weight polyethylene film having an average roughness Ra on the central line of the surface in a range of at most 0.05 μm and a kinematic friction coefficient of at most 0.2 and a prefilled syringe, in which a medicament is enclosed and sealed in an injection cylinder or two-component cylinder by the use of the sealing stopper for a syringe, and in which a surface of the rubber body is laminated with a tetrafluoroethylene resin film or ultra-high molecular weight polyethylene film having an average roughness Ra on the central line of the surface in a range of at most 0.05 μm and a kinematic friction coefficient of at most 0.2.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are to illustrate the principle and merits of the present invention in detail.

Fig. 1 (a), (b) and (c) are cross-sectional views of structures of a sealing stopper and prefilled syringe according to the present invention.

Fig. 2 is a chart with a multiplication of about 60000 times, showing measured data of surface roughness of a PTFE film obtained by a casting method used in Example of the present invention and a PTFE film obtained by a skiving method used in Comparative Example, for comparison.

Fig. 3 (a) to (h) are cross-sectional views of various shapes of the sealing stoppers according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have made various studies to develop a sealing stopper laminated with a PTFE film or UHMWPE film capable of preventing the rubber body from elution of rubber components and contamination of a medicament in contact with the rubber stopper. Accordingly, it is found that the PTFE film or UHMWPE film having the specified surface roughness and kinematic friction coefficient is effective for this purpose.

As a means for solving the above described problems, there are provided the following inventions and embodiments:

(1) A sealing stopper for a syringe, in which a surface of the rubber body is laminated with a tetrafluoroethylene resin film or ultra-high molecular weight polyethylene film having an average roughness Ra on the central line of the surface in a range of at most 0.05 μm and a kinematic friction coefficient of at most 0.2.

(2) The sealing stopper for a syringe, as described in the above (1), wherein the tetrafluoroethylene resin film is prepared by a casting shaping method comprising using, as a raw material, a suspension containing tetrafluoroethylene resin powder having a grain diameter of at most 0.01 to 1.0 μm, a dispersing agent and a solvent.

(3) The sealing stopper for a syringe, as described in the above (1), wherein the ultra-high molecular weight polyethylene film is prepared by an inflation shaping method or extrusion shaping method.

(4) A prefilled syringe, in which a medicament is enclosed and sealed in an injection cylinder or two-component cylinder by the use of the sealing stopper for a syringe, and in which a surface of the rubber body is laminated with a tetrafluoroethylene resin film or ultrahigh molecular weight polyethylene film having an average roughness Ra on the central line of the surface in a range of at most 0.05 μm and a kinematic friction coefficient of at most 0.2.

(5) A process for the production of a sealing stopper for a syringe, which comprises preparing a suspension of polytetrafluoroethylene fine grains having a maximum grain diameter in a range of 0.01 to 1.0 μm with a concentration of 40 to 50 % in a suitable solvent containing a dispersing agent, coating the resulting suspension onto a metallic belt, heating and drying the coating at a temperature of higher than the melting point of polytetrafluoroethylene to form a thin film, repeating this procedure to obtain a sintered cast film with a suitable thickness and then laminating a rubber body with the cast film.

(6) The process for the production of a sealing stopper for a syringe, as described in the above (5), wherein the thin film has a thickness of 5 to 20 μm and the sintered cast film has a thickness of 10 to 60 μm.

Referring to Fig. 1 showing a sealing stopper for a syringe (which will hereinafter be referred to as "sealing stopper") and a prefilled syringe of the present invention, a sealing stopper 1 shown in Fig. 1 (a) comprises a rubber stopper body 2 whose surface is laminated with a resin film 3. 4 designates a fitting part of a plunger not shown. In a formulation step for an injection medicament, the end of an injection cylinder 5 shown in Fig. 1 (b) is sealed by a cap 6, and an injection medicament 7 is charged for the formulation in an injection cylinder 5, followed by sealing by the sealing stopper 1 to prepare a prefilled syringe. Ordinarily, an injection needle, plunger and covers for the various parts (not shown) are adapted to the prefilled syringe, thus obtaining a finished product.

The inventors have made various studies and investigations and consequently, have found that if the resin film 3 laminated on the surface of the rubber stopper body 2 has the specified surface property, i.e. a surface roughness represented by an average roughness Ra on the central line of the surface in a range of at most 0.05 μm, measured according to JIS B0601-1982 and a kinematic friction coefficient of at most 0.2, measured according to JIS K7218-1986, very high sealing property and slidable property can be realized and from the standpoint of a resin film having both the sanitary property and chemical stability required in the field of the sealing stopper for a syringe and a prefilled syringe of the present invention, the PTFE film and UHMWPE film are most suitable, in particular, the PTFE film prepared by a casting method using the specified raw materials or the UHMWPE film prepared by the inflation shaping method or extrusion shaping method is most suitable because of capable of adjusting the surface roughness to the scope of the present invention. The present invention is based on this finding. Thus, high sealing property and slidable property (low kinematic friction resistance) can be obtained to improve the quality holding property of medicaments and make easy medical operations.

Fig. 1 (a), (b) and (c) are schematic views for illustrating a sealing stopper for a syringe (which will hereinafter be referred to as "sealing stopper") and prefilled syringe according to the present invention. As shown in Fig. 1 (a), the sealing stopper 1 has the resin film 3 consisting of PTFE or UHMWPE laminated on the surface of the rubber stopper

body 2. Since the prefilled syringe of the present invention plays also as a container for an injection liquid medicament, it is required that a resin film laminated on a rubber surface not only has physically sealing property and slidable property, but also it is hardly subject to adsorbing or elision even if contacted with a medicament for a long time and not harmful to the human body.

The reason why PTFE is particularly selected and used from various fluoro resins in the present invention is that PTFE has such a stable property that dissolving or swelling does not appear in substantially all medicaments, PTFE has such an excellent heat resistance of organic materials that at about 327 °C corresponding to the melting point, it becomes only transparent gel-like and does not show melt flow property, and the continuous application tempearure is very high, i.e. about 260 °C, a PTFE film has a surface excellent in hydrophobic property, lipophobic property and non-sticky property and PTFE has an excellent slidable property such as represented by a smaller kinematic friction coefficient as shown in Table 1 than that of other plastics. According to these advantages, physical properties and chemical properties required for a surface laminating film of a sealing stopper for a syringe can be satisfied because of being resistant to a sterilizing processing at a high temperature in a formulation process, being free from adsorption or elision even if contacted with a medicament filled inside for a long time and chemically stable and having such a high slidable property that a sealing stopper can smoothly be thrusted in a syringe during administration of a medicament.

Furthermore, the reason why UHMWPE is used as another laminating film consists in that various polyethylenes, in general, have chemical stability and high chemical resistance, very high melt viscosity and good thermal stability and UHMWPE having a molecular weight of at least one hundred million, in particular, is excellent in wear resistance, shock resistance and self lubricating property, has such a small friction coefficient similar to PTFE that it can preferably be used as a coating resin and is so excellent in radiation resistance that it can be applied to sterilization by radiation.

In Table 1 are shown kinematic friction coefficients as a coefficient for showing the degree of sliding (slidable property) of PTFE and UHMWPE for comparison with other resins, measured by JIS K7218-1986.

Table 1

Resin	Kinematic Friction Coefficient ($kg/cm^2 \cdot m/sec$)
Polytetrafluoroethylene (PTFE)	0.2
Ultrahigh Molecular Weight Polyethylene (UHMWPE)	0.2
Nylon 66	0.4
Polyoxymethylene	0.4

In the present invention, a PTFE film or UHMWPE film having an average roughness Ra on the central line of the surface in a range of at most 0.05 μm according to JIS B0601-1982 is used and that capable of satisfying this characteristic value shows a very smooth surface and allows sufficently to display elasticity of a rubber stopper.

PTFE or UHMWPE of the present invention can be produced by any one of production processes capable of giving the specified surface roughness and kinematic friction coefficient, but since the PTFE film meets with the problem of pinholes when it is subjected to slicing or skiving as described above, it is particularly preferable to employ a casting method capable of providing excellent surface properties so as to realize the above described surface roughness.

Fig. 2 is a chart showing measured data of surface roughness of a PTFE film (D-1) obtained by a casting method used in Reference Example 1 and a PTFE film (D-2) obtained by a skiving method used in Reference Example 2 for comparison, respectively measured by JIS BO601-1982, in which x-direction shows a measured length (unit mm), y-direction shows a cut-off value (unit mm) and the maximum height (Rmax) is a height difference between the maximum value and minimun value, represented with a multiplication of about 60,000 times. As is evident from Fig. 2, the surface of film D-1 is much smoother than that of D-2.

UHMWPE having a very high melting point can be formed into a thin film by a method comprising heating under pressure and skiving a primary molding in an analogous manner to PTFE as described above before obtaining a sheet or film or sintering it into a sheet. Since the skiving method has the above described problem, however, it is particularly preferable to employ an extrusion method or an inflation forming method comprising closing one end of a UHMWPE film formed in a tubular form and blowing compressed air into the tubular form from the other end thereof to inflate it, whereby to realize the specified surface roughness on the central line of the surface in a range of at most 0.05 μm according to the present invention in the similar manner to D-1 except omitting the measured chart.

As the thickness of a film to be laminated on a rubber stopper body is the thinner, the rubber elasticity can more effectively be utilized and the sealing property is the better, but handling of the film is difficult during producing and lamination working of the laminated stopper. Thus, the thickness of the PTFE film or UHMWPE film according to the present invention is generally about 0.001 mm to 0.1 mm, preferably 0.001 to 0.05 mm, more preferably 0.005 to 0.03 mm. In the real manufacturing, the void ratio is low in the case of a thickness range of 0.01 to 0.05 mm, the proportion

defective being decreased. Production of a sealing stopper with a laminated film thickness of at most 0.001 mm is difficult and this is a critical limit in the lamination working of a rubber stopper body. On the other hand, a thickness exceeding 1 mm is not preferable because of not obtaining high sealing property.

Production of a PTFE film by a casting method will specifically be illustrated. A PTFE suspension is prepared by the use of a suitable dispersing agent, the suspension having such a grain diameter that a stable suspended state can be maintained, i.e. a maximum grain diameter of 0.01 to 1.0 μm, preferably at most 0.5 μm, and a solid concentration of about 35 to 60 %. A more preferred concentration is about 40 to 50 %. As a solvent and dispersing agent, there can be used commonly used ones. As a dispersant, for example, there is used a nonionic surfactant such as Nissan Nonion HS 208 (Commercial Name, manufactured by Nippon Yushi Co., Ltd.). As a solvent, for example, water can be used. In Table 2 are shown examples of compositions of the suspensions without limiting the present invention.

Table 2

	Weight (g)/Volume (1)	Resin Concentration (weight %)	Density of Suspension
PTFE Resin	900	60	1.50
	693	50	1.39
	601	45	1.34
	515	40	1.29
	436	35	1.24
Surfactant[1)]	1 weight %		
Solvent[2)]	1 liter (total)		

(note)
1) Nissan Nonion HS 208 (Commercial Name, manufactured by Nippon Yushi Co., Ltd.)
2) water

The suspension is poured onto a high heat resistance, rust proofing belt, for example, stainless steel belt, heated in a heating furnace of closed type at a temperature of higher than the melting point of PTFE (327 °C) to evaporate water content and the subjected to sintering working for 4 to 6 hours to form a thin film. Since the feature of this method consists in directly preparing a thin film without a step of preparing a cylindrical primary work as in other working methods, there can be obtained a thin film free from pinholes or surface scratches due to the above described skiving working method. Furthermore, a very fine PTFE with a maximum grain diameter of at most 1.0 μm is herein used, thus resulting in a film product with a true specific gravity of approximately 2.14 to 2.20, which has scarcely pinholes even as a result of visual observation or pinhole investigation and exhibits very small surface roughness, i.e. excellent smoothness.

A rubber used for the sealing rubber stopper of the present invention is not particularly limited, but is exemplified by synthetic rubbers such as isoprene rubbers, butadiene rubbers, styrene butadiene rubbers, ethylene pro-pyrene rubbers, isoprene-isobutylene rubbers, nitrile rubbers, etc. and natural rubbers. The rubber used as a predominant component can be blended with additives such as fillers, cross-linking agents, etc. For the sealing stopper for a prefilled syringe according to the present invention, however, it is preferable to select a material excellent in sanitary property as well as in gaseous permeability resistance so as to stably store a liquid medicament for a long time, e.g. 3 years in a container (injection cylinder). A compounding example of such a rubber formulation is shown in the following Table 3. When a PTFE film having a high softening point is laminated, Compounding Examples 1 and 2 each using a high vulcanization temperature are suitable and when a UHMWPE film having a melting point of 135 °C is laminated, Compounding Examples 3 and 4 are suitable. In the present invention, the shape of the rubber stopper body and production process thereof are not particularly limited.

Table 3

Composition	Compounding			
	Example 1	2	3	4
Butyl Rubber[1)]	100			
Chlorinated Butyl Rubber[2)]		100		

(Note):
1) manufactured by Exxon Chemical Co., Esso Butyl # 365 (commercial name), bonded isoprene content: 1.5 mol %, Mooney viscosity: 43 to 51
2) manufactured by Exxon Chemical Co., Esso Butyl HT 1066 (commercial name), bonded chlorine content: 1.3 wt %, Mooney viscosity: 34 to 40

Table 3 (continued)

Composition	Compounding			
	Example 1	2	3	4
Isobutylene-Isoprene-Divinylbenzene Terpolymer Partially Cross-linked Butyl Rubber[3)]			100	
Acrylonitrile-Butadiene Rubber[4)]				100
Wet Process Hydrous Silica[5)]	35	30	30	20
Dipentamethylene Thiuram Tetrasulfide[6)]	2.5			
Zinc Di-n-dibutylthiocarbamate[7)]	1.5			
Active Zinc Oxide[8)]	5	4	1.5	
Stearic Acid[9)]	1.5	3		
Magnesium Oxide[10)]		1.5		
2-Di-n-butylamino-4,6-dimercapto-s-triazine[11)]		1.5		
1,1-Bis(t-butylperoxy)-3,3,5-trimethylcyclohexane[12)]			2	8
Total (weight part)	145.5	140.0	133.5	128
Vulcanization Conditions				
Temperature (°C)	175	180	150	155
Time (min)	10	10	10	10

3) manufactured by Bayer AG, Bayer Butyl XL-10000 (commercial name)

4) manufactured by Nippon Zeon Co., Nipol DN 102 (commercial name), bonded acrylonitrile content: 42 wt %, Mooney viscosity: 60

5) manufactured by Nippon Silica Kogyo Co., Nipseal ER (commercial name), pH: 7.5 to 9.0 (5 % aqueous solution)

6) manufactured by Kawaguchi Kagaku Kogyo Co., Accel TRA (commercial name), MP: at least 120 °C

7) manufactured by Kawaguchi Kagaku Kogyo Co., Accel BZ (commercial name)

8) manufactured by Seido Kagaku Kogyo Co., Active Zinc White AZO (commercial name), ZnO 93 to 96 %

9) manufactured by Kao Co., Lunack S# 30, (commercial name)

10) manufactured by Kyowa Kagaku Kogyo Co., Kyowa Mag # 150 (commercial name), specific surface area: 130 to 170 mg

11) manufactured by Sankyo Kasei Co., Jisnet DB (commercial name) MP: at least 137 °C

12) manufactured by Nippon Yushi Co., Perhexa 3M-40 (commercial name), molecular weight: 302, one minute half-life temperature: 149 °C

Lamination of a surface of a rubber stopper with a PTFE film or UHMWPE film according to the present invention can be carried out by a known technique, for example, comprising subjecting one side of a film to a chemical etching treatment, sputtering treatment or corona discharge treatment, arranging the film in a metallic mold for shaping with a rubber compound as a base material of a sealing stopper body and then vulcanizing, bonding and shaping in a predetermined shape. Fig. 3 shows various shapes, in cross section, of sealing stoppers of the present invention without limiting the same. Even if a syringe has a complicated structure, for example, in which a plurality of annular projections are formed on a slidable area of an inner wall of the syringe, the advantages of the present invention can of course be obtained. An area to be laminated includes a part in contact with an inner wall of a syringe or a part in contact with a medicament and is not intended to be limited thereto.

Since the sealing stopper of the present invention has very high slidable property, even if the sealing stopper is designed in such a size that its compressibility, i.e. sealing rpoperty becomes higher by enlarging a difference between an inner diameter of an injection cylinder and an outer diameter of the sealing stopper, as shown in Examples hereinafter described, sufficient slidable property can be obtained.

The sealing stopper of the present invention can be applied to not only plastic injection cylinders, but also glass injection cylinders. However, since glass surfaces generally have larger roughness than plastic surfaces, the sealing stopper of the present invention can be applied to the plastic injection cylinders with better sealing property and sliding proeprty.

The prefilled syringe of the present invention includes any one of syringes of prefilled type using the sealing stopper for syringes according to the present invention hereinbefore illustrated and there is no limitation concerning materials or shapes of injection cylinders and other parts, for example, caps at front ends thereof, plunger rods provided at the back end of the sealing stopper, etc. For example, as a material for an injection cylinder (icluding two-component vessel), there are generally used plastics from the standpoint of the above described surface roughness, such as cylcic olefin resins, cyclic olefin-ethylene copolymers, polyethylene terephthalate resins, polystyrene resins, etc. In particular, cyclic olefin resins and cyclic olefin-ethylene copolymers are preferably used because of having higher transparency and heat resistance and having no chemical interaction with medicaments.

Fig. 1 (c) shows a sate of fitting a sealing stopper 1 to an injection cyljnder 5. In the case of a prefilled syringe, a

medicament is previously charged in the injection cylinder 5 serving also as a vessel for storage of an injection agent 7 and the sealing stopper 1 is thrusted therein to close the injection cylinder to obtain a product. 6 designates a cap for closing an injection needle-fitted opening at the end of the injection cylinder 5. The syringe of this type includes the so-called kit articles. Since the storage period of a medicament generally extends to a long time of period, i.e. three years, in particular, sealing property, chemical resistance and chemical stability are required for the sealing stopper and during use, moreover, higher slidability and operativeness must be provided for emergency. The article of the present invention can satisfy all the requirements.

The present invention will now be illustrated in detail by the following Examples and Comparative Examples without limiting the same.

Reference Example 1

Production of PTFE Film (D-1) by Casting Method

6.01 kg of PTFE fine powder (Hostaflon TF 1760 -commercial name-, manufactured by Hoechst AG, maximum grain diameter: less than 1 μm, mean grain diameter: 0.1 μm) was added to 10 liter of Nissan Nonion HS 208 (nonionic surfactant) diluted with distilled water to 6 % and adequately suspended and dispersed by means of a homogenizer to obtain 16.01 kg of a 45 weight % PTFE suspension. The suspension was coated onto a cleaned and polished stainless steel plate to give a coating thickness of 10 μm (generally, 5-20 μm), dried for 1.5 minutes by an infrared lamp and heated at 360-380°C for about 10 minutes to evaporate the surfactant. After repeating this procedure four times (generally, 1-8 times), the suspension was sintered in a thickness of about 40 μm (0.04 mm) (generally, 10-60 μm). After the last sintering, the resulting layer was quenched with water and stripped from the metal plate to obtain a clear PTFE casting film (D-1). The number of the procedures was increased or decreased and thus, a film with a desired thickness could be obtained.

Reference Example 2

Production of PTFE Film (D-2) by Skiving Method

For comparison, a PTFE film was produced by the skiving method of the prior art, as described in the column of Prior Art (D-2). The same PTFE fine powder as that of Reference Example 1 was uniformly charged in a metallic mold having a diameter of 250 mm and height of 2000 mm and being of a polished stainless steel sheet, while passing through a stainless steel sieve of 10 mesh. The fine powder was gradually compressed to 300 kg/cm^2 at normal temperature and maintained for 25 minutes to obtain a preformed product, which was heated to 370 °C at a rate of 10 °C/min in an electric furnace and maintained at this temperature until the whole material was uniformly sintered. The sintered product was then cooled to room temperature at a temperature lowering rate of 15 °C/min to obtain a sintered article. The thus obtained sintered round rod (300 mm diameter x 500 mm h) was subjected to skiving working, thus obtaining a PTFE film with a thickness of about 40 μm or a desired thickness.

The surface roughness of thus resulting D-1 and D-2 films and an ETFE film (D-3) obtained by an extrusion method as Reference Example 3 was measured by the following measurement method using a surface roughness and shape measurement device (Surfcom 550A -commercial name-, manufactured by Tokyo Seimitsu Co.) at a magnification of 60000, a cutoff value of 0.5 mm and a measured length of 4.0 mm, thus obtaining results as shown in Table 4. This mesurement was carried out as to only the film, not after laminated, since the measurement of the laminated film was impossible from the structure of the measurement device.

Measurement Method of Roughness Depth on Film Surface

Measurement of the surface roughness was carried out according to JIS B0601-1982 using the surface roughness and shape measurement device of needle touch type (Surfcom 550A). While the needle part of the measurement device was applied to a surface of a sample and moved within a predetermined range, an average roughness (Ra) on the center line, maximum height (Rmax) and ten point average roughness (Rz) were measured to obtain a measured chart, from which Ra, Rmax and Rz were read. The measurement was carried out six times as to each sample and arithmetical average values of Ra, Rmax and Rz were obtained excluding the maximum value. Ra and Rz values represented the roughness depths of the film surface by numeral as an arithmetical average of all the roughness depth profiles from the center line.

As to each of the foregoing Samples D-1 to D-3, a film of 20 μm thick was prepared and subjected to measurement of the kinematic friction factor of the surface according to the following measurement method. Measured results and properties of the each film are shown in Table 4.

Measurement Method of Kinematic Friction Factor

The kinematic friction factor is a factor representative of a degree of sliding (slidability) of a film. According to JIS K7218-1986, the kinematic friction factor of a surface of a sample was measured using a friction and abrasion tester of Matsubara type (manufactured by Toyo Poldwin Co.) under test conditions of workpiece: SUS, load: 5kgf - 50 kgf (same load for 30 minutes every 5 kgf), speed: 12 m/min, time: 168 hours. Calculation of the kinematic friction factor was carried out by the following formula:

$$\text{Kinematic Friction Factor } (kg/cm^2 \cdot m/sec)$$

$$= \text{kinematic friction force at vertical load of 15 kgf/load 15 kgf}$$

Overall Light Percent Transmission and Haze

The overall light percent transmission and haze were measured according to JIS K7105-1981, "Test Method of Optical Properties of Plastics" using a device for measuring light transmission of integrated globe type. The haze means a ratio of scattered light to a quantity of transmitted light through a sample. The light percent transmission is a ratio of the overall light transmission and diffusion transmission to the quantity of the overall projected light.

Table 4

Film No.	Reference Example 1 D-1	Reference Example 2 D-2	Reference Example 3 D-3	Reference Example 4 D-4
Resin : Production Process	PTFE : Casting Method	PTFE : Skiving Method	ETFE : Extrusion Method	UHMWPE : Inflation Method
Central Line Average Roughness : Ra	0.036 μm	0.136 μm	0.03 μm	0.032 μm
Maximum Height : Rmax	0.910 μm	0.212 μm	0.87 μm	0.89 μm
Ten Point Average Roughness : Rz	0.396 μm	1.290 μm	0.23 μm	0.30 μm
Kinematic Friction Factor	0.07 kg/cm² · m/sec	0.10 kg/cm² · m/sec	0.38 kg/cm² · m/sec	0.20 kg/cm² · m/sec
Tensile Strength Length Direction	3.5 kg/mm²	4.2 kg/mm²	124 kg/mm²	45,000 kg/mm²
Width Direction	3.5 kg/mm²	2.0 kg/mm²	118 kg/mm²	43,500 kg/mm²
Elongation Length Direction	360 %	450 %	1057 %	350 %
Width Direction	340 %	460 %	1273 %	340 %
Overall Light Percent Transmission	92 %	88 %	99 %	55 %
Haze	33 %	82 %	1 %	38 %
Heat Shrinkage Length Direction (100°C)	0.8 %	1.5 %	0.5 %	20 %
Width Direction	0.7 %	-1.2 %	0.4 %	25 %
Heat Shrinkage Length Direction (200°C)	1.8 %	2.9 %	1.4 %	MP 135°C, impossible to measure
Width Direction	1.7 %	-1.8 %	1.2 %	