POLYPEPTIDES, ANTIBODY VARIABLE DOMAINS & ANTAGONISTS

The present invention relates to immunoglobulin single variable domains (dAbs) e.g. dAbs which are protease resistant, and also to formulations, and compositions comprising such dAbs for ocular delivery and to their uses to treat ocular diseases and conditions.

BACKGROUND OF THE INVENTION

A difficulty of treating ocular diseases and conditions has been the inefficiency of delivering therapeutic agents to the eye. When a drug is delivered to the eye it very often clears extremely rapidly from the ocular tissues. Additionally, when therapeutics are delivered topically to the eye a problem has been that they may not reach the posterior segments of the eye (the retina, vitreous and choroid). Hence, many posterior segment ocular conditions have been treated by administering drugs intravenously or by intravitreal administration. Many of these diseases, e.g. AMD, glaucoma, diabetic retinopathies cannot be treated optimally. Therefore a need exists to provide further agents which can be suitable for ocular delivery and which can treat or prevent ocular diseases and conditions.

Polypeptides and peptides have become increasingly important agents for use as medical, therapeutic and diagnostic agents. However in certain *in vivo* environments e.g. the eye and in certain physiological states, such as cancer and inflammatory states, the amount of proteases present in a tissue, organ or animal can increase. This increase in proteases can result in accelerated degradation and inactivation of endogenous proteins and of therapeutic peptides, polypeptides and proteins that are administered to treat disease. Accordingly, some agents that have potential for *in vivo* use (*e.g.*, use in treating, diagnosing or preventing disease) have only limited efficacy because they are rapidly degraded and inactivated by proteases.

Protease resistant polypeptides provide several advantages. For example, protease resistant polypeptides remaining active *in vivo* longer than protease sensitive agents and, accordingly, remaining functional for a period of time that is sufficient to produce biological effects.

VEGF is a secreted, heparin-binding, homodimeric glycoprotein existing in several alternate forms due to alternative splicing of its primary transcript (Leung et al., 1989, Science 246: 1306). VEGF is also known as vascular permeability factor (VPF) due to its ability to induce vascular leakage, a process important in inflammation.

In the eye VEGF and VEGF-receptors are known to stimulate both choroidal and retinal vessel angiogenesis and regulate the vascular permeability of such vessels. Both these features contribute to retinal damage and consequential visual acuity deterioration which results from a number of retinal inflammatory conditions, vasculopathies and maculopathies. Attempts to regulate VEGF activity or VEGF-receptor activity has previously been shown to effectively manage the vascular permeability in both animal models and human disease (Gragoudas et al., 2004: N. Engl. J. Med 351: 2805)

Targeting VEGF with currently available therapeutics is not effective in all patients. Thus, a need exists for improved agents for treating pathological conditions mediated by VEGF e.g. vascular proliferative diseases (e.g. Age related macular degeneration (AMD)).

TNF-α (Tumour Necrosis Factor-α) is a pro-inflammatory cytokine which has been implicated in a number of ophthalmic inflammatory conditions such as uveitis and AMD and in the generation of retinal vasculopathies in which there is an inflammatory component. The generation of choroidal neovascular lesions associated with age-related macular disease has been demonstrated to have an associated inflammatory component. Effective management of this associated inflammatory component has been

demonstrated to directly effect the development of the choroidal neo-angiogenic lesion and the vascular permeability both of which can impact human disease. Recent evidence in human AMD patients have suggested that the use of anti-TNFα therapeutics can impact disease in patients which are unresponsive to anti-VEGF therapies (Theodossiadis et al., 2009: Am. J. Ophthalmol. 147: 825-830).

Interleukin 1 (IL-1) is an important mediator of the immune response that has biological effects on several types of cells. Interleukin 1 binds to two receptors Interleukin 1 Receptor type 1 (IL-1R1, CD121a, p80), which transduces signal into cells upon binding IL-1, and Interleukin 1 Receptor type 2 (IL-1R1, CDw121b), which does not transduce signals upon binding IL-1 and acts as an endogenous regulator of IL-1. Another endogenous protein that regulates the interaction of IL-1 with IL-1R1 is Interleukin 1 receptor antagonist (IL-1ra). IL-1ra binds IL-1R1, but does not activate IL-1R1 to transduce signals.

Signals transduced through IL-1R1 upon binding IL-1 (*e.g.*, IL-1α or IL-1β) induce a wide spectrum of biological activities that can be pathogenic. For example, signals transduced through IL-1R1 upon binding of IL-1 can lead to local or systemic inflammation, and the elaboration of additional inflammatory mediators (*e.g.*, IL-6, Il-8, TNF). Accordingly, the interaction of IL-1 with IL-1R1 has been implicated in the pathogenesis of ocular diseases.

Certain agents that bind Interleukin 1 Receptor Type 1 (IL-1R1) and neutralize its activity (*e.g.*, IL-1ra) have proven to be effective therapeutic agents for certain inflammatory conditions.

SUMMARY OF THE INVENTION

In a first aspect the invention provides a composition which comprises or consists of an immunoglobulin single variable domain (or dAb) which can bind to a

desired target molecule (e.g. VEGF, IL-1, or TNF-α), e.g. at the site of delivery, for administration to the eye.

The invention also provides compositions which comprise or consist of an immunoglobulin single variable domain (or dAb) which can bind to a desired target molecule (e.g. VEGF, IL-1, or TNF-α, TNFR1, TNFR2, IL-1r), for use to treat, prevent or diagnose ocular diseases or conditions, such as Age related macular degeneration (AMD), Uveitis, glaucoma, dry eye, diabetic retinopathy, and diabetic macular oedema.

In an embodiment the immunoglobulin single variable domain can be protease resistant, e.g. resistant to one or more of the following: serine protease, cysteine protease, aspartate proteases, thiol proteases, matrix metalloprotease, carboxypeptidase (e.g., carboxypeptidase A, carboxypeptidase B), trypsin, chymotrypsin, pepsin, papain, elastase, leukozyme, pancreatin, thrombin, plasmin, cathepsins (*e.g.*, cathepsin G), proteinase (e.g., proteinase 1, proteinase 2, proteinase 3), thermolysin, chymosin, enteropeptidase, caspase (*e.g.*, caspase 1, caspase 2, caspase 4, caspase 5, caspase 9, caspase 12, caspase 13), calpain, ficain, clostripain, actinidain, bromelain, and separase. In particular embodiments, the protease is trypsin, elastase or leucozyme. Such protease resistant polypeptides are especially suitable for delivery to protease rich environments in vivo such as the eye.The protease can also be provided by a biological extract, biological homogenate or biological preparation. In one embodiment, the protease is one found in the eye and/or tears. Examples of such proteases found in the eye include caspases, calpains, matric metalloproteases, disintegrin, metalloproteinases (ADAMs) and ADAM with thrombospondin motifs, the proteosomes, tissue plasminogen activator, secretases, cathepsin B and D, cystatin C, serine protease PRSS1, ubiquitin proteosome pathway (UPP). In one embodiment, the protease is a non-bacterial protease. In an embodiment, the protease is an animal, e.g., mammalian, e.g., human, protease.

The composition can be delivered to different regions of the eye, e.g. to the surface of the eye, the cornea, or 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,and the composition can bind to target molecules (e.g. VEGF, IL-1, or TNF-α) in these parts of the eye. The composition can also be delivered to the peri-ocular region of the eye.

The target molecule may for example be VEGF, IL-1, or TNF-α or it can be any other desired target e.g. a target molecule present in the eye, for example on the surface of the eye, within the eye or in tear ducts or lachrymal glands, e.g. the target can be IL-1, IL-17 or TNF receptor such as TNFR1, TGFbeta, IL-6, IL-8, IL-21, IL-23, CD20, Nogo-a, Myelin associated glycoprotein (MAG) or Beta amyloid.

In one embodiment the invention provides a protease resistant immunoglobulin single variable domain (or dAb) for administration to the eye, e.g. in the form of eye drops or as a gel or e.g. in an implant. The dAb can for example bind to a target molecule present in the eye e.g. VEGF, IL-1, or TNF-α.

Administration to the eye can be for example by topical administration, e.g. in the form of eye drops; or alternatively it can be by injection into the eye.

It can be useful to target the delivery of the immunoglobulin single variable domain into particular regions of the eye such as the surface of the eye, or 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). Hence the invention further provides a method of delivering a composition directly to the eye which compises administering said composition to the eye by a method selected from: intra-ocular injection, topical delivery, eye drops, peri-ocular administration and use of a slow release formulations (such as a polymeric nano or microparticle or gel) or by using delivery devices making use of iontophoresis.

It can also be useful if the immunoglobulin single variable domain 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) an immunoglobulin single variable domain that bind to a

target molecule e.g. in the eye (e.g. toVEGF, IL-1, or TNF-α), and also (b) an ocular penetration enhancer and /or (c) a viscosity enhancer e.g. for topical delivery to the eye.

In one aspect, the immunoglobulin single variable domain to be delivered to the eye can be any one of the VEGF dAbs, disclosed in WO 2008/149146, WO 2008149147, or WO 2008149150 which bind to VEGF. For example it can be a polypeptide encoded by an amino acid sequence that is at least 80% identical to the amino acid sequence of DOM15-26-593 (shown in figure 1a: SEQ ID NO 1). In one embodiment, the percent identity is at least 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% or 100%. In one embodiment the protease resistant polypeptide is obtainable by the method described herein for isolating protease resistant polypeptides. The DOM15-26-593 for delivery to the eye may also further comprises a domain of an antibody constant region. For example it may have an amino acid sequence identical to the amino acid sequence of DOM15-26-593-Fc fusion (shown in Figure 1b: SEQ ID NO 2) or the percent identity may be at least 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% to that shown in Figure 1b: SEQ ID NO 2.

In one aspect the VEGF dAb which is encoded by an amino acid sequence that is at least 80% identical to the amino acid sequence of DOM15-26-593 (e.g. by one which is 97% identical or more) can comprise valine at position 6 and/or leucine at position 99, and/or lysine at position 30 (Kabat numbering) as described in WO 2008149150 and WO 2008149147 (the contents of which are incorporated herein by reference).

In a further aspect, the immunoglobulin single variable domain to be delivered to the eye can be any one of the anti TNFR1 dAbs disclosed in WO 2008/149144, or WO 2008/149148.

In one embodiment the immunoglobulin single variable domain which binds to α-TNF-αR1 can comprise an amino acid sequence that is at least 97% (e.g. 98%, 99% or 100% identical) identical to the amino acid sequence of Dom 1h-131-206 (shown in

figure 4; SEQ ID NO 6). Preparation and selection of Dom 1h-131-206 is described in WO2008149148.

In yet a further aspect, the immunoglobulin single variable domain to be delivered to the eye can be any one of the anti-IL-1R1 dAbs disclosed in WO 2008/149149.

In one embodiment the immunoglobulin single variable domain which binds to IL-1 can comprise an amino acid sequence that is at least 97% (e.g. 98%, 99% or 100% identical) identical to: (a) the amino acid sequence of DOM 4-130-54 (shown in figure 3; SEQ ID NO 5); or to (b) the amino acid sequence of DOM 0400 PEG (shown in figure 2; SEQ ID NO 4).

Preparation and selection of DOM 4-130-54 is described in WO 2007063311 and also WO2008149149. To prepare Dom 0400 the DOM 4-130-54 dAb sequence is taken and is mutated such that a cysteine at position 80 replaces the proline present in DOM 4-130-54, this dAb is then attached to a 40KDa linear PEG molecule (obtained from NOF Corporation, Europe) by standard maleimide coupling to the free cysteine at position 80 of the dAb.

The invention also provides for use of any of the compositions comprising or consisting of an immunoglobulin single variable domain in the manufacture of a medicament for the treatment, prevention or diagnosis of an eye condition or disease e.g. wherein said eye disease is Age related Macular Degeneration (AMD), Uveitis glaucoma, dry eye, diabetic retinopathy, or diabetic macular oedema.

The invention also provides a composition comprising or consisting of an immunoglobulin single variable domain e.g. a VEGF, IL-1, or TNF-α dAb, for use in

the treatment, prevention or diagnosis of an eye condition or disease e.g. AMD, Uveitis glaucoma, dry eye, diabetic retinopathy, or diabetic macular oedema.

In one alternative embodiment the immunoglobulin single variable domain for delivery to the eye can be one which is not the amino acid sequence of DOM15-26-593 (shown in Figure 1a; SEQ ID NO 1) or which is not the amino acid sequence of DOM15-26-593-Fc fusion (shown in Figure 1b; SEQ ID NO 2).

In another alternative embodiment the immunoglobulin single variable domain for delivery to the eye can be one which is not a molecule which comprises or consists of any of the molecules disclosed in the following applications: PCT/GB2008/050399, PCT/GB2008/050400, PCT/GB2008/050406, PCT/GB2008/050405, PCT/GB2008/050403, PCT/GB2008/050404, PCT/GB2008/050407.

In another alternative embodiment the immunoglobulin single variable domain for delivery to the eye can be one which is not the amino acid sequence of Dom1h-131-511, Dom1h-131-201, Dom1h-131-202, Dom1h-131-203, Dom1h-131-204, Dom1h-131-205 as disclosed in PCT/GB2008/050400.

In another alternative embodiment the immunoglobulin single variable domain for delivery to the eye can be one which is not the amino acid sequence of Dom4-130-202 as disclosed in PCT/GB2008/050406.

In another alternative embodiment the immunoglobulin single variable domain for delivery to the eye can be one which is not the amino acid sequence of Dom1h-131-206 as disclosed in PCT/GB2008/050405.

It can also be useful to deliver other agents to the eye in combination or association with the immunoglobulin single variable domains, for example it can be useful to deliver penetration enhancers such as sodium caprate or a viscosity agent such as Hydroxypropylmethylcellulose (HPMC).

The single immunoglobulin variable domains (dAbs) for ocular delivery (e.g. that bind to VEGF, IL-1, or TNF-α), can be formatted to have a larger hydrodynamic size, for example, by attachment of a PEG group, serum albumin, transferrin, transferrin receptor or at least the transferrin-binding portion thereof, an antibody Fc region, or by conjugation to an antibody domain. For example, the dAb monomer (e.g. VEGF dAb), can be formatted as a larger antigen-binding fragment of an antibody (e.g., formatted as a Fab, Fab', $F(ab)_2$, $F(ab')_2$, IgG, scFv). The hydrodynamic size of the dAb and its serum half-life can also be increased by conjugating or linking it to a binding domain (e.g., an antibody or antibody fragment) that binds an antigen or epitope that increases half-live *in vivo*, as described herein (see, Annex 1 of WO2006038027 incorporated herein by reference in its entirety). For example, the VEGF dAb can be conjugated or linked to an anti-serum albumin or anti-neonatal Fc receptor antibody or antibody fragment, e.g. an anti-SA or anti-neonatal Fc receptor dAb, Fab, Fab' or scFv, or to an anti-SA affibody or anti-neonatal Fc receptor affibody.

Examples of suitable albumin, albumin fragments or albumin variants for use in compositions described herein e.g. linked with VEGF-binding dAbs, are described in WO 2005/077042A2 and WO2006038027, which are incorporated herein by reference in their entirety.

Formatted dAbs (e.g. dAbs formatted by PEGylation) can have a molecular weight which is e.g. between 30KDa and 100 KDa e.g. around 50-60 KDa and can be useful for delivery to the retina and/or the choroids and/or the lachrymal fluid.

Naked (unformatted) dAbs which have a molecular weight around 15 KDa can be useful for delivery to the vitreous and/or aqueous humour and/or retina and/or choroids.

In other embodiments of the invention described throughout this disclosure, instead of the use of a single immunoglobulin variable domain or "dAb" in an antagonist or ligand of the invention, it is contemplated that the skilled addressee can

use a domain that comprises the CDRs of a dAb that binds e.g. VEGF, IL-1, or TNF-α (e.g., CDRs grafted onto a suitable protein scaffold or skeleton, e.g. an affibody, an SpA scaffold, an LDL receptor class A domain or an EGF domain) or can be a protein domain comprising a binding site for VEGF, IL-1, or TNF-α e.g., wherein the domain is selected from an affibody, an SpA domain, an LDL receptor class A domain or an EGF domain. The disclosure as a whole is to be construed accordingly to provide disclosure of antagonists, ligands and methods using such domains in place of a dAb.

Protease resistant dAbs described herein can be selected using the methods and teachings described in WO 2008149143, the contents of which are incorporated herein by reference.

In one aspect, the invention provides a protease resistant immunoglobulin single variable domain comprising e.g. a VEGF, IL-1, or TNF-α binding site, wherein the variable domain is resistant to protease when incubated with

(i) a concentration (c) of at least 10 micrograms/ml protease at 37°C for time (t) of at least one hour; or

(ii) a concentration (c') of at least 40 micrograms/ml protease at 30°C for time (t) of at least one hour. In one embodiment, the ratio (on a mole/mole basis) of protease, e.g. trypsin, to variable domain is 8,000 to 80,000 protease:variable domain, e.g. when C is 10 micrograms/ml, the ratio is 800 to 80,000 protease:variable domain; or when C or C' is 100 micrograms/ml, the ratio is 8,000 to 80,000 protease:variable domain. In one embodiment the ratio (on a weight/weight, e.g. microgram/microgram basis) of protease (e.g., trypsin) to variable domain is 16,000 to 160,000 protease:variable domain e.g. when C is 10 micrograms/ml, the ratio is 1,600 to 160,000 protease:variable domain; or when C or C' is 100 micrograms/ml, the ratio is 1,6000 to 160,000 protease:variable domain. In one embodiment, the concentration (c or c') is at least 100 or 1000 micrograms/ml protease. In one embodiment, the concentration (c or c') is at least 100 or 1000 micrograms/ml protease. Reference is made to the description herein of the conditions suitable for proteolytic activity of the protease for use when working with repertoires or libraries of peptides or polypeptides (e.g., w/w parameters). These

conditions can be used for conditions to determine the protease resistance of a particular immunoglobulin single variable domain. In one embodiment, time (t) is or is about one, three or 24 hours or overnight (*e.g.*, about 12-16 hours). In one embodiment, the variable domain is resistant under conditions (i) and the concentration (c) is or is about 10 or 100 micrograms/ml protease and time (t) is 1 hour. In one embodiment, the variable domain is resistant under conditions (ii) and the concentration (c') is or is about 40 micrograms/ml protease and time (t) is or is about 3 hours. In one embodiment, the protease is selected from trypsin, elastase, leucozyme and pancreatin. In one embodiment, the protease is trypsin. In one embodiment, the protease is a protease found in sputum, mucus (*e.g.*, gastric mucus, nasal mucus, bronchial mucus), bronchoalveolar lavage, lung homogenate, lung extract, pancreatic extract, gastric fluid, saliva or tears or the eye. In one embodiment, the protease is one found in the eye and/or tears. In one embodiment, the protease is a non-bacterial protease. In an embodiment, the protease is an animal, e.g., mammalian, e.g., human, protease.

In one embodiment, the variable domain is resistant to trypsin and/or at least one other protease selected from elastase, leucozyme and pancreatin. For example, resistance is to trypsin and elastase; trypsin and leucozyme; trypsin and pacreatin; trypsin, elastase and leucozyme; trypsin, elastase and pancreatin; trypsin, elastase, pancreatin and leucozyme; or trypsin, pancreatin and leucozyme.

In one embodiment, the variable domain is displayed on bacteriophage when incubated under condition (i) or (ii) for example at a phage library size of 10^6 to 10^{13} , e.g. 10^8 to 10^{12} replicative units (infective virions).

In one embodiment, the variable domain specifically binds VEGF, IL-1, or TNF-α following incubation under condition (i) or (ii), e.g. assessed using BiaCore TM or ELISA, e.g. phage ELISA or monoclonal phage ELISA.

In one embodiment, the variable domains specifically bind protein A or protein L. In one embodiment, specific binding to protein A or L is present following incubation under condition (i) or (ii).

In one embodiment, the variable domains may have an OD_{450} reading in ELISA, e.g. phage ELISA or monoclonal phage ELISA) of at least 0.404, e.g., following incubation under condition (i) or (ii).

In one embodiment, the variable domains display (substantially) a single band in gel electrophoresis, e.g. following incubation under condition (i) or (ii).

In another embodiment, an agent (dAb) can be locally administered to the eye via an implantable delivery device. Thus, in one embodiment, the invention provides an implantable delivery device containing e.g. the VEGF, IL-1, or TNF-α dAb, for ocular delivery

In a further aspect, the invention provides a pharmaceutical composition comprising an immunoglobulin single variable domain (e.g.VEGF, IL-1, or TNF-α dAb), and a pharmaceutically or physiologically acceptable carrier, excipient or diluent for ocular delivery.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a: Depicts the amino acid sequence of DOM15-26-593

Fig. 1b: Depicts the amino acid sequence of DOM15-26-593-Fc fusion

Fig. 1c: Depicts the amino acid sequence of an antibody Fc

Fig. 2: Depicts the amino acid sequence of DOM 0400 PEG (a pegylated anti-IL1 dAb, molecular weight about 52 KDa)

Fig. 3: Depicts the amino acid sequence of DOM4-130-54 (An anti-IL1 dAb)

Fig. 4: Depicts the amino acid sequence of Dom 1h-131-206 (An anti TNF alpha R1 dAb)

DETAILED DESCRIPTION OF THE INVENTION

Within this specification the invention has been described, with reference to embodiments, in a way which enables a clear and concise specification to be written. It is intended and should be appreciated that embodiments may be variously combined or separated without parting from the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridization techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel *et al.*, Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. which are incorporated herein by reference) and chemical methods.

As used herein, the term "antagonist of vascular endothelial growth factor (VEGF)" or "anti-VEGF antagonist" or the like refers to an agent (e.g., a molecule, a compound) which binds VEGF and can inhibit a (i.e., one or more) function of VEGF.

As used herein, "peptide" refers to about two to about 50 amino acids that are joined together via peptide bonds.

As used herein, "polypeptide" refers to at least about 50 amino acids that are joined together by peptide bonds. Polypeptides generally comprise tertiary structure and fold into functional domains.

As used herein, a peptide or polypeptide (*e.g.* a domain antibody (dAb)) that is "resistant to protease degradation" is not substantially degraded by a protease when incubated with the protease under conditions suitable for protease activity. A polypeptide (*e.g.*, a dAb) is not substantially degraded when no more than about 25%, no more than about 20%, no more than about 15%, no more than about 14%, no more than about 13%, no more than about 12%, no more than about 11%, no more than about 10%, no more than about 9%, no more than about 8%, no more than about 7%, no more than about 6%, no more than about 5%, no more than about 4%, no more than about 3%, no more that about 2%, no more than about 1%, or substantially none of the protein is degraded by protease after incubation with the protease for about one hour at a

temperature suitable for protease activity. For example at 37 or 50 degrees C. Protein degradation can be assessed using any suitable method, for example, by SDS-PAGE or by functional assay (*e.g.*, ligand binding) as described herein.

As used herein, "target ligand" refers to a ligand which is specifically or selectively bound by a polypeptide or peptide. For example, when a polypeptide is an antibody or antigen-binding fragment thereof, the target ligand can be any desired antigen or epitope. Binding to the target antigen is dependent upon the polypeptide or peptide being functional.

As used herein an antibody refers to IgG, IgM, IgA, IgD or IgE or a fragment (such as a Fab , $F(ab')_2$, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, diabody) whether derived from any species naturally producing an antibody, or created by recombinant DNA technology; whether isolated from serum, B-cells, hybridomas, transfectomas, yeast or bacteria.

As used herein, "antibody format" refers to any suitable polypeptide structure in which one or more antibody variable domains can be incorporated so as to confer binding specificity for antigen on the structure. A variety of suitable antibody formats are known in the art, such as, chimeric antibodies, humanized antibodies, human antibodies, single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy chains and/or light chains, antigen-binding fragments of any of the foregoing (*e.g.*, a Fv fragment (*e.g.*, single chain Fv (scFv), a disulfide bonded Fv), a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment), a single antibody variable domain (*e.g.*, a dAb, V_H, V_{HH}, $V_{L)}$, and modified versions of any of the foregoing (*e.g.*, modified by the covalent attachment of polyethylene glycol or other suitable polymer or a humanized V_{HH}).

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 other V regions or domains. An immunoglobulin single variable domain can be present in a format (*e.g.,* homo- or hetero-multimer) with other 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" as the term is used herein. A "single immunoglobulin variable domain" is the same as an "immunoglobulin single variable domain" as the term is used herein. A "single antibody variable domain" is the same as an "immunoglobulin single variable domain" as the term is used herein. An immunoglobulin single variable domain is in one embodiment 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, the contents of which are incorporated herein by reference in their entirety), 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. The V_{HH} may be humanized.

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. A "single antibody 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.

As used herein, the term "dose" refers to the quantity of ligand administered to a subject all at one time (unit dose), or in two or more administrations over a defined time interval. For example, dose can refer to the quantity of ligand (*e.g.*, ligand comprising an immunoglobulin single variable domain that binds target antigen) administered to a subject over the course of one day (24 hours) (daily dose), two days, one week, two

weeks, three weeks or one or more months (*e.g.*, by a single administration, or by two or more administrations). The interval between doses can be any desired amount of time.

The phrase, "half-life," refers to the time taken for the serum concentration of the ligand (e.g., dAb, polypeptide or antagonist) to reduce by 50%, *in vivo*, for example due to degradation of the ligand and/or clearance or sequestration of the ligand by natural mechanisms. The ligands of the invention can be stabilized *in vivo* and their half-life increased by binding to molecules which resist degradation and/or clearance or sequestration. Typically, such molecules are naturally occurring proteins which themselves have a long half-life *in vivo*. The half-life of a ligand is increased if its functional activity persists, *in vivo*, for a longer period than a similar ligand which is not specific for the half-life increasing molecule. For example, a ligand specific for human serum albumin (HSA) and a target molecule is compared with the same ligand wherein the specificity to HSA is not present, that is does not bind HSA but binds another molecule. For example, it may bind a third target on the cell. Typically, the half-life is increased by 10%, 20%, 30%, 40%, 50% or more. Increases in the range of 2x, 3x, 4x, 5x, 10x, 20x, 30x, 40x, 50x or more of the half-life are possible. Alternatively, or in addition, increases in the range of up to 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 150x of the half-life are possible.

As used herein, "hydrodynamic size" refers to the apparent size of a molecule (e.g., a protein molecule, ligand) based on the diffusion of the molecule through an aqueous solution. The diffusion, or motion of a protein through solution can be processed to derive an apparent size of the protein, where the size is given by the "Stokes radius" or "hydrodynamic radius" of the protein particle. The "hydrodynamic size" of a protein depends on both mass and shape (conformation), such that two proteins having the same molecular mass may have differing hydrodynamic sizes based on the overall conformation of the protein.

As referred to herein, the term "competes" means that the binding of a first target to its cognate target binding domain is inhibited in the presence of a second binding domain that is specific for said cognate target. For example, binding may be inhibited sterically, for example by physical blocking of a binding domain or by

alteration of the structure or environment of a binding domain such that its affinity or avidity for a target is reduced. See WO2006038027 for details of how to perform competition ELISA and competition BiaCore experiments to determine competition between first and second binding domains.

Calculations of "homology" or "identity" or "similarity" between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In an embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, 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. Amino acid and nucleotide sequence alignments and homology, similarity or identity, as defined herein may be prepared and determined using the algorithm BLAST 2 Sequences, using default parameters (Tatusova, T. A. *et al.*, *FEMS Microbiol Lett*, *174*:187-188 (1999).

Protease resistance:

The invention in one embodiment relates to dAbs, e.g. anti-VEGF dAbs, TNFR1 dAbs, IL-1 dAbs, for delivery to the eye, which have been selected by a method of selection for protease resistant dAbs that have a desired biological activity e.g. binding to VEGF, TNFR1 or IL-1. Two selective pressures are used in the method to produce an efficient process for selecting polypeptides that are highly stable and resistant to protease degradation, and that have desired biological activity. As described herein,

protease resistant peptides and polypeptides generally retain biological activity. In contrast, protease sensitive peptides and polypeptides are cleaved or digested by protease in the methods described herein, and therefore, lose their biological activity. Accordingly, protease resistant peptides or polypeptides are generally selected based on their biological activity, such as binding activity.

The ocular environment is one which is rich in proteases and hence use of protease resistant dAbs for ocular delivery as described herein provides several advantages. For example, variable domains that are selected for resistance to proteolytic degradation by one protease (*e.g.*, trypsin), are also resistant to degradation by other proteases (*e.g.*, elastase, leucozyme). Protease resistance can correlate with a higher melting temperature (Tm) of the peptide or polypeptide. Higher melting temperatures are indicative of more stable variable domains, antagonists, peptides and polypeptides. Resistance to protease degradation can also correlate with high affinity binding to target ligands. Thus, the methods described and referenced herein (in WO 2008149143) provide an efficient way to select, isolate and/or recover dAbs that have a desired biological activity and that are well suited for *in vivo* therapeutic and/or diagnostic ocular uses because they are protease resistant and stable. In one embodiment protease resistance can correlate with an improved PK, for example improved over a variable domain, antagonist, peptide or polypeptide that is not protease resistant. Improved PK may be an improved AUC (area under the curve) and/or an improved half-life. Protease resistance can also correlate with an improved stability of the variable domain, antagonist, peptide or polypeptide to shear and/or thermal stress and/or a reduced propensity to aggregate during nebulisation, for example improved over a variable domain, antagonist, peptide or polypeptide that is not protease resistant. In one embodiment protease resistance correlates with an improved storage stability, for example improved over an variable domain, antagonist, peptide or polypeptide that is not protease resistant. In one aspect, one, two, three, four or all of the advantages are provided, the advantages being resistance to protease degradation, higher Tm and high affinity binding to target ligand.

The methods described and referenced herein (in WO 2008/149143) can be used as part of a program to isolate protease resistant peptides or polypeptides, e.g. dAbs that can comprise, if desired, other suitable selection methods. In these situations, the methods described herein can be employed at any desired point in the program, such as before or after other selection methods are used.

In certain embodiments, the dAb for ocular delivery is selected for resistance to degradation by trypsin, elastase or leucozyme and specifically binds VEGF. In these embodiments, a library or repertoire comprising dAbs is provided and combined with trypsin, elastase or leucozyme (or a biological preparation, extract or homogenate comprising trypsin) under conditions suitable for proteolytic digestion. Trypsin, elastase or leucozyme resistant dAbs are selected that bind VEGF. For example, the protease resistant dAb is not substantially degraded when incubated at 37°C in a 0.04% (w/w) solution of protease for a period of at least about 2 hours. In another example, the protease resistant dAb is not substantially degraded when incubated at 37°C in a 0.04% (w/w) solution of protease for a period of at least about 3 hours. In another example, the protease resistant dAb is not substantially degraded when incubated at 37°C in a 0.04% (w/w) solution of protease for a period of at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 7 hours, at least about 8 hours, at least about 9 hours, at least about 10 hours, at least about 11 hours, or at least about 12 hours.

In another aspect, there is provided a method of producing a repertoire of protease resistant peptides or polypeptides (e.g., dAbs). The method comprises providing a repertoire of peptides or polypeptides; combining the repertoire of peptides or polypeptides and a protease under suitable conditions for protease activity; and recovering a plurality of peptides or polypeptides that specifically bind VEGF, whereby a repertoire of protease resistant peptides or polypeptides is produced. Proteases, display systems, conditions for protease activity, and methods for selecting peptides or polypeptides that are suitable for use in the method are described herein with respect to the other methods.

In some embodiments, 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) that comprises a repertoire of peptides or polypeptides is used, and the method further comprises amplifying or increasing the copy number of the nucleic acids that encode the plurality of selected peptides or polypeptides. Nucleic acids can be amplified using any suitable method, such as by phage amplification, cell growth or polymerase chain reaction.

In particular embodiments, there is provided a method of producing a repertoire of protease resistant polypeptides that comprise anti-VEGF dAbs. The method comprises providing a repertoire of polypeptides that comprise anti-VEGF dAbs; combining the repertoire of peptides or polypeptides and a protease (*e.g.*, trypsin, elastase, leucozyme) under suitable conditions for protease activity; and recovering a plurality of polypeptides that comprise dAbs that have binding specificity for VEGF. The method can be used to produce a naïve repertoire, or a repertoire that is biased toward a desired binding specificity, such as an affinity maturation repertoire based on a parental dAb that has binding specificity for VEGF.

Selection/Isolation/Recovery

A protease resistant peptide or polypeptide (*e.g.*, a population of protease resistant polypeptides) can be selected, isolated and/or recovered from a repertoire or library (*e.g.*, in a display system) using any suitable method. In one embodiment, a protease resistant polypeptide 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 one embodiment, the selection is based on specific binding to VEGF. In another embodiment, selection is on the basis of the selected functional characteristic to

produce a second repertoire in which members are protease resistant, followed by selection of a member from the second repertoire that specifically binds VEGF.

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 protease resistant peptides or polypeptides share a common selectable feature. For example, the protease resistant peptide or polypeptide can be selected from a library or repertoire in which substantially all protease resistant peptides or polypeptides 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 protease resistant peptides or polypeptides 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 peptides or polypeptides that contain all or substantially all of the protease resistant peptides or polypeptides that were components of the original library or repertoire. For example, peptides or polypeptides 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 peptides or polypeptides (*e.g.*, a repertoire that has been incubated with protease) can be added to the vessel and the vessel maintained under conditions suitable for peptides or polypeptides to bind the immobilized ligand. Unbound peptides or polypeptides can be washed away and bound peptides or polypeptides 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 peptides or polypeptides to the ligand on the matrix. Peptides or polypeptides that do not bind the affinity matrix can be washed away and bound peptides or polypeptides 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 that competes for binding to the ligand. In one example, a biotinylated target ligand is combined with a repertoire under conditions suitable for peptides or polypeptides in the repertoire to bind the target ligand (VEGF). Bound peptides or polypeptides are recovered using immobilized avidin or streptavidin (*e.g.*, on a bead).

In some embodiments, the generic 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.

Nucleic Acids, host cells and methods for producing protease resistant polypeptides:

The protease resistant peptide or polypeptide selected by the method described herein can also be produced in a suitable *in vitro* expression system e.g. E.coli or Pichia species e.g. P. pastoris, by chemical synthesis or by any other suitable method.

Polypeptides, dAbs & Antagonists:

As described herein, protease resistant dAbs generally bind their target ligand with high affinity.

For example, the VEGF dAb can bind VEGF with an affinity (KD; KD=K_{off} (kd)/K_{on} (ka) as determined by surface plasmon resonance) of 300 nM to 1 pM (*i.e.*, 3 x 10^{-7} to 5 x 10^{-12}M), e.g. 50 nM to 1 pM, e.g. 5 nM to 1 pM and e.g. 1 nM to 1 pM; for example K_D of 1 x 10^{-7} M or less, e.g. 1 x 10^{-8} M or less, e.g. 1 x 10^{-9} M or less, e.g. 1 x 10^{-10} M or less and e.g. 1 x 10^{-11} M or less; and/or a K_{off} rate constant of 5 x 10^{-1} s^{-1} to 1 x 10^{-7} s^{-1}, e.g. 1 x 10^{-2} s^{-1} to 1 x 10^{-6} s^{-1}, e.g. 5 x 10^{-3} s^{-1} to 1 x 10^{-5} s^{-1}, for example 5 x

10^{-1} s^{-1} or less, e.g. 1 x 10^{-2} s^{-1} or less, e.g. 1 x 10^{-3} s^{-1} or less, e.g. 1 x 10^{-4} s^{-1} or less, e.g. 1 x 10^{-5} s^{-1} or less, and e.g. 1 x 10^{-6} s^{-1} or less as determined by surface plasmon resonance.

Although we are not bound by any particular theory, peptides and polypeptides that are resistant to proteases are believed to have a lower entropy and/or a higher stabilization energy. Thus, the correlation between protease resistance and high affinity binding may be related to the compactness and stability of the surfaces of the peptides and polypeptides and dAbs selected by the method described herein.

In one embodiment, a VEGF dAb inhibits binding of VEGF at a concentration 50 (IC50) of IC50 of about 1 μM or less, about 500 nM or less, about 100 nM or less, about 75 nM or less, about 50 nM or less, about 10 nM or less or about 1 nM or less.

In certain embodiments, the VEGF dAb specifically binds VEGF, eg, human VEGF, and dissociates from human VEGF with a dissociation constant (K_D) of 300 nM to 1pM or 300nM to 5pM or 50nM to 1pM or 50nM to 5pM or 50nM to 20 pM or about 10 pM or about 15pM or about 20pM as determined by surface plasmon resonance. In certain embodiments, the polypeptide, dAb or antagonist specifically binds VEGF, eg, human VEGF, and dissociates from human VEGF with a K_{off} rate constant of 5 x 10^{-1} s^{-1} to 1 x 10^{-7} s^{-1}, e.g. 1 x 10^{-2} s^{-1} to 1 x 10^{-6} s^{-1}, e.g. 5 x 10^{-3} s^{-1} to 1 x 10^{-5} s^{-1}, for example 5 x 10^{-1} s^{-1} or less, e.g. 1 x 10^{-2} s^{-1} or less, e.g.1 x 10^{-3} s^{-1} or less, e.g. 1 x 10^{-4} s^{-1} or less, e.g. 1 x 10^{-5} s^{-1} or less, and e.g. 1 x 10^{-6} s^{-1} or less as determined by surface plasmon resonance.

. In certain embodiments, VEGF dAb specifically binds VEGF, eg, human VEGF, with a K_{on} of 1x10^{-3} $M^{-1}s^{-1}$ to 1x10^{-7} $M^{-1}s^{-1}$ or 1x10^{-3} $M^{-1}s^{-1}$ to 1x10^{-6} $M^{-1}s^{-1}$ or about 1x10^{-4} $M^{-1}s^{-1}$ or about 1x10^{-5} $M^{-1}s^{-1}$. In one embodiment, the polypeptide, dAb or antagonist specifically binds VEGF, eg, human VEGF, and dissociates from human VEGF with a dissociation constant (K_D) and a K_{off} as defined in this paragraph. In one embodiment, the polypeptide, dAb or antagonist specifically binds VEGF, eg, human

VEGF, and dissociates from human VEGF with a dissociation constant (K_D) and a K_{on} as defined in this paragraph. In some embodiments, the polypeptide or dAb specifically binds VEGF (eg, human VEGF) with a K_D and/or K_{off} and/or K_{on} as recited in this paragraph and comprises an amino acid sequence that is at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a dAb with the amino acid sequence of DOM15-26-593.

The dAb can be expressed in *E. coli* or in *Pichia* species (e.g., *P. pastoris*). In one embodiment, the ligand or dAb monomer is secreted in a quantity of at least about 0.5 mg/L when expressed in *E. coli* or in *Pichia* species (e.g., *P. pastoris*). Although, the ligands and dAb monomers described herein can be secretable when expressed in *E. coli* or in *Pichia* species (e.g., *P. pastoris*), they can be produced using any suitable method, such as synthetic chemical methods or biological production methods that do not employ *E. coli* or *Pichia* species.

In some embodiments, the polypeptide, dAb or antagonist does not comprise a *Camelid* immunoglobulin variable domain, or one or more framework amino acids that are unique to immunoglobulin variable domains encoded by *Camelid* germline antibody gene segments , eg at position 108, 37, 44, 45 and/or 47.

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Antagonists of VEGF can be monovalent or multivalent. In some embodiments, the antagonist is monovalent and contains one binding site that interacts with VEGF, the binding site provided by a polypeptide or dAb of the invention. Monovalent antagonists bind one VEGF and may not induce cross-linking or clustering of VEGF on the surface of cells which can lead to activation of the receptor and signal transduction.

Alternatively the antagonist of VEGF is multivalent. Multivalent antagonists of VEGF can contain two or more copies of a particular binding site for VEGF or contain two or more different binding sites that bind VEGF, at least one of the binding sites being provided by a dAb of the invention. For example, as described herein the antagonist of VEGF can be a dimer, trimer or multimer comprising two or more copies of a dAb that binds VEGF, or two or more different dAbs that bind VEGF.

In other embodiments, the, dAb specifically binds VEGF with a K_D described herein and inhibits tumour growth in a standard murine xenograft model (e.g., inhibits tumour growth by at least about 10%, as compared with a suitable control). In one embodiment, the polypeptide, dAb or antagonist inhibits tumour growth by at least about 10% or by at least about 25%, or by at least about 50%, as compared to a suitable control in a standard murine xenograft model when administered at about 1 mg/kg or more, for example about 5 or 10 mg/kg.

In other embodiments, the polypeptide, dAb or antagonist binds VEGF and antagonizes the activity of the VEGF in a standard cell assay with an ND_{50} of $\leq$ 100 nM.

In certain embodiments, the dAbs are efficacious in animal models of ocular disease when an effective amount is administered. Generally an effective amount is about 1 mg/kg to about 10 mg/kg (e.g., about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, or about 10 mg/kg). The dAb can be administered at a dosing frequency of e.g. once or twice daily, once or twice weekly, once or twice monthly.

Generally, the dAbs will be utilized in purified form together with pharmacologically appropriate carriers for ocular delivery. Typically, these carriers can include aqueous or alcoholic/aqueous solutions, emulsions or suspensions, any including saline and/or buffered media. Suitable physiologically-acceptable adjuvants, if necessary to keep a polypeptide complex in suspension, may be chosen from thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin and alginates. Preservatives and other additives, such as antimicrobials, antioxidants, chelating agents and inert gases, may also be present (Mack (1982) *Remington's Pharmaceutical Sciences*, 16th Edition). A variety of suitable formulations can be used, including extended release formulations. . These might comprise, implants, gels, nanoparticles, and microparticles. Drug loaded PLA nano- and microparticles have been used to deliver drug to the posterior segment of the eye after sub-conjunctival delivery of the formulation (Kompella et al IOVS 2003 44(3) 1192-1201). In particular, microspheres

are retained at the site of delivery and appear to be more appropriate for retinal drug delivery compared to nanoparticles which may be cleared more readily (Amrite et al ARVO abstract #5067/B391 2003).

The ligands (e.g., antagonists) of the present invention may be used as separately administered compositions or in conjunction with other agents. These can include various drugs for ocular delivery to the eye and/or ocular penetration enhancers and/or viscosity enhancers.

Pharmaceutical compositions can include "cocktails" of various other agents in conjunction with the ligands of the present invention, or even combinations of ligands according to the present invention having different specificities, such as ligands selected using different target antigens or epitopes, whether or not they are pooled prior to administration.

The precise dosage and frequency of administration of the dAbs to the eye will depend on the age, sex and condition of the patient, concurrent administration of other drugs, counterindications and other parameters to be taken into account by the clinician.

The dAbs of this invention 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 lyophilisation and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immunoglobulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted upward to compensate.

The compositions containing the present dAbs or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In certain therapeutic

applications, an adequate amount to accomplish at least partial inhibition, suppression, modulation, killing, or some other measurable parameter, of a population of selected cells can be defined as a "therapeutically-effective dose". Amounts needed to achieve this dosage will depend upon the severity of the disease and the general state of the patient's own immune system. The skilled clinician will be able to determine the appropriate dosing interval to treat, suppress or prevent disease.

Treatment or therapy performed using the compositions described herein is considered “effective” if one or more symptoms are reduced (e.g., by at least 10% or at least one point on a clinical assessment scale), relative to such symptoms present before treatment, or relative to such symptoms in an individual (human or model animal) not treated with such composition or other suitable control. Symptoms will obviously vary depending upon the disease or disorder targeted, but can be measured by an ordinarily skilled clinician or technician. Such symptoms can be measured, for example, by monitoring the level of one or more biochemical indicators of the disease or disorder (e.g., levels of an enzyme or metabolite correlated with the disease, affected cell numbers, etc.), by monitoring physical manifestations or by an accepted clinical assessment scale.

Similarly, prophylaxis performed using a composition as described herein is “effective” if the onset or severity of one or more symptoms is delayed, reduced or abolished relative to such symptoms in a similar individual (human or animal model) not treated with the composition.

In one embodiment, the invention is a method for treating, suppressing or preventing ocular disease or condition, selected from for example cancer (e.g. a solid tumour), inflammatory disease, autoimmune disease, vascular proliferative disease (e.g.AMD (age related macular degeneration)) comprising administering to a mammal in need thereof a therapeutically-effective dose or amount of a polypeptide, dAb which binds to VEGF or antagonist of VEGF according to the invention or to IL-1, or TNF-α

or TNF-αR. Examples of such ocular diseases or conditions include AMD, Uveitis, dry eye, diabetic retinopathy and diabetic macular oedema.

Formats:

Increased half-life is useful in *in vivo* applications of immunoglobulins, especially antibodies and most especially antibody fragments of small size. Such fragments (Fvs, disulphide bonded Fvs, Fabs, scFvs, dAbs) can suffer from rapid clearance from the body; thus, whilst they are able to reach most parts of the body rapidly, and are quick to produce and easier to handle, their *in vivo* applications have been limited by their only brief persistence *in vivo*. Hence the dAbs described herein can be modified to provide increased half-life *in vivo* and consequently longer persistence times in the body.

Methods for pharmacokinetic analysis and determination of ligand half-life will be familiar to those skilled in the art. Details may be found in *Kenneth, A et al:* Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in *Peters et al,* Pharmacokinetic analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. ex edition (1982), which describes pharmacokinetic parameters such as t alpha and t beta half lives and area under the curve (AUC).

Half lives (t½ alpha and t½ beta) and AUC can be determined from a curve of serum concentration of ligand against time. The WinNonlin analysis package (available from Pharsight Corp., Mountain View, CA94040, USA) can be used, for example, to model the curve. In a first phase (the alpha phase) the ligand is undergoing mainly distribution in the patient, with some elimination. A second phase (beta phase) is the terminal phase when the ligand has been distributed and the serum concentration is decreasing as the ligand is cleared from the patient. The t alpha half life is the half life of the first phase and the t beta half life is the half life of the second phase. Thus, in one embodiment, the present invention provides a ligand or a composition comprising a ligand according to the invention having a tα half–life in the range of 15 minutes or more. In one embodiment, the lower end of the range is 30 minutes, 45 minutes, 1 hour,

2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 10 hours, 11 hours or 12 hours. In addition, or alternatively, a ligand or composition according to the invention will have a tα half life in the range of up to and including 12 hours. In one embodiment, the upper end of the range is 11, 10, 9, 8, 7, 6 or 5 hours. An example of a suitable range is 1 to 6 hours, 2 to 5 hours or 3 to 4 hours.

In one embodiment, the dAb or a composition comprising a dAb according to the invention has a tβ half–life in the range of 30 minutes or more. In one embodiment, the lower end of the range is 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 10 hours , 11 hours, or 12 hours. In addition, or alternatively, a ligand or composition according to the invention has a tβ half–life in the range of up to and including 21 days. In one embodiment, the upper end of the range is 12 hours, 24 hours, 2 days, 3 days, 5 days, 10 days, 15 days or 20 days. In one embodiment a ligand or composition according to the invention will have a tβ half life in the range 12 to 60 hours. In a further embodiment, it will be in the range 12 to 48 hours. In a further embodiment still, it will be in the range 12 to 26 hours.

In addition, or alternatively to the above criteria, the present invention provides a dAb or a composition comprising a ligand according to the invention having an AUC value (area under the curve) in the range of 1 mg.min/ml or more. In one embodiment, the lower end of the range is 5, 10, 15, 20, 30, 100, 200 or 300 mg.min/ml. In addition, or alternatively, a ligand or composition according to the invention has an AUC in the range of up to 600 mg.min/ml. In one embodiment, the upper end of the range is 500, 400, 300, 200, 150, 100, 75 or 50 mg.min/ml. In one embodiment a ligand according to the invention will have a AUC in the range selected from the group consisting of the following: 15 to 150 mg.min/ml, 15 to 100 mg.min/ml, 15 to 75 mg.min/ml, and 15 to 50mg.min/ml.

dAbs of the invention can be formatted to have a larger hydrodynamic size, for example, by attachment of a PEG group, serum albumin, transferrin, transferrin receptor or at least the transferrin-binding portion thereof, an antibody Fc region, or by conjugation to an antibody domain. For example, dAbs can be formatted as a larger antigen-binding fragment of an antibody, or as an antibody (e.g., formatted as a Fab,

Fab', $F(ab)_2$, $F(ab')_2$, IgG, scFv). In another embodiment dAbs according to the invention can be formatted as a fusion or conjugate with another polypeptide or peptide.

Hydrodynamic size of the ligands (*e.g.*, dAb monomers and multimers) of the invention may be determined using methods which are well known in the art. For example, gel filtration chromatography may be used to determine the hydrodynamic size of a ligand. Suitable gel filtration matrices for determining the hydrodynamic sizes of ligands, such as cross-linked agarose matrices, are well known and readily available.

The size of a ligand i.e. dAb format (*e.g.*, the size of a PEG moiety attached to a dAb monomer), can be varied depending on the desired application e.g. if it is desired to have the dAb remain in the systemic circulation for a longer period of time the size of can be increased, for example by formatting as an Ig like protein.

Half-life extension by targeting an antigen or epitope that increases half-live *in vivo*

The hydrodynamic size of a ligand and its serum half-life can also be increased by conjugating or associating a dAb to a binding domain (e.g., antibody or antibody fragment) that binds an antigen or epitope that increases half-live *in vivo*, as described herein. For example, the VEGF dAb can be conjugated or linked to an anti-serum albumin or anti-neonatal Fc receptor antibody or antibody fragment, eg an anti-SA or anti-neonatal Fc receptor dAb, Fab, Fab' or scFv, or to an anti-SA affibody or anti-neonatal Fc receptor Affibody or an anti-SA avimer, or an anti-SA binding domain which comprises a scaffold selected from, but preferably not limited to, the group consisting of CTLA-4, lipocallin, SpA, an affibody, an avimer, GroEl and fibronectin (see PCT/GB2008/000453 filed 8th February 2008 for disclosure of these binding domain, which domains and their sequences are incorporated herein by reference and form part of the disclosure of the present text). Conjugating refers to a composition comprising polypeptide, dAb or antagonist of the invention that is bonded (covalently or noncovalently) to a binding domain that binds serum albumin.

Suitable polypeptides that enhance serum half-life *in vivo* include, for example, transferrin receptor specific ligand-neuropharmaceutical agent fusion proteins (see U.S.

Patent No. 5,977,307, the teachings of which are incorporated herein by reference), brain capillary endothelial cell receptor, transferrin, transferrin receptor (*e.g.*, soluble transferrin receptor), insulin, insulin-like growth factor 1 (IGF 1) receptor, insulin-like growth factor 2 (IGF 2) receptor, insulin receptor, blood coagulation factor X, αl-antitrypsin and HNF 1α. Suitable polypeptides that enhance serum half-life also include alpha-1 glycoprotein (orosomucoid; AAG), alpha-1 antichymotrypsin (ACT), alpha-1 microglobulin (protein HC; AIM), antithrombin III (AT III), apolipoprotein A-1 (Apo A-1), apolipoprotein B (Apo B), ceruloplasmin (Cp), complement component C3 (C3), complement component C4 (C4), C1 esterase inhibitor (C1 INH), C-reactive protein (CRP), ferritin (FER), hemopexin (HPX), lipoprotein(a) (Lp(a)), mannose-binding protein (MBP), myoglobin (Myo), prealbumin (transthyretin; PAL), retinol-binding protein (RBP), and rheumatoid factor (RF).

Suitable proteins from the extracellular matrix include, for example, collagens, laminins, integrins and fibronectin. Collagens are the major proteins of the extracellular matrix. About 15 types of collagen molecules are currently known, found in different parts of the body, *e.g.* type I collagen (accounting for 90% of body collagen) found in bone, skin, tendon, ligaments, cornea, internal organs or type II collagen found in cartilage, vertebral disc, notochord, and vitreous humor of the eye.

Suitable proteins from the blood include, for example, plasma proteins (*e.g.*, fibrin, α-2 macroglobulin, serum albumin, fibrinogen (*e.g.*, fibrinogen A, fibrinogen B), serum amyloid protein A, haptoglobin, profilin, ubiquitin, uteroglobulin and β-2-microglobulin), enzymes and enzyme inhibitors (*e.g.*, plasminogen, lysozyme, cystatin C, alpha-1-antitrypsin and pancreatic trypsin inhibitor), proteins of the immune system, such as immunoglobulin proteins (*e.g.*, IgA, IgD, IgE, IgG, IgM, immunoglobulin light chains (kappa/lambda)), transport proteins (*e.g.*, retinol binding protein, α-1 microglobulin), defensins (*e.g.*, beta-defensin 1, neutrophil defensin 1, neutrophil defensin 2 and neutrophil defensin 3) and the like.

Suitable proteins found at the blood brain barrier or in neural tissue include, for example, melanocortin receptor, myelin, ascorbate transporter and the like.

Suitable polypeptides that enhance serum half-life *in vivo* also include proteins localized to the kidney (*e.g.*, polycystin, type IV collagen, organic anion transporter Kl, Heymann's antigen), proteins localized to the liver (*e.g.*, alcohol dehydrogenase, G250), proteins localized to the lung (*e.g.*, secretary component, which binds IgA), proteins localized to the heart (*e.g.*, HSP 27, which is associated with dilated cardiomyopathy), proteins localized to the skin (*e.g.*, keratin), bone specific proteins such as morphogenic proteins (BMPs), which are a subset of the transforming growth factor β superfamily of proteins that demonstrate osteogenic activity (*e.g.*, BMP-2, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8), tumor specific proteins (*e.g.*, trophoblast antigen, herceptin receptor, oestrogen receptor, cathepsins (*e.g.*, cathepsin B, which can be found in liver and spleen)).

Suitable disease-specific proteins include, for example, antigens expressed only on activated T-cells, including LAG-3 (lymphocyte activation gene), osteoprotegerin ligand (OPGL; see *Nature* 402, 304-309 (1999)), OX40 (a member of the TNF receptor family, expressed on activated T cells and specifically up-regulated in human T cell leukemia virus type-I (HTLV-I)-producing cells; see *Immunol.* 165 (1):263-70 (2000)). Suitable disease-specific proteins also include, for example, metalloproteases (associated with arthritis/cancers) including CG6512 Drosophila, human paraplegin, human FtsH, human AFG3L2, murine ftsH; and angiogenic growth factors, including acidic fibroblast growth factor (FGF-1), basic fibroblast growth factor (FGF-2), vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), transforming growth factor-α (TGF α), tumor necrosis factor-alpha (TNF-α), angiogenin, interleukin-3 (IL-3), interleukin-8 (IL-8), platelet-derived endothelial growth factor (PD-ECGF), placental growth factor (P1GF), midkine platelet-derived growth factor-BB (PDGF), and fractalkine.

Suitable polypeptides that enhance serum half-life *in vivo* also include stress proteins such as heat shock proteins (HSPs). HSPs are normally found intracellularly. When they are found extracellularly, it is an indicator that a cell has died and spilled out its contents. This unprogrammed cell death (necrosis) occurs when as a result of trauma, disease or injury, extracellular HSPs trigger a response from the immune system.

Binding to extracellular HSP can result in localizing the compositions of the invention to a disease site.

Suitable proteins involved in Fc transport include, for example, Brambell receptor (also known as FcRB). This Fc receptor has two functions, both of which are potentially useful for delivery. The functions are (1) transport of IgG from mother to child across the placenta (2) protection of IgG from degradation thereby prolonging its serum half-life. It is thought that the receptor recycles IgG from endosomes. (See, Holliger *et al, Nat Biotechnol* 15(7):632-6 (1997).)

dAbs that Bind Serum Albumin

The invention in one embodiment a first dAb that binds to an ocular target molecule, e.g. VEGF, IL-1, or TNF-α, and a second dAb that binds serum albumin (SA), the second dAb binding SA with a K_D as determined by surface plasmon resonance of 1nM to 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 100, 200, 300, 400 or 500 μ M (*i.e.*, x 10^{-9} to 5 x 10^{-4}), or 100 nM to 10 μ M, or 1 to 5 μ M or 3 to 70 nM or 10nM to 1, 2, 3, 4 or 5μ M. For example 30 to 70 nM as determined by surface plasmon resonance. In one embodiment, the first dAb (or a dAb monomer) binds SA (*e.g.*, HSA) with a K_D as determined by surface plasmon resonance of approximately 1, 50, 70, 100, 150, 200, 300 nM or 1, 2 or 3 μ M. In one embodiment, for a dual specific ligand comprising a first anti-SA dAb and a second dAb to VEGF, the affinity (eg K_D and/or K_{off} as measured by surface plasmon resonance, eg using BiaCore) of the second dAb for its target is from 1 to 100000 times (e.g. 100 to 100000, or 1000 to 100000, or 10000 to 100000 times) the affinity of the first dAb for SA. In one embodiment, the serum albumin is human serum albumin (HSA). For example, the first dAb binds SA with an affinity of approximately 10 μM, while the second dAb binds its target with an affinity of 100 pM. In one embodiment, the serum albumin is human serum albumin (HSA). In one embodiment, the first dAb binds SA (eg, HSA) with a K_D of approximately 50, for example 70, 100, 150 or 200 nM. Details of dual specific ligands are found in WO03002609, WO04003019 and WO04058821.

The dAbs of the invention can in one embodiment comprise a dAb that binds serum albumin (SA) with a K_D as determined by surface plasmon resonance of 1nM to 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 100, 200, 300, 400 or 500 μ M (*i.e.*, x 10-9 to 5 x 10-4), or 100 nM to 10 μ M, or 1 to 5 μ M or 3 to 70 nM or 10nM to 1, 2, 3, 4 or 5μ M. For example 30 to 70 nM as determined by surface plasmon resonance. In one embodiment, the first dAb (or a dAb monomer) binds SA (*e.g.*, HSA) with a K_D as determined by surface plasmon resonanceof approximately 1, 50, 70, 100, 150, 200, 300 nM or 1, 2 or 3 μ M. In one embodiment, the first and second dAbs are linked by a linker, for example a linker of from 1 to 4 amino acids or from 1 to 3 amino acids, or greater than 3 amino acids or greater than 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids. In one embodiment, a longer linker (greater than 3 amino acids) is used to enhance potency (K_D of one or both dAbs in the antagonist).

In particular embodiments, the dAb binds human serum albumin and competes for binding to albumin with a dAb selected from the group consisting of

MSA-16, MSA-26 (See WO04003019 for disclosure of these sequences, which sequences and their nucleic acid counterpart are incorporated herein by reference and form part of the disclosure of the present text),

DOM7m-16 (SEQ ID NO: 473), DOM7m-12 (SEQ ID NO: 474), DOM7m-26 (SEQ ID NO: 475), DOM7r-1 (SEQ ID NO: 476), DOM7r-3 (SEQ ID NO: 477), DOM7r-4 (SEQ ID NO: 478), DOM7r-5 (SEQ ID NO: 479), DOM7r-7 (SEQ ID NO: 480), DOM7r-8 (SEQ ID NO: 481), DOM7h-2 (SEQ ID NO: 482), DOM7h-3 (SEQ ID NO: 483), DOM7h-4 (SEQ ID NO: 484), DOM7h-6 (SEQ ID NO: 485), DOM7h-1 (SEQ ID NO: 486), DOM7h-7 (SEQ ID NO: 487), DOM7h-22 (SEQ ID NO: 489), DOM7h-23 (SEQ ID NO: 490), DOM7h-24 (SEQ ID NO: 491), DOM7h-25 (SEQ ID NO: 492), DOM7h-26 (SEQ ID NO: 493), DOM7h-21 (SEQ ID NO: 494), DOM7h-27 (SEQ ID NO: 495), DOM7h-8 (SEQ ID NO: 496), DOM7r-13 (SEQ ID NO: 497), DOM7r-14 (SEQ ID NO: 498), DOM7r-15 (SEQ ID NO: 499), DOM7r-16 (SEQ ID NO: 500), DOM7r-17 (SEQ ID NO: 501), DOM7r-18 (SEQ ID NO: 502), DOM7r-19 (SEQ ID NO: 503), DOM7r-20 (SEQ ID NO: 504), DOM7r-21 (SEQ ID NO: 505),

DOM7r-22 (SEQ ID NO: 506), DOM7r-23 (SEQ ID NO: 507), DOM7r-24 (SEQ ID NO: 508), DOM7r-25 (SEQ ID NO: 509), DOM7r-26 (SEQ ID NO: 510), DOM7r-27 (SEQ ID NO: 511), DOM7r-28 (SEQ ID NO: 512), DOM7r-29 (SEQ ID NO: 513), DOM7r-30 (SEQ ID NO: 514), DOM7r-31 (SEQ ID NO: 515), DOM7r-32 (SEQ ID NO: 516), DOM7r-33 (SEQ ID NO: 517) (See WO2007080392 for disclosure of these sequences, which sequences and their nucleic acid counterpart are incorporated herein by reference and form part of the disclosure of the present text; the SEQ ID No's in this paragraph are those that appear in WO2007080392),

dAb8 (dAb10), dAb 10, dAb36, dAb7r20 (DOM7r20), dAb7r21 (DOM7r21), dAb7r22 (DOM7r22), dAb7r23 (DOM7r23), dAb7r24 (DOM7r24), dAb7r25 (DOM7r25), dAb7r26 (DOM7r26), dAb7r27 (DOM7r27), dAb7r28 (DOM7r28), dAb7r29 (DOM7r29), dAb7r29 (DOM7r29), dAb7r31 (DOM7r31), dAb7r32 (DOM7r32), dAb7r33 (DOM7r33), dAb7r33 (DOM7r33), dAb7h22 (DOM7h22), dAb7h23 (DOM7h23), dAb7h24 (DOM7h24), dAb7h25 (DOM7h25), dAb7h26 (DOM7h26), dAb7h27 (DOM7h27), dAb7h30 (DOM7h30), dAb7h31 (DOM7h31), dAb2 (dAbs 4,7,41), dAb4, dAb7, dAb11, dAb12 (dAb7m12), dAb13 (dAb 15), dAb15, dAb16 (dAb21, dAb7m16) , dAb17, dAb18, dAb19, dAb21, dAb22, dAb23, dAb24, dAb25 (dAb26, dAb7m26), dAb27, dAb30 (dAb35), dAb31, dAb33, dAb34, dAb35, dAb38 (dAb54), dAb41, dAb46 (dAbs 47, 52 and 56), dAb47, dAb52, dAb53, dAb54, dAb55, dAb56, dAb7m12, dAb7m16, dAb7m26, dAb7r1 (DOM 7r1), dAb7r3 (DOM7r3), dAb7r4 (DOM7r4), dAb7r5 (DOM7r5), dAb7r7 (DOM7r7), dAb7r8 (DOM7r8), dAb7r13 (DOM7r13), dAb7r14 (DOM7r14), dAb7r15 (DOM7r15), dAb7r16 (DOM7r16), dAb7r17 (DOM7r17), dAb7r18 (DOM7r18), dAb7r19 (DOM7r19), dAb7h1 (DOM7h1), dAb7h2 (DOM7h2), dAb7h6 (DOM7h6), dAb7h7 (DOM7h7), dAb7h8 (DOM7h8), dAb7h9 (DOM7h9), dAb7h10 (DOM7h10), dAb7h11 (DOM7h11), dAb7h12 (DOM7h12), dAb7h13 (DOM7h13), dAb7h14 (DOM7h14), dAb7p1 (DOM7p1), and dAb7p2 (DOM7p2) (see PCT/GB2008/000453 filed 8th February 2008 and published as WO 2008/096158 for disclosure of these sequences, which sequences and their nucleic acid counterpart are incorporated herein by reference and form part of the disclosure of the present text). Alternative names are shown in

brackets after the dAb, e.g. dAb8 has an alternative name which is dAb10 i.e. dAb8 (dAb10).

In certain embodiments, the dAb binds human serum albumin and comprises an amino acid sequence that has at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% amino acid sequence identity with the amino acid sequence of a dAb selected from the group consisting of

MSA-16, MSA-26,

DOM7m-16 (SEQ ID NO: 473), DOM7m-12 (SEQ ID NO: 474), DOM7m-26 (SEQ ID NO: 475), DOM7r-1 (SEQ ID NO: 476), DOM7r-3 (SEQ ID NO: 477), DOM7r-4 (SEQ ID NO: 478), DOM7r-5 (SEQ ID NO: 479), DOM7r-7 (SEQ ID NO: 480), DOM7r-8 (SEQ ID NO: 481), DOM7h-2 (SEQ ID NO: 482), DOM7h-3 (SEQ ID NO: 483), DOM7h-4 (SEQ ID NO: 484), DOM7h-6 (SEQ ID NO: 485), DOM7h-1 (SEQ ID NO: 486), DOM7h-7 (SEQ ID NO: 487), DOM7h-22 (SEQ ID NO: 489), DOM7h-23 (SEQ ID NO: 490), DOM7h-24 (SEQ ID NO: 491), DOM7h-25 (SEQ ID NO: 492), DOM7h-26 (SEQ ID NO: 493), DOM7h-21 (SEQ ID NO: 494), DOM7h-27 (SEQ ID NO: 495), DOM7h-8 (SEQ ID NO: 496), DOM7r-13 (SEQ ID NO: 497), DOM7r-14 (SEQ ID NO: 498), DOM7r-15 (SEQ ID NO: 499), DOM7r-16 (SEQ ID NO: 500), DOM7r-17 (SEQ ID NO: 501), DOM7r-18 (SEQ ID NO: 502), DOM7r-19 (SEQ ID NO: 503), DOM7r-20 (SEQ ID NO: 504), DOM7r-21 (SEQ ID NO: 505), DOM7r-22 (SEQ ID NO: 506), DOM7r-23 (SEQ ID NO: 507), DOM7r-24 (SEQ ID NO: 508), DOM7r-25 (SEQ ID NO: 509), DOM7r-26 (SEQ ID NO: 510), DOM7r-27 (SEQ ID NO: 511), DOM7r-28 (SEQ ID NO: 512), DOM7r-29 (SEQ ID NO: 513), DOM7r-30 (SEQ ID NO: 514), DOM7r-31 (SEQ ID NO: 515), DOM7r-32 (SEQ ID NO: 516), DOM7r-33 (SEQ ID NO: 517) (the SEQ ID No's in this paragraph are those that appear in WO2007080392),

dAb8, dAb 10, dAb36, dAb7r20, dAb7r21, dAb7r22, dAb7r23, dAb7r24, dAb7r25, dAb7r26, dAb7r27, dAb7r28, dAb7r29, dAb7r30, dAb7r31, dAb7r32, dAb7r33, dAb7h21, dAb7h22, dAb7h23, Ab7h24, Ab7h25, Ab7h26, dAb7h27, dAb7h30, dAb7h31, dAb2, dAb4, dAb7, dAb11, dAb12, dAb13, dAb15, dAb16,

dAb17, dAb18, dAb19, dAb21, dAb22, dAb23, dAb24, dAb25, dAb26, dAb27, dAb30, dAb31, dAb33, dAb34, dAb35, dAb38, dAb41, dAb46, dAb47, dAb52, dAb53, dAb54, dAb55, dAb56, dAb7m12, dAb7m16, dAb7m26, dAb7r1, dAb7r3, dAb7r4, dAb7r5, dAb7r7, dAb7r8, dAb7r13, dAb7r14, dAb7r15, dAb7r16, dAb7r17, dAb7r18, dAb7r19, dAb7h1, dAb7h2, dAb7h6, dAb7h7, dAb7h8, dAb7h9, dAb7h10, dAb7h11, dAb7h12, dAb7h13, dAb7h14, dAb7p1, and dAb7p2.

For example, the dAb that binds human serum albumin can comprise an amino acid sequence that has at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% amino acid sequence identity with DOM7h-2 (SEQ ID NO:482), DOM7h-3 (SEQ ID NO:483), DOM7h-4 (SEQ ID NO:484), DOM7h-6 (SEQ ID NO:485), DOM7h-1 (SEQ ID NO:486), DOM7h-7 (SEQ ID NO:487), DOM7h-8 (SEQ ID NO:496), DOM7r-13 (SEQ ID NO:497), DOM7r-14 (SEQ ID NO:498), DOM7h-22 (SEQ ID NO:489), DOM7h-23 (SEQ ID NO:490), DOM7h-24 (SEQ ID NO:491), DOM7h-25 (SEQ ID NO:492), DOM7h-26 (SEQ ID NO:493), DOM7h-21 (SEQ ID NO:494), DOM7h-27 (SEQ ID NO:495) (the SEQ ID No's in this paragraph are those that appear in WO2007080392),

dAb8, dAb 10, dAb36, dAb7h21, dAb7h22, dAb7h23, Ab7h24, Ab7h25, Ab7h26, dAb7h27, dAb7h30, dAb7h31, dAb2, dAb4, dAb7, dAb11, dAb12, dAb13, dAb15, dAb16, dAb17, dAb18, dAb19, dAb21, dAb22, dAb23, dAb24, dAb25, dAb26, dAb27, dAb30, dAb31, dAb33, dAb34, dAb35, dAb38, dAb41, dAb46, dAb47, dAb52, dAb53, dAb54, dAb55, dAb56, dAb7h1, dAb7h2, dAb7h6, dAb7h7, dAb7h8, dAb7h9, dAb7h10, dAb7h11, dAb7h12, dAb7h13 and dAb7h14.

In certain embodiments, the dAb binds human serum albumin and comprises an amino acid sequence that has at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% amino acid sequence identity with the amino acid sequence of a dAb selected from the group consisting of

DOM7h-2 (SEQ ID NO:482), DOM7h-6 (SEQ ID NO:485), DOM7h-1 (SEQ ID NO:486), DOM7h-7 (SEQ ID NO:487), DOM7h-8 (SEQ ID NO:496), DOM7h-22 (SEQ ID NO:489), DOM7h-23 (SEQ ID NO:490), DOM7h-24 (SEQ ID NO:491),

DOM7h-25 (SEQ ID NO:492), DOM7h-26 (SEQ ID NO:493), DOM7h-21 (SEQ ID NO:494), DOM7h-27 (SEQ ID NO:495) (the SEQ ID No's in this paragraph are those that appear in WO2007080392),

dAb7h21, dAb7h22, dAb7h23, Ab7h24, Ab7h25, Ab7h26, dAb7h27, dAb7h30, dAb7h31, dAb2, dAb4, dAb7, dAb38, dAb41, dAb7h1, dAb7h2, dAb7h6, dAb7h7, dAb7h8, dAb7h9, dAb7h10, dAb7h11, dAb7h12, dAb7h13 and dAb7h14.

In more particular embodiments, the dAb is a V_K dAb that binds human serum albumin and has an amino acid sequence selected from the group consisting of

DOM7h-2 (SEQ ID NO:482), DOM7h-6 (SEQ ID NO:485), DOM7h-1 (SEQ ID NO:486), DOM7h-7 (SEQ ID NO:487), DOM7h-8 (SEQ ID NO:496) (the SEQ ID No's in this paragraph are those that appear in WO2007080392),

dAb2, dAb4, dAb7, dAb38, dAb41, dAb54, dAb7h1, dAb7h2, dAb7h6, dAb7h7, dAb7h8, dAb7h9, dAb7h10, dAb7h11, dAb7h12, dAb7h13 and dAb7h14. ,

In more particular embodiments, the dAb is a V_H dAb that binds human serum albumin and has an amino acid sequence selected from dAb7h30 and dAb7h31.

In more particular embodiments, the dAb is dAb7h11 or dAb7h14.

In other embodiments, the dAb, ligand or antagonist binds human serum albumin and comprises one, two or three of the CDRs of any of the foregoing amino acid sequences, eg one, two or three of the CDRs of dAb7h11 or dAb7h14.

Suitable *Camelid* V_{HH} that bind serum albumin include those disclosed in WO 2004/041862 (Ablynx N.V.) and in WO2007080392 (which V_{HH} sequences and their nucleic acid counterpart are incorporated herein by reference and form part of the disclosure of the present text), such as Sequence A (SEQ ID NO:518), Sequence B (SEQ ID NO:519), Sequence C (SEQ ID NO:520), Sequence D (SEQ ID NO:521), Sequence E (SEQ ID NO:522), Sequence F (SEQ ID NO:523), Sequence G (SEQ ID NO:524), Sequence H (SEQ ID NO:525), Sequence I (SEQ ID NO:526), Sequence J (SEQ ID NO:527), Sequence K (SEQ ID NO:528), Sequence L (SEQ ID NO:529), Sequence M (SEQ ID NO:530), Sequence N (SEQ ID NO:531), Sequence O (SEQ ID

NO:532), Sequence P (SEQ ID NO:533), Sequence Q (SEQ ID NO:534), these sequence numbers corresponding to those cited in WO2007080392 or WO 2004/041862 (Ablynx N.V.). In certain embodiments, the *Camelid* V_{HH} binds human serum albumin and comprises an amino acid sequence that has at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% amino acid sequence identity with ALB1disclosed in WO2007080392 or with any one of SEQ ID NOS:518-534, these sequence numbers corresponding to those cited in WO2007080392 or WO 2004/041862.

In some embodiments, the dAb composition comprises an anti-serum albumin dAb that competes with any anti-serum albumin dAb disclosed herein for binding to serum albumin (*e.g.*, human serum albumin).

Conjugation to a half-life extending moiety (eg, albumin)

In one embodiment, a (one or more) half-life extending moiety (eg, albumin, transferrin and fragments and analogues thereof) is conjugated or associated with the VEGF-binding (or IL-1, or TNF-α binding or TNF-αR binding) dAb. Examples of suitable albumin, albumin fragments or albumin variants for use in a VEGF (or IL-1, or TNF-α or TNF-αR) -binding format are described in WO 2005077042, which disclosure is incorporated herein by reference and forms part of the disclosure of the present text. In particular, the following albumin, albumin fragments or albumin variants can be used in the present invention:

- SEQ ID NO:1 (as disclosed in WO 2005077042, this sequence being explicitly incorporated into the present disclosure by reference);
- Albumin fragment or variant comprising or consisting of amino acids 1-387 of SEQ ID NO:1 in WO 2005077042;
- Albumin, or fragment or variant thereof, comprising an amino acid sequence selected from the group consisting of: (a) amino acids 54 to 61 of SEQ ID NO:1 in WO 2005077042; (b) amino acids 76 to 89 of SEQ ID NO:1 in WO 2005077042; (c) amino acids 92 to l00 of SEQ ID NO:1 in WO 2005077042; (d)

amino acids 170 to 176 of SEQ ID NO:1 in WO 2005077042; (e) amino acids 247 to 252 of SEQ ID NO:1 in WO 2005077042; (f) amino acids 266 to 277 of SEQ ID NO:1 in WO 2005077042; (g) amino acids 280 to 288 of SEQ ID NO:1 in WO 2005077042; (h) amino acids 362 to 368 of SEQ ID NO:1 in WO 2005077042; (i) amino acids 439 to 447 of SEQ ID NO:1 in WO 2005077042 (j) amino acids 462 to 475 of SEQ ID NO:1 in WO 2005077042; (k) amino acids 478 to 486 of SEQ ID NO:1 in WO 2005077042; and (l) amino acids 560 to 566 of SEQ ID NO:1 in WO 2005077042.

Further examples of suitable albumin, fragments and analogs for use in a VEGF binding format are described in WO 03076567, which disclosure is incorporated herein by reference and which forms part of the disclosure of the present text. In particular, the following albumin, fragments or variants can be used in the present invention:

- Human serum albumin as described in WO 03076567, eg, in figure 3 (this sequence information being explicitly incorporated into the present disclosure by reference);
- Human serum albumin (HA) consisting of a single non-glycosylated polypeptide chain of 585 amino acids with a formula molecular weight of 66,500 (See, Meloun, *et al.*, *FEBS Letters 58*:136 (1975); Behrens, *et al., Fed. Proc. 34*:591 (1975); Lawn, *et al.*, *Nucleic Acids Research 9*:6102-6114 (1981); Minghetti, *et al.*, *J. Biol. Chem. 261*:6747 (1986));
- A polymorphic variant or analog or fragment of albumin as described in Weitkamp, *et al., Ann. Hum. Genet. 37*:219 (1973);
- An albumin fragment or variant as described in EP 322094, eg, HA(1-373., HA(1-388), HA(1-389), HA(1-369), and HA(1-419) and fragments between 1-369 and 1-419;
- An albumin fragment or variant as described in EP 399666, eg, HA(1-177) and HA(1-200) and fragments between HA(1-X), where X is any number from 178 to 199.

Where a (one or more) half-life extending moiety (eg, albumin, transferrin and fragments and analogues thereof) is used to format the dAbs of the invention, it can be conjugated using any suitable method, such as, by direct fusion, for example by using a single nucleotide construct that encodes a fusion protein, wherein the fusion protein is encoded as a single polypeptide chain with the half-life extending moiety located N- or C-terminally to the dAb. Alternatively, conjugation can be achieved by using a peptide linker between moieties, eg, a peptide linker as described in WO 03076567 or WO 2004003019 (these linker disclosures being incorporated by reference in the present disclosure to provide examples for use in the present invention). Typically, a polypeptide that enhances serum half-life *in vivo* is a polypeptide which occurs naturally *in vivo* and which resists degradation or removal by endogenous mechanisms which remove unwanted material from the organism (*e.g.*, human). For example, a polypeptide that enhances serum half-life *in vivo* can be selected from proteins from the extracellular matrix, proteins found in blood, proteins found at the blood brain barrier or in neural tissue, proteins localized to the kidney, liver, lung, heart, skin or bone, stress proteins, disease-specific proteins, or proteins involved in Fc transport.

The dAbs of the invention can be formatted as a fusion protein that contains a first immunoglobulin single variable domain that is fused directly to a second immunoglobulin single variable domain. If desired such a format can further comprise a half-life extending moiety. For example, the ligand can comprise a first immunoglobulin single variable domain that is fused directly to a second immunoglobulin single variable domain that is fused directly to an immunoglobulin single variable domain that binds serum albumin.

Generally the orientation of the polypeptide domains that have a binding site with binding specificity for a target, and whether the ligand comprises a linker, is a matter of design choice. However, some orientations, with or without linkers, may provide better binding characteristics than other orientations. All orientations (*e.g.*, dAb1-linker-dAb2; dAb2-linker-dAb1) are encompassed by the invention are ligands

that contain an orientation that provides desired binding characteristics can be easily identified by screening.

dAbs according to the invention, including dAb monomers, dimers and trimers, can be linked to an antibody Fc region, comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding ligands linked as a single nucleotide sequence to an Fc region may be used to prepare such polypeptides.

In embodiments of the invention the dAbs can be encoded by codon optimized nucleotide sequences e.g. optimized for expression by *Pichia pastoris* or *E. coli* E.G. as described in WO2008149147.

EXEMPLIFICATION

Example 1:
Topical delivery of DOM15-26-593 (myc tagged anti-VEGF dAb) to eyes of rabbits:

DOM 15-26-593 can be selected and prepared as described in WO2008149147 and has the amino sequence shown in Figure 1a (SEQ ID NO 1).

Myc tagged DOM15-26-593 (- the Dom 15-26-593 dAb with amino acid sequence shown in figure 1a (SEQ ID NO 1) was prepared and used as a c-myc-tagged anti-VEGF dAb in this experiment) was prepared as an endotoxin free preparation at a 2 mg/ml concentration formulated in a 50 mM sodium acetate buffer (pH 7.0) supplemented with 104 mM sodium chloride, 0.02% (w/v) Tween 80, 0.5% (w/v) Sodium caprate and either 0.3% or 1.5% (w/v) Hydroxypropyl methylcellulose (HPMC). Adult Chinchilla Bastard rabbits were obtained from Charles River, Germany. The animals were allowed to acclimatize before use. The left eyes of six female rabbits were dosed every 20 minutes over a four hour period with 50 microlitres of 2 mg/ml solution of anti-VEGF dAb. Each dose was placed in the subconjunctival sac. Three

rabbits received the anti-VEGF dAb formulated in 0.3% HPMC and three with the drug formulated in 1.5% HPMC. Two hours after the last dose the animals were culled. As close as possible to the time that euthanasia had been confirmed both eyes from each animal were enucleated. Each eye was washed in PBS to remove any excess drug from the surface. Samples of aqueous and vitreous humour were collected and stored frozen (-20°C) prior to analysis. The samples of aqueous and vitreous humour were tested for concentration of DOM15-26-593 (anti-VEGF-dAb) present using a sandwich ELISA assay where the dAb was captured on recombinant human VEGF protein coated plates and detected using an antibody with specificity for a c-myc tag.

The VEGF dAb ELISA assay described above was performed as follows:
The assay uses recombinant human VEGF (rVEGF, obtained from R&D Systems) coated onto the surface of Immunsorb plates (obtained from Nunc) to capture the VEGF dAb. The plates were washed to remove any unbound dAb. Bound dAb was subsequently detected using an antibody to the Myc tag of the VEGF dab (obtained from Sigma). Excess antibody was removed by washing and the bound anti-myc antibody detected using an anti-mouse IgG peroxidase conjugate (Sigma). The assay was developed using TMB solution and stopped using acid. The signal from the assay is proportional to the amount of dAb. The stages in the assay are summarized as follows:

Coating the Plate:

1. Sufficient rVEGF was prepared at 0.25µg/mL to coat the plates (5 ml for each ELISA plate) was prepared. This was done by for each plate, by adding 25 µL of stock VEGF to 5 mL of carbonate coating buffer (0.2M sodium carbonate-bicarbonate coating buffer solution pH 9.4 (Pierce, Cat No: 28382)) and mixing by inversion.
2. 50µL of rVEGF (0.25µg/mL) solution was added to each well of a Immunsorb 96-well ELISA plate using a multichannel pipette.
3. The plate was covered with a plastic lid and stored at 4°C for approximately 42 hours.

Washing and blocking plates:

4. The plates were removed from 4°C storage

5. Each plate was washed 6 times with PBS+0.1% Tween 20.

6. 100µL of assay blocking buffer (1% BSA/PBS) was added to all wells of each plate.

7.The plates were incubated at room temperature with agitation for 1 hour.

Preparation of Samples and Standards:

8. Standards and samples were diluted in assay diluent (0.1% BSA/0.05% Tween20/PBS) while the plates were blocking. The standard (reference material i.e Dom15-26-593) was serially (10-fold) diluted to produce a log dilution curve.

Addition of Samples:

9. Blocked plates were washed (as in 6 above).

10. 50µl of diluted sample or standard was added to appropriate wells. 50 µl/well assay diluent was added to wells to act as negative controls.

11. Plates were incubated for 2 hours at room temperature with agitation.

12. Plates were washed 6 times and blotted dry (as in 6 above).

13. 50µL of anti-myc antibody was added (9E10 Sigma M5546) diluted 1:500 (in assay diluent: 0.1% BSA/0.05% Tween20/PBS) to all wells (i.e. add 10µL of anti-myc antibody (9E10) to 5 ml assay diluent for each plate).

14. Plates were incubated on the rocker for at least 1 hour at room temperature.

15. Plates were washed 6 times and blotted dry (as in 6 above).

16. 50µL of anti-mouse Ig HRP at 1:10000 was added (Sigma A9309) to all wells. (i.e. dilute stock antibody 1 : 10 by adding 5µL of anti-mouse Ig HRP antibody to 45µL of assay diluent (0.1% BSA/0.05% Tween20/PBS). For each plate add 5 µL of the 1 : 10 diluted stock to 5ml assay diluent.

17. Plates were incubated on the rocker for at least 1 hour at room temperature.

18. Plates were washed 6 times and blotted dry (as in 6 above).

19. 50µL of TMB substrate was added to all wells. As the development of this assay is quite fast, it is advisable to add TMB to no more than 3 plates at a time. TMB can be used directly from the fridge or at room temperature.

20. The reaction was stopped (once sufficient colour has developed) by adding 50μL of 1M HCl to every well.

21. Plates were read on a 96 well plate reader at 450nm.

Results:

Results are shown in Table 1.

The dosing schedule was well tolerated with no signs of redness, irritancy or abnormal animal behaviour observed. The results of the ELISA assay carried out to investigate the level of anti-VEGF dAb (DOM15-26-593) present in vitreous and aqueous humour samples obtained from treated and contralateral (non-treated) eyes showed that most of the dAb detected was present in the vitreous humour of the treated eyes. The rabbit (animal 3) that had the highest concentration in the vitreous humour also had detectable levels of anti-VEGF dAb present in the aqueous humour of the treated eye.

It was observed that the dAb formulated in 1.5% HPMC (which was a more viscous solution) appeared to be retained in the eye following each dose more effectively than the more fluid 0.3% HPMC containing formulation. The rabbits dosed with the lower HPMC concentration appeared to lose some of the later dosing material by blinking it out.

Table 1: Concentrations of anti-VEGF dAb (DOM15-26-593) present in vitreous and aqueous humour from topically treated and contralateral eyes. Treatment consisted of 12 doses (each consisting of a 50 μL volume of a 2 mg/ml solution) administered in to the subconjunctival sac (every 20 minutes over a 4 hour period)

Rabbit no. (% HPMC)	**Vitreous humour (ng/ml) Treated eye**	**Vitreous humour (ng/ml) Contralateral eye**	**Aqueous humour (ng/ml) Treated eye**	**Aqueous humour (ng/ml) Contralateral eye**
1 (1.5%)	8 ng/ml	ND*	ND*	ND*
2 (1.5%)	5 ng/ml	ND*	ND*	ND*

3 (1.5%)	14 ng/ml	ND*	6 ng/ml	ND*
4 (0.3%)	5 ng/ml	4 ng/ml	5 ng/ml	ND*
5 (0.3%)	4 ng/ml	4 ng/ml	ND*	ND*
6 (0.3%)	7 ng/ml	ND*	ND*	ND*

ND* = Not Detected (</= 2 ng/ml)

Conclusions:

The dose of anti-VEGF dAb was placed in the conjunctival sac. It was expected that some of the dAb may penetrate through the cornea and would subsequently be detected in the aqueous humour. Surprisingly the majority of the anti-VEGF dAb detected was present in the vitreous humour and this observation would be consistent with the anti-VEGF dAb entering the eye by diffusion from the eye socket across the sclera and choroidal membranes in order to enter the posterior chamber.

Hydroxypropylcellulose (HPMC) had been included in the formulation as a viscosity enhancer. The 1.5% formulation appeared to be retained in the treated eye more effectively. The more fluid 0.3% formulation was less well retained and this may contribute to movement of anti-VEGF dAb to the contralateral eyes observed in two out of the three rabbits in this group.

Example 2:

Pharmacokinetics of DOM15-26-593 following intravitreal administration to eyes of rabbits:

An experiment was carried out to investigate the duration that the anti-VEGF immunoglobulin single variable domain antibody (anti-VEGF dAb) DOM15-26-593 was retained in the eye following direct injection of the DOM15-26-593 into vitreous humour. Dom 15-26-593 dAb with amino acid sequence shown in figure 1a (SEQ ID NO 1) was prepared (2 mg/ml concentration formulated in a 50 mM sodium acetate

buffer (pH 7.0) supplemented with 104 mM sodium chloride, 0.02% (w/v) Tween 80), and used as a c-myc-tagged anti-VEGF dAb in this experiment. Adult Chinchilla Bastard rabbits were obtained from Charles River, Germany. The animals were allowed to acclimatize before use. Each rabbit was anaesthetized and 10 microlitres of a 2 mg/ml solution (solution prepared as described in Example 1) (total 20 μg) of c-myc tagged anti-VEGF dAb (DOM15-26-593) was injected directly into the vitreous humour of the left eye. The rabbits were euthanized at various times (2, 24 and 30 hours) after the injection and both eyes were enucleated and samples of aqueous and vitreous humour were collected. These samples were stored frozen (-20°C) prior to analysis. The samples of aqueous and vitreous humour were tested for concentration of DOM15-26-593 present using a sandwich ELISA assay where the dAb was captured on recombinant VEGF protein coated plates and detected using an antibody with specificity for a c-myc tag.

Results:

The concentrations of DOM15-26-593 (anti-VEGF dAb) are shown in the Table 2 below:

Table 2: Concentrations of anti-VEGF dAb (DOM15-26-593) present in vitreous and aqueous humour from intravitreally dosed and contralateral eyes. Treatment consisted of a single intravitreal injection (10 μL of a 2 mg/ml solution) to the left eye – rabbits were allowed to recover from anesthesia and were culled at 2, 24 and 30 hours after dosing.

Rabbit no.	**Sample time (hours)**	**Vitreous humour (ng/ml) Treated eye**	**Vitreous humour (ng/ml) Contralateral eye**	**Aqueous humour (ng/ml) Treated eye**	**Aqueous humour (ng/ml) Contralateral eye**
1	2	121.48	ND*	0.75	ND*
2	2	115.43	ND*	ND*	ND*

3	24	88.61	0.10	1.58	ND*
4	24	157.79	ND*	ND*	ND*
5	30	88.90	ND*	ND*	ND*
6	30	17.31	ND*	0.14	ND*

ND* = not detected (< 0.1 ng/ml)

Concentrations rounded to 2 decimal places.

Conclusions:

The results of the experiment indicated that the concentration of DOM15-26-593 was maintained at levels approximating to the injected concentration at 24 hours after dosing. The domain antibody is present in vitreous humour at 24 and 30 hours after dosing.

Low concentrations of DOM15-26-593 (anti-VEGF dAb) were detected in the aqueous humour of some of the treated eye. However, there was minimal transfer of DOM15-26-593 to the contralateral untreated eye.

Example 3:

Rat laser-induced choroidal neovascularization (CNV) model:

Experimental choroidal neovascularization (CNV) was induced unilaterally in groups of five 2-4 month old female Dark Agouti (DA) rats. Laser light photocoagulation (PC) was used to rupture Bruch's membrane of anaesthetized rats. 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. Five lesions (532 nm, 150 mW, 0.2 second, 200 μm diameter) were made in a single eye of each experimental animal. Lesions were made in a peripapillary distributed and standardized fashion centered on the optic nerve at 500μm radius and avoiding major vessels. 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. Laser spots that did not result in the formation of a bubble were

excluded from the analysis. Immediately after laser CNV induction, each animal was dosed intravitreally with a 5μL volume (centered on the optic disc). (This volume was selected as it was calculated that there would be sufficient volume to cover the retinal area where the lesions had been made). The dAb was formulated as a 2 mg/ml concentration in a 50 mM sodium acetate buffer (pH 5.5) supplemented with 104 mM sodium chloride, 0.02% (w/v) Tween 80). The 5 μL volume contained 50 μg of anti-VEGF dAb (DOM15-26-593; with the amino acid sequence shown in figure 1a; SEQ ID NO 1), 50 μg of anti-VEGF DOM15-26-593-Fc fusion (with the amino acid sequence shown in figure 1b; SEQ ID NO 2) (or no compound (vehicle only, negative controls). *In vivo* image data of CNV and associated leakage was generated using confocal high-resolution SLO Fluorescein Angiography (0.2ml 10% intra-abdominally injected Fluorescein Sodium, FS) and OCT (Heidelberg Spectralis, Heidelberg, Germany) at 7 and 14 days after lesion generation and injections. 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 fluorescein angiographic images. The artero-venous phase was recorded immediately after FS injection. Fluorescein angiograms were thereafter recorded one minute after injection and again four minutes after injection. The effect of drug treatment was evaluated by semi-quantitative assessment of late-phase fluorescein angiography. Leakage was defined as the presence of a hyperfluorescent lesion that increased in size with time in the late-phase angiogram. The intensity and area of staining in late-phase fluorescein angiography was graded by two examiners in a masked fashion. When the two scores given for a particular lesion did not coincide, the higher score was used for the analysis. Such discrepant scoring was observed in <10% of lesions analyzed, and the discrepancy was never by more than one grade. The study was carried out in a masked manner and the substances were only unmasked once all the data had been collected.

Results:

Results are shown below in Table 3.

At 7 and 14 days after induction of choroidal neovascularization (CNV) using laser burns to rat retina, fluoresecein angiography was used to observe each lesion. The

lesions were graded as follows: Grade 0 = no leakage, Grade 1 = Small leakage, Grade 2 = Medium leakage and Grade 3 = Large leakage. The results for groups of rats treated intravitreally with anti-Vascular Endothelium Growth Factor domain antibody (anti-VEGF dAb, DOM15-26-593), DOM15-26-593-FC fusion and for negative control vehicle dosed groups are tabulated below. The results indicate that treatment with anti-VEGF dAb (DOM15-26-593) or DOM15-26-593-FC fusion reduced the extent of neovascularization and leakage compared with control (sham-treated) rats.

Table 3: CNV lesion scores in rat eyes at 7 and 14 after induction by photocoagulation.

Substance	**Lesions at Day 7**	**Lesions at Day 14**
Anti-VEGF dAb-Fc** (DOM15-26-593 –FC FUSION)	G0 = 19 G1 = 1	G0 = 7 G1 = 7 G2 = 6
Anti-VEGF dAb** (DOM15-26-593)	G0 = 14 G1 = 6	G0 = 6 G1 = 7 G2 = 5 G3 = 2
Negative control (Vehicle)	G0 = 1 G1 = 5 G2 = 7 G3 = 7	 G1 = 5 G2 = 11 G3 = 4

** = data at both time points statistically significant from controls ($P < 0.05$)

Conclusions:

The results indicated that DOM15-26-593 –Fc fusion, (anti-VEGF dAb-Fc) was efficacious in a rat model where experimental choroidal neovascularization (CNV) induced by laser photocoagulation of the RPE-choroid was characterized by fluorescence angiography. Results for DOM15-26-593 –Fc fusion were significantly better than the control vehicle dosed group at both 7 and 14 days. This group appeared to retain slightly more activity than the anti-VEGF dAb (DOM15-26-593) group. However, anti-VEGF dAb (DOM15-26-593) was also efficacious (significantly better than the control at both 7 and 14 days post-laser induced injury).

These results indicate that anti-VEGF dAb (DOM15-26-593) and anti-VEGF dAb-Fc were efficacious in an experimental rat CNV model. This demonstration of efficacy in an in vivo rodent model of ophthalmic disease indicates that the domain antibodies may be beneficial in the treatment of Choroidal Neovascularisation in Age-related Macular Degeneration (AMD).

Example 4:

Topical delivery of an anti-TNF-α antibody, an Fc-formatted anti-VEGF dAb, a pegylated anti-IL-1 dAb and an anti-IL-1 dAb to rabbits:

Method

Female, adult Chinchilla Bastard rabbits were obtained from Charles River, Germany. The animals were allowed to acclimatize before use. A blood sample was collected from the marginal ear vein of every rabbit five days prior to commencement of dosing. The blood was allowed to clot at room temperature and was centrifuged (12000 rpm/2 minutes) to separate the serum. The serum was transferred to fresh tubes and stored frozen (-20°C).

Preparation and selection of DOM 4-130-54 is described in WO 2007063311 and also WO2008149149. To prepare Dom 0400 the DOM 4-130-54 dAb sequence is taken and is mutated such that a cysteine at position 80 replaces the proline present in DOM 4-130-54, this dAb is then attached to a 40KDa linear PEG molecule (obtained from NOF Corp., Europe) by standard maleimide coupling to the free cysteine at position 80 of the dAb.

Domain antibodies (dAbs) with specificity for IL-1 in either a naked format (DOM4-130-54; IL-1 naked dAb, 12.026 kDa; with amino acid sequence shown in figure 3; SEQ ID NO 5) or a pegylated format (DOM0400PEG; IL-1 pegylated dAb, 52.032 kDa; with amino acid sequence sequence shown in figure 2; SEQ ID NO 4) were formulated at 8.5 and 10.4 mg/ml respectively in 20 mM succinate, 5% sorbitol, pH 6.0.

An Fc-formatted α-VEGF dAb (VEGF 15-26-593 with amino acid sequence shown in figure 1b; SEQ ID NO 2) was formulated at 9.1 mg/ml in 50 mM phosphate, 1% L-arginine, 0.05 mM EDTA.0.02% polysorbate and 0.3% NaCl pH 7.0. Monoclonal antibody with specificity for TNF-α (commercially available) was reconstituted from a freeze-dried preparation at 10 mg/ml using sterile distilled water. The left eyes of groups of four rabbits were dosed five times daily (at 3 hour intervals) over a period of 4.2 days. Animals were allowed a rest period of 12 hours (overnight) between each dosing day. Each dose consisted of 25 microlitres of a solution of the relevant compound placed under the top eye lid. The animals were held still for at least 30 seconds after dosing. At various times prior to and during the dosing schedule samples of lachrymal (tear) fluid were collected by placing a small absorbent strip of paper under the eyelid to absorb some fluid. The area of paper impregnated with tear fluid was placed into a tube containing 200 μL of phosphate buffered saline. The tube was centrifuged (12000 rpm/2 minutes), the paper removed and the recovered sample stored frozen (-20°C) prior to analysis.

One hour after the last dose a blood sample was collected from the marginal ear vein of every rabbit. The blood was allowed to clot so that serum could be separated and stored by the methods described above. Immediately afterwards the animals were euthanized. As close as possible to the time that euthanasia had been confirmed both eyes from each animal were enucleated. Each eye was washed in PBS to remove any excess drug from the surface. Samples of aqueous and vitreous humour were collected and stored frozen (-20°C) prior to analysis. Vitreous humour was subjected to a single freeze/thaw cycle before being tested in an assay. Eyes were dissected and the retina/choroid collected. Retina/choroid samples were weighed and 100 microlitres of lysis buffer (10 mM Tris pH 7.4; 0.1% SDS; with proteinase inhibitor cocktail, (Roche)) was added to each 15 mg of retina/choroid tissue. The samples were homogenized using ultrasonic disruption (Covaris S2 Sonolab Single) using a 2 minute cycle of repeated high and low frequency bursts. Samples of retina/choroid were centrifuged (12000 rpm/2minutes) in a

microfuge (Heraeus). Supernatants were transferred to fresh tubes and stored frozen (-20°C).

The drug content of each sample was tested and measured using sandwich format ELISA assays. The α-TNF-α antibody was captured using plates coated with recombinant human TNF-α protein (Peprotech) and detected using an alkaline phosphatase conjugated anti-human IgG (Fc specific) antibody (Sigma). The IL-1 and pegylated IL-1 dAbs were captured using plates coated with recombinant human IL-1 Receptor Type 1 Fc (Axxora) and detected using Protein L-peroxidase (Sigma). VEGF-Fc formatted dAb was captured using an in-house preparation of recombinant VEGF protein and detected with an anti-human IgG (Fc specific) Alkaline phosphatase conjugated antibody (Sigma).

Results

In all cases drug dosing was well tolerated with no signs of redness, irritancy or abnormal animal behaviour.

The results of the various formats of domain antibodies and for the α-TNF-α antibody in aqueous and vitreous humour and in retina/choroid are shown in the following tables. Results are shown as for the mean concentrations (from three independent assays where each sample was tested in triplicate) +/- Standard Deviation (shown in brackets)

Table 4: Concentration in Aqueous Humour after topical dosing (ng/ml)

	1	1	2	2	3	3	4	4
	Left	Right	Left	Right	Left	Right	Left	Right
α-TNF-α antibody	ND	ND	2.2 (2.0)	2.3 (0.7)	ND	ND	ND	ND
VEGF-Fc dAb	7.6 (7.5)	ND	2.0 (1.3)	1.9 (1.0)	1.1 (0.4)	ND	ND	ND
IL-1 pegylated DOM0400PEG	ND	ND	1.7 (2.9)	1.7 (2.9)	ND	ND	ND	ND

IL-1 DOM4-130-54	131.2 (53.0)	3.0 (1.2)	2.3 (0.3)	2.3 (0.8)	1.7 (0.3)	1.9 (0.4)	1.6 (0.2)	1.3 (0.04)

ND = Not detected – below limit of quantitation (in at least 2 out of 3 repeat assays)

Results in this table are rounded to one decimal place.

Table 5: Concentration in Vitreous Humour after topical dosing (ng/ml):

	1	1	2	2	3	3	4	4
	Left	Right	Left	Right	Left	Right	Left	Right
α-TNF-α antibody	ND	ND	ND	ND	ND	ND	ND	ND
VEGF-Fc dAb	ND	ND	ND	ND	ND	ND	ND	ND
IL-1 pegylated DOM0400PEG	ND	6.7 (11.5)	15.0 (21.8)	1.7 (2.9)	ND	ND	5.0 (5.0)	1.7 (2.9)
IL-1 DOM4-130-54	2.5 (0.6)	2.1 (0.5)	2.3 (0.6)	2.6 (0.9)	1.6 (0.1)	1.8 (0.2)	2.7 (2.7)	2.1 (0.9)

ND = Not detected - below limit of quantitation (in at least 2 out of 3 repeat assays)

Results in this table are rounded to one decimal place.

Table 6: Concentration in Retina/Choroid after topical dosing

(ng/ml in samples where 100μL lysis buffer has been added to 15 mg of tissue):

	1	1	2	2	3	3	4	4
	Left	Right	Left	Right	Left	Right	Left	Right
α-TNF-α antibody	ND	ND	8.9 (3.6)	ND	7.3 (2.6)	ND	ND	ND
VEGF-Fc dAb	7.5 (5.8)	29.5 (14.2)	1.1 (0.7)	2.0 (0.4)	7.6 (1.2)	2.2 (0.001)	0.6 (0.2)	ND
IL-1 pegylated DOM0400PEG	3.3 (2.9)	3.3 (2.9)	11.7 (7.6)	5.0 (5.0)	5.0 (5.0)	3.3 (2.9)	10 (5.0)	5.0 (5.0)
IL-1 DOM4-130-54	91.9 (90.3)	4.6 (1.4)	3.4 (0.5)	2.7 (1.3)	5.3 (0.5)	3.4 (0.3)	3.9 (1.2)	3.3 (0.7)

ND = Not detected – below limit of quantitation (in at least 2 out of 3 repeat assays)

Results in this table are rounded to one decimal place.

Lachrymal fluid (tear) samples were collected from rabbits just prior to doses 20 and 21 results for concentrations of drug present are shown in Tables 4 and 5 respectively. Material dosed was detected in all of the left (dosed) eyes (although there was quite a lot of variation in concentration detected between individual rabbits) and some transfer to most of the contralateral (right not dosed) eyes had also occurred. Dosing material was still present in the eye at 12 hours after dose 20. DOM0400PEG (pegylated IL-1 dAb) and VEGF-Fc (15-26-593) appeared to be retained in tears at higher concentrations over the 12 hour period between doses 20 and 21 than the naked IL-1 dAb (DOM4-130-54).

The results for the concentrations of the various formats of domain antibodies and for the antibody in lachrymal fluid (tears) are shown in the following tables (only the Left eye was dosed):

Table 7: Concentration in Lachrymal fluid samples collected prior to dose 20 (3 hours after previous dose) (μg/ml). Standard deviation is in brackets:

	1	1	2	2	3	3	4	4
	Left	Right	Left	Right	Left	Right	Left	Right
α-TNF-α antibody	13.0 (1.0)	18.6 (2.2)	211.9 (46.2)	ND	249.3 (40.2)	0.5 (0.3)	6.7 (0.7)	ND
VEGF-Fc dAb	28.6 (4.7)	ND	47.7 (7.0)	ND	19.4 (1.2)	0.3 (0.06)	32.5 (4.6)	0.2 (0.02)
IL-1 pegylated DOM0400PEG	1.0 (0.2)	0.3 (0)	3.4 (0.6)	0.5 (0.2)	25.0 (11.1)	0.3 (0)	21.1 (6.4)	ND
IL-1 DOM4-130-54	7.5 (4.3)	0.8 (0.1)	3.9 (0.7)	0.2 (0.02)	3.8 (0.6)	ND	5.7 (1.8)	0.3 (0.06)

Results in this table are rounded to one decimal place.

Table 8: Concentration in Lachrymal fluid samples collected prior to dose 21 (12 hours after previous dose): Concentration (μg/ml). Standard deviation is in brackets:

	1	1	2	2	3	3	4	4

	Left	Right	Left	Right	Left	Right	Left	Right
α-TNF-α antibody	17.2 (2.4)	5.3 (3.0)	81.7 (6.1)	0.3 (0.2)	2.9 (0.3)	ND	24.2 (1.2)	ND
VEGF-Fc dAb	21.2 (1.3)	1.3 (1.9)	12.5 (0.7)	ND	6.7 (0.5)	0.1 (0.03)	27.0 (5.0)	0.06 (0.02)
IL-1 pegylated DOM0400PEG	0.4 (0.2)	ND	43.1 (15.8)	0.3 (0)	102.3 (3.2)	44.2 (39.1)	1.5 (2.6)	0.3 (0)
IL-1 DOM4-130-54	5.6 (3.4)	0.9 (0.1)	3.6 (0.9)	ND	2.1 (0.2)	ND	3.0 (0.7)	0.1 (0.01)

Results in this table are rounded to one decimal place.

The results for the concentrations of the various formats of domain antibodies and for the antibody in prebleeds and in serum are shown in the following table:

Table 9: Concentration in prebleeds and serum collected just prior to euthanasia Concentration (ng/ml) with Standard Deviation in brackets:

	1	1	2	2	3	3	4	4
	Prebleed	Serum	Prebleed	Serum	Prebleed	Serum	Prebleed	Serun
α-TNF-α antibody	ND	ND	62.6 (49.0)	ND	220.1* (115.0)	102.7 (115.6)	ND	152.4 (130.4
VEGF-Fc dAb	ND	2.1 (1.8)	1.8 (1.3)	16.9 (6.5)	ND	16.9 (15.6)	ND	15.6 (0.5)
IL-1 pegylated DOM0400PEG	ND	3.33 (5.8)	ND	3.33 (5.8)	ND	3.33 (5.8)	ND	3.33 (5.8)
IL-1 DOM4-130-54	3.5 (0.9)	3.2 (0.6)	3.5 (1.2)	3.4 (1.2)	3.8 (1.7)	3.2 (0.9)	4.5 (1.9)	4.2 (1.9)

Results in this table are rounded to one decimal place.

* Rabbit 3 in the α-TNF-α antibody treated group had a prebleed that was red in colour owing to lysis and this may have contributed to the apparently high result.

Example 5:

Topical Delivery of α-TNF-αR1 dAb

Methods

Adult male Chinchilla Bastard rabbits were obtained from Charles River, Germany. The animals were allowed to acclimatize before use. A blood sample was collected from the

marginal ear vein of every rabbit seven days prior to commencement of dosing. The blood was allowed to clot at room temperature before centrifugation (12000 rpm/2 minutes) to separate the serum. The serum was transferred to fresh tubes and stored frozen (-20°C).

A domain antibody (dAb) with specificity for TNF-αR1 (i.e. an anti-TNFα receptor type 1dAb) which is Dom 1h-131-206 with amino acid sequence shown in figure 4; SEQ ID NO 6; Preparation and selection of Dom 1h-131-206 is described in WO2008149148) was formulated in phosphate buffered saline at 10 mg/ml. The left eyes of a group of four rabbits were dosed 10 times on a single day (at hourly intervals). Each dose consisted of 50 microlitres of 10 mg/ml α-TNF-αR1 dAb solution placed under the top eye lid. The animals were held still for at least 30 seconds after dosing. At various times prior to and during the dosing schedule samples of lachrymal (tear) fluid were collected by placing a small absorbent strip of paper under the eyelid to absorb some fluid. The area of paper impregnated with tear fluid was placed into a tube containing 200 μL of phosphate buffered saline. The tube was centrifuged (12000 rpm/2 minutes), the paper removed and the recovered sample stored frozen (-20°C) prior to analysis.

One hour after the last dose a blood sample was collected from the marginal ear vein of every rabbit. The blood was allowed to clot so that serum could be separated by the methods described above. Immediately afterwards the animals were euthanized. As close as possible to the time that euthanasia had been confirmed both eyes from each animal were enucleated. Each eye was washed in PBS to remove any excess drug from the surface. Samples of aqueous and vitreous humour were collected and stored frozen (-20°C) prior to analysis. Vitreous humour was subjected to a single freeze/thaw cycle before being tested in an assay. Eyes were dissected and the retina/choroid was collected. The retina/choroid samples were weighed and 900 microlitres of lysis buffer (10 mM Tris pH 7.4; 0.1% SDS; with proteinase inhibitor cocktail, (Roche)) was added to each sample. The samples were homogenized using ultrasonic disruption (Covaris S2

Sonolab Single) using a 2 minute cycle of repeated high and low frequency bursts. Samples of retina/choroid were centrifuged (12000 rpm/2 minutes) in a microfuge (Heraeus). Supernatants were transferred to fresh tubes and stored frozen (-20°C). The samples were tested for concentration of α-TNF-αR1 dAb by a sandwich ELISA assay where the dAb was captured using plates coated with recombinant human TNF R1/TNFRSF1A/Fc chimera (R+D Systems) and detected with specificity for human IgG (F(ab)2) fragments (Thermo). This antibody was not conjugated, so an anti-goat/sheep-HRP reagent (Sigma) was used to detect bound antibody.

Results

Drug dosing was well tolerated with no signs of redness, irritancy or abnormal animal behaviour observed.

Concentrations of α-TNF-αR1 dAb in ocular fluids and serum are shown for samples tested in triplicate. The α-TNF-αR1 dAb was detected in all of the ocular samples tested.

Table 10: Concentrations of α-TNF-αR1 dAb in ocular samples:

	Aqueous Humour (ng/ml +/- s.e.)	**Vitreous Humour (ng/ml+/- s.e.)**	**Retina/Choroid (ng/100 mg+/- s.e.)**
Rabbit 1 Left (dosed) eye	2.6 +/- 0.4	0.7 +/- 0.2	209.1 +/- 7.5
Rabbit 1 Right (un-treated)	0.7 +/- 0.3	0.1 +/- 0.1	3.5 +/- 0.7
Rabbit 2 Left (dosed) eye	10.8 +/- 5.4	0.4 +/- 0.2	229.4 +/- 42.0
Rabbit 2 Right (un-treated)	0.3 +/- 0.1	0.4 +/- 0.1	63.9 +/- 1.8
Rabbit 3 Left (dosed) eye	43.6 +/- 6.0	4.3 +/- 2.6	1086.6 +/- 20.7
Rabbit 3 Right (un-treated)	12.3 +/- 1.6	1.3 +/- 0.4	25.1 +/- 0.6
Rabbit 4 Left (dosed) eye	12.3 +/- 1.4	0.7 +/- 0.2	88.1 +/- 4.2

Rabbit 4 Right (un-treated)	0.5 +/- 0.1	0.2 +/- 0.03	74.3 +/- 4.3

Results in Table 10 are rounded to one decimal place.

S.E. = Standard Error

Lachrymal fluid (tear) samples were collected from rabbits just prior to doses 2, 6, 10 together with 1 hour following the final dose and concentrations of α-TNF-αR1 dAb detected in the samples are shown in Table 8. α-TNF-αR1 dAb was detected in all of the left (dosed) eyes and some transfer to most of the contralateral (right not dosed) eyes had also occurred.

Table 11: Concentrations of α-TNF-αR1 dAb in lachrymal fluid (tear) samples:

	Left (dosed) eye (Mean data for 4 rabbits) (µg/ml +/- S.E.)	**Right (contralateral) eye (Mean data for 4 rabbits) (µg/ml +/- S.E.)**
Prior to Second dose	19.74 +/- 6.27	0.1 +/- 0.01
Prior to Sixth dose	20.75 +/- 5.15	1.77 +/- 0.47
Prior to Tenth dose	21.83 +/- 5.81	1.11 +/- 0.41
1h after Tenth dose	27.03 +/- 6.98	0.60 +/- 0.36

Results in Table 11 are rounded to two decimal places.

S.E. = Standard Error

Blood was collected for serum prior to the first dose and at the time of euthanasia. The resulting data is shown in Table 9. Low concentrations of α-TNF-αR1 dAb were detected in serum obtain from each of the four rabbits 1 hour following the final dose.

Table 12: Concentrations of α-TNF-αR1 dAb in serum samples:

	Prior to first dose	**1h following final dose**

	(ng/ml +/- s.e.)	(ng/ml +/- s.e.)
Rabbit 1	ND	0.36 +/- 0.21
Rabbit 2	ND	0.44 +/- 0.18
Rabbit 3	ND	0.51 +/- 0.08
Rabbit 4	ND	1.02 +/- 0.12

Results in Table 12 are rounded to two decimal places.

ND = Not detected

S.E. = Standard Error

CLAIMS

1. A composition comprising an immunoglobulin single variable domain that binds to a target molecule, for ocular delivery.

2. The composition according to claim 1, wherein said immunoglobulin single variable domain is resistant to a protease, wherein the protease is selected from the group consisting of: ocular protease, caspases, calpains, matric metalloproteases, disintegrins, metalloproteinases (ADAMs) and ADAM with thrombospondin motifs, proteosomes, tissue plasminogen activator, secretases, cathepsin B, cathepsin D, cystatin C, serine protease PRSS1, and ubiquitin proteosome pathway (UPP), for ocular delivery.

3. A composition according to any preceding claim, which comprises an immunoglobulin single variable domain which binds to a target molecule selected from the group of: VEGF, TNFα , TNFαR, IL-1, IL-1r, TNFαR1, TGFbeta, IL-6, IL-8 IL-17, IL-21, IL-23, CD20, Nogo-a , Myelin associated glycoprotein (MAG) and Beta amyloid, for ocular delivery.

4. The composition according to claim 3, wherein said immunoglobulin single variable domain which binds to VEGF comprises an amino acid sequence that is at least 97% identical to (a) the amino acid sequence of DOM15-26-593 (shown in SEQ ID NO 1) or (b) the amino acid sequence of DOM15-26-593-Fc (SEQ ID NO 2).

5. The composition according to claim 4, wherein the immunoglobulin single variable domain comprises valine at position 6, wherein numbering is according to Kabat.

6. The composition according to claim 4, wherein the immunoglobulin single variable domain comprises leucine at position 99, wherein numbering is according to Kabat.

7. The composition according to claim 4, wherein the immunoglobulin single variable domain comprises lysine at position 30, wherein numbering is according to Kabat.

8. The composition according to claim 4, wherein the immunoglobulin single variable domain comprises an amino acid sequence that is identical to a sequence selected from: (a) the amino acid sequence of DOM15-26-593 (shown in SEQ ID NO 1) , (b) the amino acid sequence of DOM15-26-593-Fc (shown in SEQ ID NO 2).

9. The composition according to claim 3, wherein said immunoglobulin single variable domain which binds to IL-1 comprises an amino acid sequence that is at least 97% identical to: (a) the amino acid sequence of DOM 4-130-54 (shown in SEQ ID NO 5); or to (b) the amino acid sequence of DOM 0400 PEG (shown in SEQ ID NO 4).

10. The composition according to claim 9, wherein said immunoglobulin single variable domain which binds to IL-1 comprises an amino acid sequence that is identical to (a) the amino acid sequence of DOM 4-130-54 (shown in SEQ ID NO 5); or to (b) the amino acid sequence of DOM 0400 PEG (shown in SEQ ID NO 4).

11. The composition according to claim 3, wherein said immunoglobulin single variable domain which binds to α-TNF-αR1and comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of Dom 1h-131-206 (shown in SEQ ID NO 6).

12. The composition according to claim 3, wherein said immunoglobulin single variable domain which binds to α-TNF-αR1and comprises an amino acid sequence that is identical the amino acid sequence of Dom 1h-131-206 (shown in SEQ ID NO 6).

13. A composition according to any one of the previous claims, which further comprises a domain of an antibody constant region, wherein the antibody constant region is an antibody Fc region.

14. The composition according to claim 9, wherein said antibody Fc region has the amino acid Fc sequence shown in SEQ ID NO 3.

15. The composition according to claim 9, wherein the immunoglobulin single variable domain is present as a fusion with an Fc and has an amino acid sequence identical to the amino acid sequence of the DOM15-26-593-Fc fusion (shown in SEQ ID NO 2).

16. A composition comprising a naked immunoglobulin single variable domain which binds to a target molecule for delivery to one or more of the ocular regions selected from: the vitreous humour, the aqueous humour, the retina and the choroid.

17. The composition according to claim 16, wherein the target molecule is selected from VEGF, a VEGF antagonist, TNFα , TNFα receptor, IL-1, α-TNF-αR1, , IL-6, IL-8, IL-17, IL-21, IL-23, Nogo-a , Myelin associated glycoprotein (MAG) and Beta amyloid.

18. A composition according to claim 16 or 17, wherein said immunoglobulin single variable domain is resistant to a protease, wherein the protease is selected from the group of: ocular protease, caspases, calpains, matric metalloproteases, disintegrins, metalloproteinases (ADAMs) and ADAM with thrombospondin motifs, proteosomes, tissue plasminogen activator, secretases, cathepsin B, cathepsin D, cystatin C, serine protease PRSS1, and ubiquitin proteosome pathway (UPP).

19. A composition according to claim 16 or 17, wherein said immunoglobulin single variable domain is selected from the group of: (a) an immunoglobulin single variable domain which binds to VEGF and which comprises an amino acid sequence that is at least 97% identical to (i) the amino acid sequence of DOM15-26-593 (shown in SEQ ID NO 1) or (ii) the amino acid sequence of DOM15-26-593-Fc (shown in SEQ ID NO 2), (b) an immunoglobulin single variable domain which binds to IL-1 and comprises an amino acid sequence that is at least 97% identical to (i) the amino acid sequence of DOM 4-130-54 (shown in SEQ ID NO 5); or to (ii) the amino acid sequence of DOM 0400 PEG (shown in SEQ ID NO 4); (c) an immunoglobulin single variable domain which binds to α-TNF-αR1and comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of Dom 1h-131-206 (shown in SEQ ID NO 6).

20. A composition comprising a formatted immunoglobulin single variable domain which binds to a target molecule for delivery to one or more of the ocular regions selected from: the retina, the choroid and the lachrymal fluid.

21. The composition according to claim 20, wherein the target molecule is selected from: VEGF, a VEGF antagonist, TNFα, TNFα receptor, IL-1, α-TNF-αR1, IL-17, IL-21, IL-23, Nogo-a , Myelin associated glycoprotein (MAG) and Beta amyloid.

22. The composition according to claim 20 or 21, wherein said immunoglobulin single variable domain is resistant to a protease, wherein the protease is selected from the group of: ocular protease, caspases, calpains, matric metalloproteases, disintegrins, metalloproteinases (ADAMs) and ADAM with thrombospondin motifs, proteosomes, tissue plasminogen activator, secretases, cathepsin B, cathepsin D, cystatin C, serine protease PRSS1, and ubiquitin proteosome pathway (UPP).

23. The composition according to claim 16 or 17, wherein said immunoglobulin single variable domain is selected from the group of: (a) an immunoglobulin single variable domain which binds to VEGF and which comprises an amino acid sequence that is at least 97% identical to (i) the amino acid sequence of DOM15-26-593 (shown in SEQ ID NO 1) or (ii) the amino acid sequence of DOM15-26-593-Fc (shown in SEQ ID NO 2), (b) an immunoglobulin single variable domain which binds to IL-1 and comprises an amino acid sequence that is at least 97% identical to (i) the amino acid sequence of DOM 4-130-54 (shown in SEQ ID NO 5); or to (ii) the amino acid sequence of DOM 0400 PEG (shown in SEQ ID NO 4); (c) an immunoglobulin single variable domain which binds to α-TNF-αR1and comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of Dom 1h-131-206 (shown in SEQ ID NO 6).

24. A composition according to claims 20-23, wherein the immunoglobulin single variable domain has a molecular weight of around 50 KDa.

25. A composition according to claim 20-24, wherein the immunoglobulin single variable domain is formatted by pegylation or fusion to an antibody Fc.

26. A composition according to any one of the preceding claims, which further comprises one or more enhancers chosen from: an ocular penetration enhancer and a viscosity enhancer.

27. A composition according to any one of the preceding claims, which further comprises a pharmaceutically or physiologically acceptable carrier, diluent, or excipient.

28. A method of delivering a composition according to any one of claims 1-27, directly to the eye, which comprises administering said composition to the eye by a method selected from the group of: topical delivery to the eye such as by eye drops or intra-ocular injection, peri-ocular administration, or by a slow release formulation.

29. A method for treating, preventing or diagnosing an eye condition which comprises administering a composition according to any one of claims 1-27, directly to the eye, by a method selected from the group consisting of: intra-ocular injection, intra-vitreal injection, topical delivery to the eye such as by eye drops, or by peri-ocular administration, and a slow release formulation.

30. A method according to claim 28 or 29, wherein the composition is administered to a one or more regions of the eye selected from the group of: surface of the eye, tear ducts, lachrymal glands, intra-ocular region, anterior chamber, posterior chamber and the vitreous humour.

31. A method of delivering a composition according to claim 16-19 and 26-27, to one or more regions of the eye selected from the group of: the vitreous humour, the aqueous humour, the retina, and the choroid, which comprises administering said composition to the eye by topical delivery such as by using eye drops.

32. A method of delivering a composition according to claim 20-27 to one or more regions of the eye selected from the group of: the retina, the choroid, and the lachrymal fluid, which comprises administering said composition to the eye by topical delivery such as by using eye drops.

33. A process for producing a pharmaceutical composition which comprises: (a) mixing a composition of any one of claim 1-27 with (b) a pharmaceutically acceptable carrier, diluent or excipient.

34. A process according to claim 33, wherein said pharmaceutical composition is for treating, preventing or diagnosing an eye condition or disease.

35. A process according to claim 34, wherein said eye condition or disease is selected from the group of: AMD, Uveitis, glaucoma, dry eye, diabetic retinopathy, diabetic macular oedema and Uveitis.