

METHODS OF TREATING NEOVASCULAR EYE DISEASES

Technical field

- 5 The present invention relates, in general terms, to methods of treating eye diseases, and more specifically to methods of treating neovascular eye diseases using PRL3 inhibitors such as PRL3-binding antibodies.

Background

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Neovascular eye diseases such as neovascular or wet age-related macular degeneration (AMD), diabetic retinopathy (DR) and diabetic macular edema (DME) are major causes of irreversible blindness globally. The World Health Organisation reported 196 million AMD patients and 146 million DR/DME patients worldwide in 2019, with these numbers trending
15 upwards.

- Anti-VEGF therapies, such as treatment with anti-VEGF antibodies, are commonly used to treat neovascular eye diseases, but approximately 40% of wet AMD and DME patients do not respond well to such therapies. This means that patients continue to experience persistent
20 fluid exudation, new or unresolved haemorrhage and progression of lesion fibrosis in the eyes, which results in poor vision recovery. In addition, current anti-VEGF therapies are administered via the intravitreal route, which has risks of ocular side effects such as inflammation and conjunctival haemorrhage. With an increasing aged population in many societies and a rising incidence of diabetes with its ocular complications, there remains a
25 need for new therapeutic targets and treatment strategies for neovascular eye diseases.

Accordingly, it would be desirable to overcome or alleviate at least one of the above-described problems, or at least to provide a useful alternative.

30 Summary

Disclosed herein is a method of treating a neovascular eye disease or condition in a subject, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof to the subject.

Disclosed herein is a PRL3-binding antibody or antigen-binding fragment thereof, for use in treating a neovascular eye disease or condition in a subject.

- 5 Disclosed herein is the use of a PRL3-binding antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating a neovascular eye disease or condition in a subject.

- Disclosed herein is a method of inhibiting or reducing intraocular neovascularisation in a
10 subject, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof to the subject.

- Disclosed herein is a method of enhancing an anti-VEGF therapy in a subject with a neovascular eye disease, the method comprising administering an effective amount of a
15 PRL3-binding antibody or antigen-binding fragment thereof simultaneously or sequentially with an effective amount of the anti-VEGF therapy to the subject.

- Disclosed herein is a method of determining the likelihood of developing a neovascular eye disease or condition in a subject, the method comprising detecting the level of PRL3 in a
20 sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to develop a neovascular eye disease or condition.

- Disclosed herein is a method of detecting a neovascular eye disease or condition in a subject,
25 the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to have a neovascular eye disease or condition.

- Disclosed herein is a method of treating a neovascular eye disease or condition in a subject,
30 the method comprising a) detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to develop have a neovascular eye disease; and b) treating the subject found likely to have a neovascular eye disease.

Disclosed herein is a method of identifying a subject with a neovascular eye disease or condition who is likely to be responsive to treatment with a PRL3-binding antibody or antigen-binding fragment thereof, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to
5 a reference indicates that the subject is likely to be responsive to treatment with a PRL3-binding antibody or antigen-binding fragment thereof.

Disclosed herein is an ophthalmic pharmaceutical composition comprising a PRL3 inhibitor.

10 **Brief description of the drawings**

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

15 **Figure 1** shows that PRL3 expression, but not PRL1, in CD31⁺/Ter119⁺ mouse (E9.5) embryonic blood vessels.

Figure 2 shows that PRL3 overexpression in diseased RPE-choroid of CNV mouse model and diseased retina of OIR model but not in normal mice. R: Retina; C: RPE-choroid; B16F0
20 and B16F10 are positive and negative controls for PRL3 immunoblot respectively. CNV: Choroidal neovascularization mouse model; OIR: Oxygen-induced retinopathy mouse model.

Figure 3 shows anti-PRL3 antibody treatment (IV and IVT) in a CNV model reduced
25 leakage and improved recovery of CNV lesions. (a) Experimental timeline of drug treatment (IVT and IV) in laser induced CNV model. (b) Representative FFA and OCT images of control (PBS/mouse IgG) and treated (mouse anti-PRL3 antibody) groups (Day 7 versus Day 1). (c) Quantification of the % recovery of the CNV lesions after drug treatment. IV
30 treatment with mouse anti-PRL3 antibody and humanised anti-PRL3 antibody (PRL3-zumab) showed significant recovery of CNV lesions compared to PBS/IgG control group (mouse anti-PRL3 antibody: 65.3% versus PRL3-zumab: 44% versus PBS/IgG: -67.7%). IV treatment with mouse anti-PRL3 antibody is 86% better than IVT delivery (IV: 65.3% versus IVT: 35.1%, p=0.003). Efficacy of IVT treatment with mouse anti-PRL3 antibody is comparable to that of Aflibercept (Eylea) (Eylea: 38.1% versus anti-PRL3 antibody: 35.1%).

'n' indicates the number of CNV spots in analysis. All images shown are representative and data are mean $\pm$ s.d. $**P < 0.01$ (One-way ANOVA, Kruskal-Wallis test). FFA: Fundus Fluorescein Angiography; OCT: Optical Coherence Tomography; IVT: Intravitreal; IV: Intravenous

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Figure 4 shows that mouse anti-PRL3 antibody attenuates Src phosphorylation which could reduce vascular leakage. Western blot of RPE-choroid tissues shows the attenuation of Src phosphorylation (Y416) effected by mouse anti-PRL3 antibody treatment.

10 **Figure 5** shows that mouse anti-PRL3 antibody concentration in vitreous humor via IV was significantly higher than that of via IVT due to a larger drug dose in the IV regime. This demonstrates that antibody could accumulate in the vitreous humor at a higher concentration when the antibody is administered via IV compared to IVT 7 days post treatment. $n \geq 3$ mice per group and data are mean $\pm$ s.d. for each group. $****P < 0.0001$ (Student's *t*-test)

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Figure 6 shows that mouse anti-PRL3 antibody concentration via IV was significantly higher in the blood compared to that of via IVT at 24h, 72h and 168h timepoints post treatment due to a larger drug dose in the IV regime. $n \geq 3$ mice per group and data are mean $\pm$ s.d. for each group. $****P < 0.0001$ (Student's *t*-test)

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Figure 7 shows that mouse anti-PRL3 antibody concentration (IV) was significantly higher in the vitreous humor of CNV mice compared to that of normal mice 7 days post treatment. $n \geq 3$ mice per group and data are mean $\pm$ s.d. for each group. $***P < 0.001$ (Student's *t*-test)

25 **Figure 8** shows that VEGF treatment upregulates PRL3 expression in HRMEC in a time-dependent fashion with the highest PRL3 expression at 72h timepoint.

Figure 9 shows that PRL3 overexpression results in an increased endothelial proliferation in HRMECs. HRMEC-PRL3 exhibit a higher rate of cell proliferation compared to

30 HRMEC-Vector ($p=0.018$). $*P < 0.05$ (Student's *t*-test)

Figure 10 shows that PRL3 overexpression results in an enhanced endothelial migration in HRMECs. HRMEC-PRL3 have a greater cell migratory ability compared to HRMEC-Vector ($p=0.0499$). $*P < 0.05$ (Student's *t*-test)

Figure 11 shows that PRL3 overexpression results in an increased permeability of endothelial monolayer. There is an increased permeability in HRMEC-PRL3 monolayer compared to HRMEC-Vector ($p=0.043$). HRMEC-PRL3 monolayer have diminished ZO-1 cell surface expression compared to HRMEC-Vector ($p=0.032$) by immunofluorescence. Both HRMEC-Vector and HRMEC-PRL3 have reporter GFP gene for successful retroviral infection of the plasmids. In the PRL3 panel, there is a mixture of PRL3 overexpressing and wildtype cells, and stronger ZO-1 expression is seen in the wildtype cells (white arrows point to intact ZO-1 expression). $*P < 0.05$ (Student's *t*-test)

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Figure 12 shows that Endothelial PRL3 overexpression results in a significant increase in the phosphorylation of Src, Akt, ERK1/2 and paxillin, and a significant reduction in VE-Cadherin, ZO-1 and VEGFR2. a) Representative western blots b) Quantification of Western blotting analysis. Data are mean $\pm$ s.d. $***P < 0.001$, $**P < 0.01$, $*P < 0.05$ (Student's *t*-test). All data are representative images and western blots of $n \geq 3$ for each experiment.

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Figure 13 shows that PRL3-zumab is not cytotoxic to ocular cell lines (a. Human retinal microvascular endothelial cell line, b. Human retinal pigment epithelial cell line, c. Human glial cell line) up to a high dose of 100 $\mu\text{g/ml}$ for 48 hours.

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Figure 14 shows that IVT administration of PRL3-zumab in rabbit model showed excellent ocular safety. Toxicology studies of IVT administration (0.084mg, 0.175mg and 0.35mg) of PRL3-zumab in New Zealand White Rabbits have demonstrated no significant change in IOP and central corneal thickness ($n=3$ in each group).

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Figure 15 shows that eyedrop administration of PRL3-zumab in rabbit model showed excellent ocular safety. Toxicology studies of eyedrop administration (0.079mg and 0.35mg) of PRL3-zumab in New Zealand White Rabbits have demonstrated no significant change in IOP and central corneal thickness ($n=3$ in each group).

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Figure 16 is a schematic representation of the potential role of endothelial PRL3 in angiogenic eye diseases.

Figure 17 shows that PRL3 overexpression desensitizes endothelial response to

Bevacizumab treatment. HRMEC-Vector control responded to Bevacizumab treatment in a dose-dependent manner from 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml to 10mg/ml. But there was no observed changes in cell proliferation in HRMEC-PRL3 with Bevacizumab treatment up to a high dose of 10mg/ml. Data are mean $\pm$ s.d. ***P < 0.001, **P < 0.01, *P < 0.05 (Student's t-test). HRMEC-Vector: Vector control HRMEC cells, HRMEC-PRL3: PRL3-overexpressing HRMEC cells.

Detailed description

10 The inventors have discovered that the protein PRL3 is upregulated in diseased choroid and retinal tissues in animal models of ocular neovascularisation, but not in normal eye tissue, and can be used as a biomarker for neovascular eye diseases and conditions. It is shown that PRL3 overexpression promotes endothelial proliferation, migration and permeability independent of VEGF stimulation, and may contribute to vascular leakage and other
15 pathologies associated with neovascular eye diseases. PRL3 overexpression also desensitises endothelial cells to anti-VEGF therapy and may be responsible for reduced therapeutic response in some patients undergoing anti-VEGF therapy. Therapies which inhibit PRL3 expression or activity may serve as alternative or complementary treatment options for patients with neovascular eye disease alongside conventional anti-VEGF
20 therapy.

The inventors show that antibody therapy of neovascular eye diseases using PRL3-binding antibodies can reduce vascular leakage and improve healing of ocular neovascular lesions. Surprisingly, the antibodies can be administered intravenously and are able to extravasate
25 from the leaky ocular vasculature and persist in eye tissue for several days after administration. Intravenous administration is advantageous over the more conventional intravitreal route as it provides greater flexibility for dosing and reduces the risk of ocular complications following administration. For example, the intravitreal route only allows for about 100 μ l of drug to be administered, thus necessitating fairly high doses (e.g., 120 mg/ml
30 for an antibody), which may be difficult to formulate and may not be well-tolerated by patients. Additionally, the intravitreal route carries the risk of ocular complications such as subconjunctival hemorrhage, increased intra-orbital pressure, damage to the retinal pigment epithelium, and cataract formation, and may compound existing injury from the eye disease. In contrast, antibodies can be provided at doses of up to about 300 mg or more when

administered intravenously, with minimal risk of associated ocular complications.

Accordingly, this disclosure provides methods of treating neovascular eye diseases or conditions using PRL3 inhibitors and in particular anti-PRL3 antibodies. Also provided are combination therapies comprising a PRL3 inhibitor and an anti-VEGF therapy, for use in treating neovascular eye diseases, particularly in subjects with poor response to anti-VEGF therapy. Also provided are methods that use PRL3 as a biomarker, for example, to detect neovascular eye diseases or to select subjects for therapy.

- 10 Disclosed herein is a method of treating a neovascular eye disease or condition in a subject, the method comprising administering an effective amount of a PRL3 inhibitor to the subject. Disclosed herein is a method of inhibiting or reducing intraocular neovascularisation in a subject, the method comprising administering an effective amount of a PRL3 inhibitor to the subject. Disclosed herein is a PRL3 inhibitor, for use in treating a neovascular eye disease or condition, or inhibiting or reducing intraocular neovascularisation in a subject. Disclosed herein is the use of a PRL3 inhibitor in the manufacture of a medicament for treating a neovascular eye disease or condition, or for inhibiting or reducing intraocular neovascularisation in a subject.
- 15
- 20 In one embodiment, the PRL3 inhibitor is a PRL3-binding antibody or antigen-binding fragment thereof.

- As used herein, "PRL3" refers to the phosphatase of regenerating liver 3 protein. PRL3 is one of three members (PRL1, PRL2 and PRL3) in the PRL family. The three PRLs form a subgroup of the protein tyrosine phosphatase (PTP) family, thus PRL3 is also known as protein tyrosine phosphatase type 4A3 (PTP4A3). While their normal cellular functions are largely unknown, the PRL family is known to be involved in cancer progression. PRL3, in particular, is highly-expressed in leukaemia and in many types of solid tumours. However, the role of PRL3 in the development of neovascular eye diseases has not been previously
- 25
- 30 been shown.

As used herein, "VEGF" refers to a vascular endothelial growth factor capable of inducing angiogenesis or an angiogenic process. The term "VEGF" includes the various subtypes of VEGF (also known as vascular permeability factor (VPF) and VEGF-A) that arise by, e.g.,

alternative splicing of the *VEGFA* gene including VEGF-121, VEGF-145, VEGF-165, VEGF-189 and VEGF-206. Further, as used herein, the term “VEGF” includes VEGF-related angiogenic factors such as PlGF (placenta growth factor), VEGF-B, VEGF-C and VEGF-D, which act through a cognate VEGF receptor (i.e., VEGFR-1/Flt-1, VEGFR-2/Flk-1 or VEGFR-3/Flt4) to induce angiogenesis or an angiogenic process.

The terms “treating”, “treatment” and the like include relieving, reducing, alleviating, ameliorating or otherwise inhibiting the effects of the neovascular eye disease or condition for at least a period of time. It is also to be understood that terms “treating”, “treatment” and the like do not imply that the disease, or a symptom thereof, is permanently relieved, reduced, alleviated, ameliorated or otherwise inhibited and therefore also encompasses the temporary relief, reduction, alleviation, amelioration or otherwise inhibition of the disease, or of a symptom thereof. Treatment may result in, for example, a reduction in ocular vascularity, improving visual acuity and a return or approach to normal visual acuity.

As used herein a “therapeutically effective amount” or “effective amount” is an amount that is non-toxic to the subject and sufficient to effect desired outcomes in a subject (i.e., achieve therapeutic efficacy). For purposes of this disclosure, a therapeutically effective amount of an inhibitor, antibody, polypeptide or composition is an amount that is sufficient to palliate, ameliorate, stabilise, reverse, prevent, slow or delay the progression of a disease state. A therapeutically effective amount can be administered in one or more administrations.

It is recognised that the effective amount of an inhibitor, antibody, polypeptide or composition will vary depending on the route of administration, the selected pharmacologically active agent or composition, and the species to which the drug or pharmacologically active agent is administered. It is also recognised that one of skill in the art will determine appropriate effective amounts by taking into account such factors as metabolism, bioavailability, and other factors that affect levels of a drug or polypeptide or composition following administration for different routes of administration.

The terms “patient”, “subject”, “host” or “individual” used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the

subphylum Chordata including primates (e.g., humans, monkeys and apes, and includes species of monkeys such from the genus *Macaca* (e.g., cynomolgus monkeys such as *Macaca fascicularis*, and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), as well as marmosets (species from the genus *Callithrix*), squirrel monkeys (species from the genus *Saimiri*) and tamarins (species from the genus *Saguinus*), as well as species of apes such as chimpanzees (*Pan troglodytes*)), rodents (e.g., mice rats, guinea pigs), lagomorphs (e.g., rabbits, hares), bovines (e.g., cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., pigs), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), avians (e.g., chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars etc.), marine mammals (e.g., dolphins, whales), reptiles (snakes, frogs, lizards etc.), and fish. A preferred subject is a human in need of treatment for a neovascular eye disease. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

A “sample” as used herein includes any biological specimen that may be extracted, untreated, treated, diluted or concentrated from a subject. A sample includes within its scope a collection of similar fluids, cells, or tissues (e.g., surgically resected tissue, biopsies, including fine needle aspiration), isolated from a subject, as well as fluids, cells, or tissues present within a subject. Any suitable methods for obtaining a biological sample can be employed; exemplary methods include, e.g., phlebotomy, fine needle aspiration and surgical biopsy. The sample may be pooled from multiple aliquots. The method may involve obtaining the sample, or the method may be performed on a sample previously obtained from the subject.

The term “sequence identity” as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a “percentage of sequence identity” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G and I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

“Similarity” refers to the percentage number of amino acids that are identical or constitute conservative substitutions as defined in Tables A and B. Similarity may be determined using sequence comparison programs such as GAP (Deveraux et al., 1984, *Nucleic Acids Research* 12: 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, which can be generally sub-classified as follows:

Table A. Amino acid sub-classification.

Sub-classes	Amino acids
Acidic	Aspartic acid, Glutamic acid
Basic	Noncyclic: Arginine, Lysine; Cyclic: Histidine
Charged	Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine
Small	Glycine, Serine, Alanine, Threonine, Proline
Polar/neutral	Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine
Polar/large	Asparagine, Glutamine
Hydrophobic	Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan
Aromatic	Tryptophan, Tyrosine, Phenylalanine
Residues that influence chain orientation	Glycine and Proline

Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement

of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional polypeptide can readily be determined by assaying its activity. Conservative substitutions are shown in Table B under the heading of exemplary and preferred substitutions. Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. After the substitutions are introduced, the variants are screened for biological activity.

Table B. Exemplary and Preferred Amino Acid Substitutions.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn, His, Lys,	Asn
Glu	Asp, Lys	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleu	Leu
Leu	Norleu, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Ile, Phe	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe

Val	Ile, Leu, Met, Phe, Ala, Norleu	Leu
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Neovascular eye diseases and conditions

As used herein, a “neovascular eye disease or condition” refers to any disease or condition
5 caused by or associated with aberrant growth or proliferation of blood vessels (angiogenesis)
in the eye, inflammation of the ocular vasculature, or leakage from vessels of the eye. The
disease or condition may be characterised by, for example, corneal, retinal, choroidal, uveal,
iris or surface neovascularisation. The neovascularisation may be an overformation of blood
vessels or formation of blood vessels in an unwanted area of the eye, such as in an avascular
10 region. Neovascularisation may be caused by occlusion or blockage of blood vessels in the
eye, or may be secondary to eye trauma or injury, or inflammatory conditions like diabetes,
autoimmune disease, conjunctivitis, episcleritis, keratitis, optic neuritis, retinal vasculitis,
scleritis, or uveitis.

15 In some embodiments, the neovascular eye disease or condition is characterised by aberrant
blood vessel growth and/or vascular leakage. Non-limiting examples of neovascular eye
diseases or conditions which may be treated using the methods of the present disclosure
include wet age-related macular degeneration (AMD), diabetic retinopathy (DR), diabetic
macular edema (DME), central retinal vein occlusion (CRVO), sickle cell retinopathy,
20 Stargardt’s disease, neovascular glaucoma, ocular ischemia, retinopathy of prematurity,
corneal neovascularisation, retinal neovascularisation, and choroidal neovascularisation.

In one embodiment, the neovascular eye disease or condition is characterised by an increased
expression or activity of PRL3 in eye tissue, for example, in choroidal and/or retinal tissue.
25 PRL3 expression or activity may be elevated in any cell type of the eye tissue, for example,
in endothelial cells or endothelial cell precursors.

PRL3 as a biomarker

30 Methods of this disclosure relate to the use of PRL3 as a biomarker for diagnosis of
neovascular eye diseases or conditions, and for patient selection for treatment with PRL3
inhibitors or antibodies.

Disclosed herein is a method of determining the likelihood of developing a neovascular eye disease or condition in a subject, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to develop a neovascular eye disease or
5 condition.

Disclosed herein is a method of detecting a neovascular eye disease or condition in a subject, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject
10 is likely to develop a neovascular eye disease or condition.

In one embodiment, the sample is an ocular tissue sample, e.g., a sample from the choroid, retina or vitreous cavity.

15 A “reference”, “control”, “reference sample”, or “control sample”, as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one embodiment, a reference is obtained from a healthy and/or non-diseased part of the body (e.g., tissue or cells) of the same subject or individual. For example, healthy and/or non-diseased cells or tissue adjacent to a diseased eye. In another embodiment, a reference is
20 obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference is obtained from a healthy and/or non-diseased part of the body (e.g., tissues or cells) of an individual who is not the subject or individual. In another embodiment, a reference is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual. The reference may be population-
25 average levels for a biomarker (e.g., PRL3) in healthy cells or tissues.

As used herein, the term “increase” or “increased” with reference to a biomarker such as PRL3 refers to a statistically significant and measurable increase in the biomarker as compared to a reference. The increase may be an increase of at least about 10%, at least
30 about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, or more.

PRL3 may be detected using any method known in the art for detecting a protein. Non-limiting examples of such methods include immunological methods using labelled antibodies or antibody fragments that bind to PRL3; protein function or activity assays; protein binding assays using non-immunological means such as using labelled aptamers, 5 lectins or other binding agents; and mass spectrometry. In one embodiment, PRL3 is detected using a PRL3-binding antibody or antigen-binding fragment thereof of this disclosure.

Disclosed herein is a method of treating a neovascular eye disease or condition in a subject, 10 the method comprising a) detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to have a neovascular eye disease or condition; and b) treating the subject found likely to have a neovascular eye disease or condition.

15 In one embodiment, the method comprises treating the subject with a PRL3 inhibitor. In one embodiment, the method comprises treating the subject with a PRL3-binding antibody or antigen-binding fragment thereof.

Disclosed herein is a method of identifying a subject with a neovascular eye disease or 20 condition who is likely to be responsive to treatment with a PRL3 inhibitor or a PRL3-binding antibody or antigen-binding fragment thereof, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to be responsive to treatment with a PRL3 inhibitor or a PRL3-binding antibody or antigen-binding fragment thereof.

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In some embodiments, the method further comprises treating the subject found likely to be responsive to treatment with a PRL3 inhibitor or a PRL3-binding antibody or antigen-binding fragment thereof.

30 PRL3 inhibitors

PRL3 inhibitors herein may be any substance which reduces the expression or activity of PRL3, and include but not are limited to small molecules, peptides (e.g., allosteric antagonists or substrate mimetics), proteins (e.g., antibodies, antibody fragments, antibody

mimics, antibody-like scaffolds), nucleic acids (e.g., aptamers, antisense oligonucleotides, short interfering RNA, short hairpin RNA) and conjugates of two or more of such. The inhibitor may be a nucleic acid encoding an inhibitory peptide or polypeptide. The inhibitor may inhibit enzyme activity directly (e.g., by preventing interaction with a substrate or by
5 altering the structure of the enzyme) or indirectly (e.g., by affecting the subcellular localisation of the enzyme or enzyme interaction with a cofactor or subunit). Methods herein may comprise administering two or more PRL3 inhibitors to the subject.

In some embodiments, the PRL3 inhibitor is a small molecule. Exemplary small molecule
10 inhibitors include compounds described in Rivas, D. R. et al. (*Sci Rep* 11, 10302 (2021). doi: 10.1038/s41598-021-89668-5), such as the compounds bardoxolone, bithionol, docusate sodium, eltromobag, eltromobag olamine and embelin; thienopyridone and thienopyridone analogues and derivatives described in PCT/US2019/061003, PCT/US2022/024927, Lazo J. S. et al. (*J Pharmacol Exp Ther.* 2019 Dec; 371(3):652-662.
15 doi: 10.1124/jpct.119.262188) and Daouti S. et al. (*Cancer Res.* 2008 Feb 15;68(4):1162-9. doi: 10.1158/0008-5472.CAN-07-2349), such as the compounds JMS-053, JMS-038, NRT-870-59, EJR-866-75 and EJR-866-81; and rhodanine derivatives described in Min, G. et al. (*Bioorg Med Chem Lett.* 2013, 23(13): 3769-3774. doi: 10.1016/j.bmcl.2013.04.092), such as the compounds CG-707 and BR-1.

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PRL3 antibodies

In preferred embodiments of the methods herein, the PRL3 inhibitor is a PRL3-binding antibody or antigen-binding fragment thereof. The PRL3-binding antibody or antigen-
25 binding fragment thereof preferably exhibits specific binding to PRL3, i.e., the antibody or antigen-binding fragment thereof binds PRL3 with greater affinity and/or with greater duration than it binds to other targets. An antibody or antigen-binding fragment thereof of this disclosure may bind PRL3 preferentially over PRL1 and PRL2. The antibody or antigen-binding fragment thereof may bind PRL3 but not PRL1 or PRL2. In one embodiment, the
30 extent of binding of an antibody to a non-PRL3 target is less than about 10% of the binding of the antibody to PRL3 as measured, e.g., by a radioimmunoassay (RIA).

The PRL3-binding antibody or antigen-binding fragment thereof may have a dissociation constant (K_d) when binding PRL3 of less than about 1 μ M, less than about 100 nM, less than

about 10 nM, less than about 1 nM, less than about 100 pM, or less than about 10 pM. Binding affinity can be measured by methods known in the art, such as using a radiolabelled antigen binding assay (RIA) performed with the Fab portion of the antibody and an antigen molecule.

5

The antibody or antigen-binding fragment thereof may bind PRL3 with a K_d in the range of about 5 pM to about 8 pM, preferably about 6 pM to about 7 pM, more preferably about 6 pM (c.g., 6.3 pM). The antibody or antigen-binding fragment thereof may have an off-rate of between about $1 \times 10^{-6} \text{ s}^{-1}$ to about $1 \times 10^{-5} \text{ s}^{-1}$, for example about $7 \times 10^{-5} \text{ s}^{-1}$.

10

In some embodiments, the PRL3-binding antibody or antigen-binding fragment thereof is humanised. Humanised antibodies are antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. The process of humanisation is usually applied to monoclonal
15 antibodies developed for administration to humans. The process of humanisation can be necessary when the process of developing a specific antibody involves generation in a non-human immune system, such as mice, as such antibodies may be immunogenic when administered to human patients. Humanisation may involve substitution of selective amino acids in the Fab portion of the molecule. Alternatively, humanisation may involve insertion
20 of the appropriate CDR coding segments into a human antibody scaffold.

In some embodiments, the antibody or antigen-binding fragment thereof binds to an epitope comprising the amino acid sequence KAKFYN (SEQ ID NO: 2) and/or HTHKTR (SEQ ID NO: 3). The antibody or antigen-binding fragment thereof may be capable of binding both
25 epitope sequences.

The antibody or antigen-binding fragment thereof may comprise at least one heavy chain variable region (V_H) incorporating CDRs with the amino acid sequences of SEQ ID NO: 4–6. The antibody or antigen-binding fragment thereof may comprise a V_H comprising an
30 amino acid sequence as set forth in any one of SEQ ID NO: 10–19, or an amino acid sequence having at least 70% sequence identity to at least one region other than a CDR of the V_H amino acid sequence as set forth in any one of SEQ ID NO: 10–19. The antibody or antigen-binding fragment thereof may comprise a V_H comprising an amino acid sequence

having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to at least one region other than a CDR of the V_H amino acid sequence as set forth in any one of SEQ ID NO: 10–19. The antibody or antigen-binding
5 fragment thereof may comprise a V_H which is distinguished from a V_H amino acid sequence as set forth in any one of SEQ ID NO: 10–19 by a deletion, substitution or addition of one or more (e.g., 1, 2, 3, 4, 5 or more) amino acids in at least one region other than a CDR (e.g., in at least one framework region, such as in 1, 2, 3 or 4 framework regions, of the V_H). The antibody or antigen-binding fragment thereof preferably comprises a V_H comprising an
10 amino acid sequence as set forth in any one of SEQ ID NO: 20–23, more preferably a V_H comprising all the amino acid sequences as set forth in SEQ ID NO: 20–23.

The antibody or antigen-binding fragment thereof may comprise at least one light chain variable region (V_L) incorporating CDRs with the amino acid sequences of SEQ ID NO:
15 7–9. The antibody or antigen-binding fragment thereof may comprise a V_L comprising an amino acid sequence as set forth in any one of SEQ ID NO: 24–32, or an amino acid sequence having at least 70% sequence identity to at least one region other than a CDR of the V_L amino acid sequence as set forth in any one of SEQ ID NO: 24–32. The antibody or antigen-binding fragment thereof may comprise a V_L comprising an amino acid sequence
20 having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to at least one region other than a CDR of the V_L amino acid sequence as set forth in any one of SEQ ID NO: 24–32. The antibody or antigen-binding fragment thereof may comprise a V_L which is distinguished from a V_L amino acid sequence
25 as set forth in any one of SEQ ID NO: 24–32 by a deletion, substitution or addition of one or more (e.g., 1, 2, 3, 4, 5 or more) amino acids in at least one region other than a CDR (e.g., in at least one framework region, such as in 1, 2, 3 or 4 framework regions, of the V_L). The antibody or antigen-binding fragment thereof preferably comprises a V_L comprising an amino acid sequence as set forth in any one of SEQ ID NO: 33–35, more preferably a V_L
30 comprising all the amino acid sequences as set forth in SEQ ID NO: 33–35.

In one embodiment, the antibody or antigen-binding fragment thereof comprises a) a heavy chain variable region (V_H) comprising the VHCDR1 amino acid sequence of

GYTFTNYMH (SEQ ID NO: 4), the VHCDR2 amino acid sequence of WIYPGNVNTYYNEKFRG (SEQ ID NO: 5) and the VHCDR3 amino acid sequence of EEKNYPWFAY (SEQ ID NO: 6); and b) a light chain variable region (V_L) comprising the VLCDR1 amino acid sequence of KASQSVEDDGENYMN (SEQ ID NO: 7), the VLCDR2 amino acid sequence of AASNLES (SEQ ID NO: 8) and the VLCDR3 amino acid sequence of QQSNEPFT (SEQ ID NO: 9). Reference to PRL3-zumab herein is a reference to a humanised PRL3-binding antibody with the CDR sequences in SEQ ID NO: 4–9.

In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a V_H comprising an amino acid sequence as set forth in any one of SEQ ID NO: 10–19, and a V_L comprising an amino acid sequence as set forth in any one of SEQ ID NO: 24–32; or b) a V_H comprising an amino acid sequence having at least 70% sequence identity to at least one region other than a CDR of the V_H amino acid sequence as set forth in any one of SEQ ID NO: 10–19, and a V_L comprising an amino acid sequence having at least 70% sequence identity to at least one region other than a CDR of the V_L amino acid sequence as set forth in any one of SEQ ID NO: 24–32.

In some embodiments, the antibody or antigen-binding fragment thereof comprises: a) a V_H which is distinguished from a V_H amino acid sequence as set forth in any one of SEQ ID NO: 10–19 by a deletion, substitution or addition of one or more (e.g., 1, 2, 3, 4, 5 or more) amino acids in at least one region other than a CDR (e.g., in at least one framework region, such as in 1, 2, 3 or 4 framework regions, of the V_H); and b) a V_L which is distinguished from a V_L amino acid sequence as set forth in any one of SEQ ID NO: 24–32 by a deletion, substitution or addition of one or more (e.g., 1, 2, 3, 4, 5 or more) amino acids in at least one region other than a CDR (e.g., in at least one framework region, such as in 1, 2, 3 or 4 framework regions, of the V_L).

Other suitable PRL3-binding antibodies are described in Sun, S. et al (*J Cancer*. 2023 Aug 21; 14(13):2585–2595. doi: 10.7150/jca.81702). Humanised PRL3-binding antibodies suitable for the methods herein are described in PCT/SG2017/050300.

Human PRL3 amino acid sequence

MARMNRPAPVEVS YKHMRLITHNPTNATLSTFIEDLK KYGATTVVRVCEVTYDK

TPLEKDGITVVDWPFDDGAPPPGKVVEDWLSLVKAKFCEAPGSCVAVHCVAGLG
RAPVLVALALIESGMKYEDAIQFIRQKRRGAINSKQLTYLEKYRPKQRLRFKDPHT
HKTRCCVM
(SEQ ID NO: 1)

5

Exemplary PRL3 epitope sequences

KAKFYN (SEQ ID NO: 2)

HTHKTR (SEQ ID NO: 3)

10 Exemplary Humanised PRL3 antibody CDR sequences

VHCDR1: GYTFTNYMH (SEQ ID NO: 4)

VHCDR2: WIYPGNVNTYYNEKFRG (SEQ ID NO: 5)

VHCDR3: EEKNYPWFAY (SEQ ID NO: 6)

VLCDR1: KASQSVEDDGENYMN (SEQ ID NO: 7)

15 VLCDR2: AASNLES (SEQ ID NO: 8)

VLCDR3: QQSNEPFT (SEQ ID NO: 9)

Exemplary humanised PRL3 antibody V_H sequences

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQLEWMGWIYP

20 GNVNTYYNEKFRGRVTITADTSASTAYMLSSLRSEDVAVYYCASEEKNYPWFAY
WGQGLVTS

(SEQ ID NO: 10)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQLEWIGWIYPG

25 NVNTYYNEKFRGRATLTADKSASTAYMLSSLRSEDVAVYYCASEEKNYPWFAYW
GQGLVTS

(SEQ ID NO: 11)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQLEWIGWIYPG

30 NVNTYYNEKFRGRATITADTSASTAYMLSSLRSEDVAVYYCASEEKNYPWFAYW
GQGLVTS

(SEQ ID NO: 12)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQLEWIGWIYPG

NVNTYYNEKFRGKATLTADKSASTAYMLSSLRSEDVAVYYCASEEKNYPWFAY
WGQGLVTS
(SEQ ID NO: 13)

5 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVKQRPQGLEWIGWIYPG
NVNTYYNEKFRGKATLTADKSASTAYMLSSLRSEDVAVYYCASEEKNYPWFAY
WGQGLVTS
(SEQ ID NO: 14)

10 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQRPQGLEWIGWIYPG
NVNTYYNEKFRGKATITADKSASTAYMLSSLRSEDVAVYYCASEEKNYPWFAYW
GQGLVTS
(SEQ ID NO: 15)

15 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQGLEWIGWIYP
GNVNTYYNEKFRGKATITADTSASTAYMELSSLRSEDVAVYFCASEEKNYPWFAY
WGQGLVTS
(SEQ ID NO: 16)

20 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQRPQGLEWIGWIYP
GNVNTYYNEKFRGKATITADKSASTAYMELSSLRSEDVAVYFCASEEKNYPWFAY
WGQGLVTVSSAS
(SEQ ID NO: 17)

25 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQGLEWIGWIYP
GNVNTYYNEKFRGKATITADTSASTAYMELSSLRSEDVAVYFCASEEKNYPWFAY
WGQGLVTVSSAS
(SEQ ID NO: 18)

30 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQGLEWIGWIYP
GNVNTYYNEKFRGKATLTADKSASTAYMELSSLRSEDVAVYFCASEEKNYPWFA
YWGQGLVTVSSAS
(SEQ ID NO: 19)

Exemplary core V_H sequences

VQSGAEVKKPGASVKVSCKASGYTFTNYYMHWV (SEQ ID NO: 20)

WIYPGNVNTYYNEKFR (SEQ ID NO: 21)

ASTAYMELSSLRSE (SEQ ID NO: 22)

5 ASEEKNYPWFAYWGQGLVT (SEQ ID NO: 23)

Exemplary humanised antibody V_L sequences

DTQLTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGSPKLLIYAASN

LESGIPARFSGSGGTDFLTISLPEDFATYYCQQSNEDPFTFGSGTKLEIK

10 (SEQ ID NO: 24)

DTQLTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGSPKLLIYAASN

LESGIPARFSGSGGTDFLTISLPEDFATYYCQQSNEDPFTFGQGTKLEIK

(SEQ ID NO: 25)

15

DTQLTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGSPKLLIYAASN

LESGVPSRFSGSGGTDFLTISLPEDFATYYCQQSNEDPFTFGQGTKLEIK

(SEQ ID NO: 26)

20 DTQLTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGSPKLLIYAASN

LESGIPSRFSGSGGTDFLTISLPEDFATYYCQQSNEDPFTFGPGTKVDIK

(SEQ ID NO: 27)

DTQLTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGSPKLLIYAASN

25 LESGIPSRFSGSGGTDFLTISLPEDFATYYCQQSNEDPFTFGPGTKVDIK

(SEQ ID NO: 28)

DTVLTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGSPKLLIYAASN

LESGIPSRFSGSGGTDFLTISLPEDFATYYCQQSNEDPFTFGPGTKVDIK

30 (SEQ ID NO: 29)

DIQMTQSPSSLSASVGDRVITICKASQSVEDDGENYMNWYQQKPGKSPKLLIYAA

SNLESGIPARFSGSGGTDFLTISLQPEDFATYYCQQSNEDPFTFGSGTKLEIKRT

(SEQ ID NO: 30)

DIQMTQSPSSLSASVGDRVTITCKASQSVEDDGENYMNWYQQKPGKAPKLLIYAA
 SNLESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSNEDPFTFGPGTKVDIKRT
 (SEQ ID NO: 31)

5

DTQLTQSPSSLSASVGDRVTITCKASQSVEDDGENYMNWYQQKPGKAPKLLIYAA
 SNLESGIPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSNEDPFTFGPGTKVDIKRT
 (SEQ ID NO: 32)

10 Exemplary core V_L sequences

QSPSSLSASVGDRVT (SEQ ID NO: 33)

KASQSVEDDGENYMNWYQQK (SEQ ID NO: 34)

SGSGSGTDFTLTISLQPEDFATYYCQQSNEDPFT (SEQ ID NO: 35)

15 The PRL3-binding antibody may be an IgA, IgD, IgE, IgM or IgG, preferably an IgG.

In some embodiments, the antibody or antigen-binding fragment thereof may further comprise a human constant region, for example, selected from IgG1, IgG2, IgG3 and IgG4.

In some embodiments, the antibody or antigen-binding fragment thereof may further
 20 comprise a murine constant region, for example, selected from IgG1, IgG2A, IgG2B and IgG3.

The antibody may be a whole antibody or an antigen-binding fragment that includes an Fc domain. The antibody or antigen-binding fragment thereof may include one or both of a C_H1
 25 and a C_H2 domain. Preferably, the antibody includes a C_H2 domain. The antibody may contain both a C_H1 and a C_H2 domain. Preferably, the antibody is not a Fab', F(ab')₂ fragment, and/or is not an scFv and/or is not a minibody. Preferably, the antibody is an IgG immunoglobulin.

30 Whole antibodies, and F(ab')₂ fragments are "bivalent". By "bivalent" is meant that the said antibodies and F(ab')₂ fragments have two antigen-binding domains. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen-binding domains. Synthetic PRL3-binding antibodies may also be made using phage display technology as is well known in the art. Antibodies may be produced by a process of affinity maturation in

which a modified antibody is generated that has an improved affinity to the antigen, compared to an unmodified parent antibody. Affinity-matured antibodies may be produced by procedures known in the art, e.g., Marks et al, *Rio/Technology* 10:779-783 (1992); Barbas et al. *Proc Nat. Acad. Sci. USA* 91 :3809-3813 (1994); Schier et al. *Gene* 169: 147-155
5 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):331 0-15 9 (1995); and Hawkins et al, *J. Mol. Biol.* 226:889-896 (1992).

In some embodiments, the anti-PRL3 antibodies of this disclosure are bispecific antibodies comprising a first antigen-binding site that specifically binds to PRL3 and a second antigen-
10 binding site that specifically binds to VEGF.

PRL3-binding antibodies or antigen-binding fragments thereof of this disclosure may be expressed in, and preferably secreted from, any suitable host cell, e.g., a bacterial cell, a fungal cell, an insect cell, or a mammalian cell (e.g., 293 cells, PerC6, CHO, BHK, Cos,
15 HeLa cells).

Antibodies according to the present invention may be detectably labelled or, at least, capable of detection. For example, the antibody may be labelled with a radioactive atom or a coloured molecule or a fluorescent molecule or a molecule which can be readily detected in any other
20 way. Suitable detectable molecules include fluorescent proteins, luciferase, enzyme substrates, and radiolabels. The antibody or antigen-binding fragment thereof may be directly labelled with a detectable label or it may be indirectly labelled. For example, the antibody or antigen-binding fragment thereof may have bound to it biotin and binding of labelled streptavidin to the biotin may be used to indirectly label the antibody or antigen-
25 binding fragment.

In some embodiments, the antibody or antigen-binding fragment is conjugated to a radioisotope or a cytotoxin. Non-limiting examples of cytotoxins include chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic
30 enzymes; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase I inhibitors, topoisomerase II inhibitors, antimetabolites, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase

angiogenesis inhibitors, immunotherapeutic agents, pro-apoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signalling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. The cytotoxin may be conjugated to the antibody or antigen-binding fragment according to methods established in the art, with or without a linker, for example, at the N- or C-terminus of the antibody.

The radioisotope may be, for example, ^{18}F , ^{32}P , ^{33}P , ^{45}Ti , ^{47}Sc , ^{52}Fe , ^{59}Fe , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{77}As , ^{86}Y , ^{90}Y , ^{89}Sr , ^{89}Zr , ^{90}Y , ^{94}Tc , ^{94}Tc , ^{99}Tc , ^{99}Mo , ^{105}Pd , ^{105}Rh , ^{111}Ag , ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{142}Pr , ^{143}Pr , ^{149}Pm , ^{153}Sm , $^{154-158}\text{Gd}$, ^{161}Tb , ^{166}Dy , ^{166}Ho , ^{169}Er , ^{175}Lu , ^{177}Lu , ^{186}Re , ^{188}Re , ^{189}Re , ^{194}Ir , ^{198}Au , ^{199}Au , ^{211}At , ^{211}Pb , ^{212}Bi , ^{212}Pb , ^{213}Bi , ^{223}Ra or ^{225}Ac . The radioisotope may further comprise a chelating agent which forms a complex with the radioisotope. Chelating agents include, but are not limited to, glucoheptonate, gluconate, glucarate, citrate, tartarate, ethylenedicycysteine (EC), nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), hydroxyethylethylenediaminetriacetic acid (HEDTA), diethylenetriaminopentaacetic acid (DTPA), trans-1,2-diaminocyclohexanetetraacetic acid (CDTA), hydrazinonicotinamide (HYNIC), mercaptoacetyltriglycine (MAG3), 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA), and 1,4,7-triazacyclononanetriacetic acid (NOTA). The antibody may be conjugated to the radioisotope or chemical compound via the chelating agent, for example, at the N- or C-terminus of the antibody.

Subjects

The subject to be treated may be a non-human mammal, but is more preferably a human. In preferred embodiments, the subject has been diagnosed with a neovascular eye disease or condition, or has been determined to be at risk of developing a neovascular eye disease or condition.

In some embodiments, the subject has been determined to have an increased level of PRL3 from a sample obtained from the subject, such as an ocular tissue sample.

Methods herein may comprise administering a PRL3 inhibitor or PRL3-binding antibody or antigen-binding fragment thereof to a subject who is undergoing or who has undergone an anti-VEGF therapy for the neovascular disease or condition.

In one embodiment, the PRL3 inhibitor or PRL3-binding antibody or antigen-binding fragment thereof is administered to a subject who is non-responsive to the anti-VEGF therapy. Advantageously, PRL3 inhibitor therapy may be provided as a complementary or
5 alternative option to anti-VEGF therapy for neovascular eye diseases or conditions, especially for subjects who are refractory to anti-VEGF therapy.

Routes of administration

- 10 PRL3 inhibitors, including PRL3 antibodies and antigen binding fragments thereof, and other therapeutic agents, medicaments and pharmaceutical compositions of this disclosure may be formulated for administration by a number of routes, including but not limited to, parenteral, intravenous, intraocular, topical (to the eye), intramuscular, and oral.
- 15 The PRL3 inhibitors, antibodies, or antigen-binding fragments thereof may be formulated in fluid or solid form. Fluid formulations may be formulated for administration by injection or topical application to a selected region of the human or animal body.

In some embodiments, the antibody or antigen-binding fragment thereof is administered
20 intravenously or locally at or near the eye. Local administration may be via the extraocular (topical), periocular or intraocular routes.

In one embodiment, the antibody or antigen-binding fragment thereof is administered topically to the eye (e.g., via eye drops, contact lens). In one embodiment, the antibody or
25 antigen-binding fragment thereof is administered periocularly (such as to the sub-conjunctival, sub-Tenon's, or orbital floor regions). In one embodiment, the antibody or antigen-binding fragment thereof is administered intraocularly (such as intracameral, intravitreal, suprachoroidal or supraciliary administration).

30 In some embodiments, the antibody or antigen-binding fragment thereof is administered intraocularly into the vitreous cavity. Intravitreal administration may be via intravitreal injection or implant (e.g., a controlled release implant).

In one embodiment, the antibody or antigen-binding fragment thereof is administered

intravenously. Intravenous administration has the advantage that it is not limited by the volume and dose of intraocular delivery, and also avoids the risk of ocular complications such as subconjunctival hemorrhage, increased intra-orbital pressure, retinal epithelial tears and cataract formation, etc.

5

Pharmaceutical compositions

Inhibitors, antibodies, antigen-binding fragments and polypeptides of the present disclosure may be formulated as pharmaceutical compositions (such as infusible or ophthalmic
10 compositions) for clinical use and may comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

By “pharmaceutically acceptable carrier” is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e., the material may be
15 administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, colouring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like.

20 Representative pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be
25 known to one of ordinary skill in the art (see, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient(s), its use in the pharmaceutical compositions is contemplated.

30 More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and will preferably be

preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of
5 a coating such as lecithin and/or by the maintenance of the required particle size.

In specific embodiments, an inhibitor or antibody of the present disclosure may be conjugated to a vehicle for cellular delivery. In these embodiments, the inhibitor or antibody may be encapsulated in a suitable vehicle to either aid in the delivery of the inhibitor to target
10 cells, to increase the stability of the inhibitor or antibody, or to minimise potential toxicity of the inhibitor or antibody. As will be appreciated by a skilled person, a variety of vehicles are suitable for delivering an inhibitor or antibody of the present disclosure. Non-limiting examples of suitable structured fluid delivery systems may include nanoparticles, liposomes, microemulsions, micelles, dendrimers and other phospholipid-containing systems. Methods
15 of incorporating inhibitors or antibodies of the present disclosure into delivery vehicles are known in the art. Although various embodiments are presented below, it will be appreciated that other methods known in the art to incorporate an antibody or antibody fragment, as described herein, into a delivery vehicle are contemplated.

20 The present disclosure also provides methods for the production of pharmaceutically useful antibody or antibody fragment compositions. Such methods of production may comprise isolating and mixing an antibody, antigen-binding fragment or polypeptide as defined herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

25 The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, eye drops, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Suitable pharmaceutical compositions may be administered systemically (e.g.,
30 intravenously, orally), or locally to the eye (e.g., intraocularly, topically). A preferred mode of administration is parenteral (e.g., intravenous, intraocular, topical).

In some embodiments, the compositions or pharmaceutical compositions herein are in the form of injectable, infusible or instillable solutions.

The solutions may be formulated for systemic administration (e.g., intravenous infusion) or local administration to the eye (e.g., intraocular injection, topical instillation).

- 5 In some embodiments, compositions and pharmaceutical compositions herein are ophthalmic compositions suitable for administration to the eye, for example, via extraocular (topical), periocular or intraocular routes.

Disclosed herein is an ophthalmic pharmaceutical composition comprising a PRL3 inhibitor.

- 10 In some embodiments, the ophthalmic pharmaceutical composition comprises a PRL3-binding antibody or antigen-binding fragment thereof.

- In some embodiments, the ophthalmic pharmaceutical composition is formulated for topical administration to the eye. In one embodiment, the topical formulation is in the form of an
15 eye drop. Other formulations for topical application to the eye are also possible, such as gels, ointments, sprays, drug-impregnated contact lenses, and extraocular implants and inserts.

In some embodiments, the ophthalmic pharmaceutical composition is an injectable formulation suitable for periocular or intraocular injection.

20

In some embodiments, the ophthalmic pharmaceutical composition is formulated for periocular administration to the eye. In one embodiment, the periocular formulation is in the form of an injectable formulation, insert or implant.

- 25 In some embodiments, the ophthalmic pharmaceutical composition is formulated for intraocular administration to the eye. In one embodiment, the intraocular formulation is in the form of an injectable formulation, insert or implant. Injectable formulations may be for intracameral, intravitreal, suprachoroidal or supraciliary administration.

- 30 Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. An antigen-binding molecule of the present disclosure can be administered on multiple occasions. Intervals between single dosages can

be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood or ocular levels of the antigen-binding molecule or PRL3 in the subject. Alternatively, the antigen-binding molecule can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending
5 on the half-life of the polypeptide in the patient.

It may be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit
10 contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutically acceptable carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding
15 such an active compound for the treatment of sensitivity in individuals.

In some embodiments, the antibody or antigen-binding fragment thereof is administered intravenously as an infusion or injection at a dose of about 1 mg/kg to about 12 mg/kg (i.e., one mg of antibody or antigen-binding fragment for every kg weight of the subject). The
20 intravenous dose may be 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, about 10 mg/kg, about 11 mg/kg, or about 12 mg/kg. The dosing schedule can vary from e.g., once a week to once every 2, 3, 4, 5, 6, 7, or 8 weeks.

25 It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens may be adjusted over time according to individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the
30 scope or practice of the claimed composition.

Combination therapy

PRL3 inhibitors may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. The administration of a combination of a PRL3 inhibitor and one more partner drugs can provide an additive or synergistic beneficial effect for treating the neovascular disease or condition, for example, by enhancing therapeutic response to one or more of the drugs, or by reducing the likelihood of non-response or a reduced response to one or more of the drugs. Additional beneficial effects may include fewer side effects, and a lower effective dose compared with a monotherapy applying only the PRL3 inhibitor or only the partner drug(s).

- 10 In embodiments of methods herein, the PRL3 inhibitor is administered as a combination therapy with a second drug for treating a neovascular eye disease. In one embodiment, the PRL3 inhibitor is administered in combination with an anti-VEGF therapy. The PRL3 inhibitor may be a PRL3-binding antibody or antigen-binding fragment thereof of this disclosure. Advantageously, the PRL3 inhibitor can reverse the desensitisation effect of PRL3 on anti-VEGF therapy, thus enhancing response to the anti-VEGF therapy.

Disclosed herein is a method of enhancing an anti-VEGF therapy in a subject with a neovascular eye disease or condition, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof simultaneously or sequentially with an effective amount of the anti-VEGF therapy to the subject.

Disclosed herein is a method of treating a subject with a neovascular eye disease or condition, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof in combination with an effective amount of an anti-VEGF agent to the subject.

Disclosed herein is a pharmaceutical combination comprising a PRL3-binding antibody or antigen-binding fragment thereof, and an anti-VEGF agent.

- 30 Disclosed herein is a pharmaceutical combination as defined herein, for use as a medicament.

Disclosed herein is a pharmaceutical combination as defined herein, for use in treating a neovascular eye disease or condition in a subject.

Disclosed herein is the use of a pharmaceutical combination as defined herein in the manufacture of a medicament for treating a neovascular eye disease or condition in a subject.

5 Disclosed herein a PRL3-binding antibody or antigen-binding fragment thereof, for use in treating a neovascular eye disease or condition in a subject, wherein an effective amount of the PRL3-binding antibody or antigen-binding fragment thereof is to be administered in combination with an effective amount of an anti-VEGF agent to the subject.

10 Disclosed herein an anti-VEGF agent, for use in treating a neovascular eye disease or condition in a subject, wherein an effective amount of the anti-VEGF agent is to be administered in combination with an effective amount of an PRL3-binding antibody or antigen-binding fragment thereof to the subject.

15 Disclosed herein is the use of a PRL3-binding antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating a neovascular eye disease or condition in a subject, wherein an effective amount of the PRL3-binding antibody or antigen-binding fragment thereof is to be administered in combination with an effective amount of an anti-VEGF agent to the subject.

20

Disclosed herein is the use of an anti-VEGF agent in the manufacture of a medicament for treating a neovascular eye disease or condition in a subject, wherein an effective amount of the anti-VEGF agent is to be administered in combination with an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof to the subject.

25

As used herein, an “anti-VEGF agent” refers to a compound that inhibits the expression or activity of VEGF. The agent may reduce or inhibit expression of functional VEGF by, e.g., altering the processing of VEGF mRNA or inducing degradation of VEGF mRNA. Alternatively, the agent may induce VEGF degradation, inhibit binding of VEGF to a VEGF
30 receptor (e.g., VEGFR1 or VEGFR2), or otherwise prevent signaling from a VEGF receptor. An “anti-VEGF therapy” refers to any course of treatment which uses an anti-VEGF agent. Anti-VEGF therapies herein may comprise two or more anti-VEGF agents.

Anti-VEGF agents include, for example, anti-VEGF antibodies (e.g., ranibizumab

[Lucentis®], bevacizumab [Avastin®], brolucizumab [Beovu®], faricimab [Vabysmo®]; anti-VEGF receptor antibodies (e.g., anti-VEGFR1 antibodies, anti-VEGFR2 antibodies, etc.); anti-VEGF aptamers (e.g., pergaptanib [Macugen®]); compounds that inhibit, regulate, and/or modulate tyrosine kinase signal transduction (e.g., sorafenib [Nexavar®], sunitinib [Sutent®], tivozanib [Fotivda®]); VEGF receptor-based chimeric molecules or VEGF-inhibiting fusion proteins (e.g., aflibercept [Eylea®] and other VEGF-Traps); oligonucleotides that inhibit VEGF expression at the nucleic acid level, e.g., antisense RNAs and RNA interference agents; and retinoids.

10 The term “pharmaceutical combination” as used herein refers to either a fixed combination in one dosage unit form, or a non-fixed combination or a kit of parts for combined administration where two or more therapeutic agents may be administered independently at the same time or separately within time intervals, especially where such administration provides therapeutically effective levels of the two or more agents in the body of the patient.

15 The terms “a combination” and “in combination with” are not intended to imply that the therapy or the therapeutic agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope described herein. The therapeutic agents in the combination can be administered concurrently with, prior to, or subsequent to, one or more other additional therapies or therapeutic agents. The therapeutic agents or therapeutic protocol can be administered in any order. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. It will further be appreciated that the additional therapeutic agent utilised in the combination may be administered together or separately in different compositions. In general, it is expected that therapeutic agents utilised in combination be utilised at levels that do not exceed the levels at which they are utilised individually. In some embodiments, the levels utilised in combination are lower than those utilised individually.

25 The individual combination partners of a combination therapy may be administered via the same route (e.g., all intravitreally) or via different routes (e.g., one intravitreally and another intravenously or topically).

Simultaneous administration refers to administration of the PRL3 inhibitor and the anti-VEGF agent together, for example as a combination preparation, or immediately after each

other and optionally via the same route of administration, e.g., both intravitreally or to the same vein or blood vessel.

Sequential administration refers to administration of one of the PRL3 inhibitor or anti-VEGF agent followed after a given time interval by separate administration of the other combination partner. It is not required that the two agents be administered by the same route. The time interval may be any pre-determined time interval, but is preferably one that provides for a cooperative effect of the PRL3 inhibitor and the anti-VEGF agent in the subject.

10

In some embodiments, the PRL3 inhibitor (such as the anti-PRL3 antibody or antigen-binding fragment thereof) is intravitreally administered in combination with the anti-VEGF agent. In some embodiments, the PRL3 inhibitor (such as the anti-PRL3 antibody or antigen-binding fragment thereof) is administered as a single combined dosage formulation with the anti-VEGF agent. In some embodiments, the PRL3 inhibitor (such as the anti-PRL3 antibody or antigen-binding fragment thereof) is administered in combination with the anti-VEGF agent, and at least the PRL3 inhibitor is administered intravenously. The PRL3 inhibitor may be administered simultaneously or sequentially (e.g., before or after) with the anti-VEGF therapy.

20

In some embodiments, the PRL3 inhibitor or PRL3-binding antibody or antigen-binding fragment thereof is administered simultaneously or sequentially with an anti-VEGF therapy. In sequential administration, the PRL3 inhibitor or PRL3-binding antibody or antigen-binding fragment thereof may be administered about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours, about 72 hours, or more than 72 hours before or after administration of the anti-VEGF therapy. Simultaneous administration refers to administration of the anti-VEGF therapy and administration of the PRL3 inhibitor or PRL3-binding antibody or antigen-binding fragment thereof within less than 5 minutes of each other if both are administered in separate dosage form. Alternatively, both the anti-VEGF therapy and the PRL3 inhibitor or PRL3-binding antibody or antigen-binding fragment thereof may be administered as a single combined dosage formulation.

30

The effective dosage of each of the combination partners employed may vary depending on

the particular compound or pharmaceutical composition employed, the mode of administration, the disease or condition being treated, and the severity of the disease or condition being treated. Thus, the dosage regimen of a combination of this disclosure is selected in accordance with a variety of factors including the route of administration and the condition of the patient. A physician of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to alleviate, counter or arrest the progress of the condition.

Kits

The present disclosure also extends to kits for detecting PRL3 in a sample (e.g., an ocular sample) obtained from a subject. The kit may allow the detection of a neovascular eye disease or condition in a subject when the level of PRL3 in the sample is increased compared to a reference.

The kit may comprise one or more reagents or materials for detecting PRL3 in the sample from the subject. For example, the kit may comprise labelled antigen-binding molecules (e.g., antibodies or aptamers) for binding to and detecting PRL3. Alternatively or additionally, the kit may comprise labelled PRL3 substrates for generating a signal upon enzymatic action by PRL3. In one embodiment, the kit comprises a PRL3-binding antibody or antigen-binding fragment thereof as defined herein.

The kit may also include appropriate reagents for detection of labels, positive and negative controls, washing solutions, blotting membranes, microtitre plates, dilution buffers and the like. For example, a protein detection kit may include (i) at least one PRL3 molecule (which may be used as a positive control), and (ii) one or more antigen-binding molecules that bind specifically to PRL3. The antigen-binding molecule may be a PRL3-binding antibody or antigen-binding fragment thereof as defined herein. The antigen-binding molecules may be suitably detectably labelled. The kit can also feature various devices (e.g., one or more) and reagents (e.g., one or more) for performing a detection assay herein, and/or printed instructional material for using the kit to quantify the level of PRL3. The reagents described herein, which may be optionally associated with detectable labels, can be presented in the format of a microfluidics card, a chip or chamber, or a microarray.

Also provided herein is a composition comprising (a) a sample obtained from a subject; and (b) a PRL3-binding antibody or antigen-binding fragment thereof for detecting PRL3 in the sample. The sample may be an ocular tissue sample.

5 It will be appreciated that the combination partners of combination therapies herein may also be presented as a kit for use in the treatment of neovascular eye disease. The kit may comprise a package where the combination partners are supplied separately for co-administration with instructions for use in the particular therapy.

10 As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form "a," "an," and "the" include plural references
15 unless the context clearly dictates otherwise. For example, the term "an agent" includes a plurality of agents, including mixtures thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will
20 be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially
25 of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

30 The section headings used herein are solely for subject matter organisation and are not intended to limit the subject matter described.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour
5 to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications, which fall within the spirit
10 and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Unless otherwise defined, all technical and scientific terms used herein have the same
15 meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit
20 the scope of the generality hereinbefore described.

EXAMPLES

Methods

25

Whole mount mouse embryo staining

E9.5 embryos were fixed in 2% paraformaldehyde for 30 minutes and washed with washing buffer (0.1% Triton-PBS). The fixed embryos were then immersed in ascending
30 concentration of methanol from 50% to 75% and to 100% for 20 minutes each. After which, the embryos were incubated in 1%-2% of H₂O₂ in methanol for 45 minutes and then immersed in descending concentration of methanol from 75% to 50% and to 25% for 20 minutes each. These embryos were then washed twice with 0.1% Triton-PBS and subsequently blocked with blocking buffer (2% Skim milk + 0.2% Triton-X in PBS) for 2

hours at 4°C. These were then incubated in primary antibodies diluted in blocking buffer (CD31, 1:200; Ter119, 1:200; vWF, 1:200; PRL3, 1:200) overnight at 4°C. The next day, these were washed with washing buffer thrice for 10 minutes each and then incubated with respective secondary antibodies (1:1000) for 3 hours at room temperature. After which, these
5 were washed with washing buffer thrice, once with PBS and once with 1XTBS for 10 minutes each. Finally, the embryos were incubated with DAB (7.5 mg DAB + 2µl H₂O₂ + 15 ml TBS) for 5 minutes until brown coloration appeared and washed thrice with PBS. The stained samples were then ready for imaging.

10 Choroidal Neovascularisation model

In the CNV mouse model, eight-week-old C57BL/6/J male mice were used. The mice were anaesthetised using a combination of ketamine (150 mg/kg body weight) and xylazine (10 mg/kg body weight) and pupils dilated using a topical administration of 1 drop of 1%
15 tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) and 1 drop of 1% phenylephrine (Bausch and Lomb Pharmaceuticals, Inc., Tampa, FL, USA) ophthalmic solutions. Laser induction was done using the MICRON IV retinal imaging system (50 µm spot size, 0.05 second duration, 150 mW) in the posterior pole of the retina. 4 spots were given to each eye. One week after laser burn, mice were sacrificed for the neural retina and
20 retinal pigment epithelium for western blotting and flat mount staining.

Drug treatment was given by intravitreal (IVT) or intravenous (IV) routes. For IVT, standard-of-care dose of 2.5 µg of drug (Eylea and mouse anti-PRL3 antibody) was carefully injected under the dissection microscope. It is administered on Day 1 after FFA and OCT
25 images are taken. For IV, 100 µg of drug (PRL3-zumab and mouse anti-PRL3 antibody) is administered by tail vein injection on Day 1 and Day 4. PBS/mouse IgG are used as negative control.

Oxygen-Induced Retinopathy model

30

In short, nursing pups and postnatal day 7 (P7) were housed in a 75% oxygen chamber for 5 days and then in room air subsequently for 5 days. After which, the mice were sacrificed, and the retinae and retinal pigment epithelia were harvested for western blotting analyses.

Fundus fluorescein angiography

Digital color fundus images were taken using a MICRON IV comprehensive system for rodent retinal imaging (Phoenix Research Laboratories, Pleasanton, CA, USA). Mice were
5 anesthetised and pupils were dilated as described above. After dilation, both eyes of the mice will be covered with a layer of viscid gel to aid in ocular imaging and also to prevent drying of the cornea during procedure. The whole procedure took about 15–20 minutes per mouse in a dim room. Mice were immediately placed near a red heat lamp after procedure to maintain body temperature. Digital coloured retinal photographs were captured from the
10 mice during pupil dilation. For FFA, mice were given, via intraperitoneal injection, 10% sodium fluorescein dye at a dose of 0.01 mL per 5 to 6g body weight and fundus images were taken using MICRON IV. FFA was performed 24 hours and 6 days post laser induction and these were set as Day 1 baseline and Day 7 measurements. The images were then analysed using ImageJ (1.53c) software in which the vascular leakage area was marked out
15 and measured by excluding the value of large vessels (with gray value more than 100). Using spot to spot comparison, the vascular leakage area of each spot in Day 7 images was compared against the same spot in Day 1 images. The difference between the two measurements was expressed as a percentage of healing.

Image-guided Optical Coherence Tomography

To perform OCT, mice are anaesthetised and their eyes dilated (as described above). An OCT module + objective lens is attached to the MICRON IV imaging system. Using live colored fundus image as a guide, a stacked image is generated for multiple retina layers.
25

Cell culture

Human Retinal Microvascular Endothelial Cells (HRMECs) (angio-proteomie (cAP-0010)) were cultured on flasks coated with Quick Coating Solution (angio-proteomie (cAP-01)), in
30 Endothelial Growth Medium (LONZA (CC-3162)).

SDS-PAGE and Western blotting

Mouse tissues were homogenised in RIPA buffer with phosphatase inhibitors and protease inhibitors (Roche). Protein concentration was quantified using BCA dye and spectrophotometer at absorbance 450nm. 70µg of protein per sample was loaded and 15% Tris-tricine gel was run for PRL3 detection, and 8% Tris-glycine gel for the detection of other markers. Primary antibodies: mouse PRL3 (318) (in-house, 1:1000), phospho-Src (CST6943, 1:1000), Src (CST2109, 1:1000), phospho-ERK1/2 (CST4370, 1:2000), ERK1/2 (CST4695, 1:1000), phospho-paxillin (CST2541, 1:1000), paxillin (CST2542, 1:1000), phospho-Akt (CST4060, 1:2000), Akt (CST4691, 1:1000), VEGFR2 (ab39638, 1:500), ZO-1 (ThermoFisher Scientific 33-9100, 1:1000), VE-Cadherin (ab205336, 1:1000), GAPDH (Sigma CB1001, 1:3000).

Quantification of mouse anti-PRL3 antibody concentration in mouse serum and vitreous humour samples

Serum samples were collected at pre dose, 24 and 72 hours after IVT and at the end of the experiment. Vitreous samples were collected at the end of experiment. Mouse Anti-PRL3 antibody concentration in serum and vitreous were analysed by ELISA method. Briefly, 96-well plates coated overnight with GST-PRL3 (1 ng) were blocked with 3% bovine serum albumin in PBS-0.05% Tween-20 prior to incubate with serum sample (1:20000) for 1 h at 37°C. After extensive washing, HRP-conjugated anti-mouse antibody (Jackson) was added for 1 h at 37°C. Colorimetric development was performed using a Turbo-TMB substrate (Pierce) and stopped by acidification with 2M H₂SO₄. Absorbance was measured at 450 nm using a plate reader (Tecan).

VEGF treatment of HRMECs

200,000 HRMECs were seeded in a well of 6-well plate. After the cells have attached overnight, the cells were starved with endothelial basal medium (LONZA) for 3 h at 37°C in 5% CO₂ atmosphere. Recombinant human VEGF protein (RnD Systems) was then added in the media to make up the final concentration of 50 ng/ml. The cells were harvested 24 h, 48h and 72h after VEGF treatment for protein analysis.

Retroviral infection of HRMECs

To establish HRMEC cells overexpressing PRL3 (HRMEC-PRL3) and a control cell line (HRMEC-Vector), HRMEC-WT cells are transduced with retrovirus that were made by transfecting Human Embryonic Kidney 293T (HEK293T) cells along with the packaging vector. The pEGFP-C1-PRL3 construct was described previously. PRL3 cDNA was amplified from pEGFP-C1-PRL3 plasmid and cloned into the retroviral vector. The conditioned media (with the retrovirus) was concentrated, aliquoted and stored at -80°C until usage. The virus was then titrated in Endothelial Growth Media-2 (EGM2) and added to HRMECs. The successful transduction could be observed by the GFP signal. HRMECs were harvested and PRL3 overexpression was confirmed by Western blotting.

10

Endothelial proliferation assay

HRMEC proliferation (HRMEC-WT, HRMEC-PRL3) were assessed using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)-based CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's instructions. Briefly, 2×10^3 cells were seeded in complete media into triplicate wells of a 96-well plate and allowed to attach overnight. The medium was then replaced with complete media containing PBS (0.1%; control) or PRL3-zumab (4, 20, 40, 80, 100 µg/ml) and left to incubate for another 48 h at 37°C in 5% CO₂ atmosphere. The media were subsequently aspirated and replaced with 100 µL fresh media containing MTS (Promega) and formazan development was done for 2 h at 37°C and 5% CO₂ before measuring absorbance at 490 nm in a spectrophotometer (Tecan).

20

Endothelial migration assay

25

2×10^3 cells (HRMEC-WT, HRMEC-PRL3) were seeded in the upper chamber of the transwell plate (Corning #3422) in 200 µL of serum-free EBM2 with 500 µL of EGM2 (with FBS) in the lower chamber as chemoattractant. Incubate cells for 24 h at 37°C and 5% CO₂. Non-migrating cells on the upper chamber were scraped off with a cotton swab. Migrated cells (at the bottom of the membrane) were stained with 0.1% crystal violet for 30 minutes. Thereafter, the membrane was washed with distilled water for 30 seconds to remove residual dye and then air dried overnight. Images were taken at 20× magnification and cells were counted.

30

Endothelial monolayer permeability assay

Using the endothelial 24-well transwell permeability assay kit (Cell Biologics, CB6929), 5×10^3 cells (HRMEC-WT, HRMEC-PRL3) were seeded on the 6.5 mm transwell insert membrane in 200 μ L of EGM2. 1 mL of EGM2 was added to the experimental wells. The transwell inserts were transferred to the wells containing 1ml EGM2 and incubated for 4 days at 37°C and 5% CO₂ until the cells grew to 100% confluence. Cells were starved with EBM2 for 3 h and 300 μ L of fresh EBM2 (including 5 μ L of streptavidin-HRP) was added to the top insert chamber of each well. 1 ml of EBM2 was added to the wells of a new 24-well plate and the inserts were transferred to the wells and incubated at 37°C and 5% CO₂ for 24 h. 20 μ L of media were transferred from the lower chamber to the wells of 96-well plate. Each sample was aliquoted in triplicates. 50 μ L of TMB substrate was added into each well and incubated in a shaker for 2 minutes until the blue coloration appeared and then the reaction was terminated by adding 50 μ L of stop solution at which it turns yellow. Absorbance reading at 450 nm was recorded in a spectrophotometer (Tecan).

Toxicology study of PRL3-zumab (IV) in Cynomolgus Monkeys

The study involves biweekly intravenous bolus injection of PRL3-zumab in 40 cynomolgus monkeys at 4 different dose levels (0, 4, 12, 36 mg/kg/dose) with 5 males and 5 females in each level followed by 8-week of recovery period. Eyes were examined once at predose for all animals and once during the last week of the dosing period. Prior to the examination, a mydriatic containing 1.0% Tropicamide was administered to dilate the pupils. All animals were examined via slit lamp and indirect ophthalmoscope. This study was performed by Wuxi Aptec.

Toxicology study of PRL3-zumab (IVT) in New Zealand White Rabbits

This study involves one dose of PRL3-zumab via intravitreal delivery in a total of 9 rabbits at 3 dose levels (0.084 mg, 0.175 mg, 0.35 mg per 35 μ L). All animals were examined using slit lamp, fundoscope and pachymetry. This study was conducted by Singapore Eye Research Institute (SERI).

Toxicology study of PRL3-zumab (Eyedrop) in New Zealand White Rabbits

This study involves two doses of PRL3-zumab daily via eyedrop delivery in a total of 6 rabbits at 2 dose levels (0.079 mg and 0.35 mg per 35 μ l). All animals were examined using slit lamp, fundoscope and pachymetry. This study was conducted by Singapore Eye
5 Research Institute (SERI).

Results

PRL3 expression is observed in embryonic blood vessels (Figure 1) but it is switched off in
10 adulthood under normal physiological condition (Figure 2). However, under pathological conditions (such as pathological angiogenesis) in the eyes, PRL3 expression is upregulated, as shown in diseased choroid-retinal pigment endothelium (RPE) in a CNV (choroidal neovascularisation) mouse model, and in diseased retina in an OIR (oxygen-induced retinopathy) mouse model (Figure 2).

15 *In vitro* studies using human retinal microvascular endothelial cells (HRMECs) were conducted. VEGF treatment upregulated PRL3 expression in HRMECs in a time-dependent fashion, with the highest PRL3 expression being at 72 hr (Figure 8). PRL3 overexpression in HRMECs following retroviral transduction led to an increased proliferation (Figure 9),
20 increased migration (Figure 10) and increased permeability of monolayer (Figure 11). PRL3-overexpressing HRMECs also have differential expression of effector molecules in VEGF signalling pathway, namely an increased phosphorylation of Src, Akt, ERK1/2 and Paxillin, as well as a reduced expression of VE-Cadherin, ZO-1 and VEGFR2 (Figure 12). Furthermore, PRL3 overexpression desensitizes the response of HRMECs to an anti-VEGF
25 antibody (bevacizumab), even at a high antibody dose of 10 mg/ml.

Mouse anti-PRL3 antibody treatment, administered intravitreally (IVT) or intravenously (IV) in a CNV model, demonstrated reduction in vascular leakage and enhanced recovery of CNV lesions (Figure 3). IV delivery of mouse anti-PRL3 antibody yielded a 86% better
30 efficacy than IVT route due to a larger drug dose permitted via IV route (Figure 3). IV treatment using PRL3-zumab also demonstrated efficacy compared to control (Figure 3). Mouse anti-PRL3 antibody treatment attenuates Src phosphorylation which could be one way attributed to the reduction of vascular leakage and improved healing (Figure 4). At 7 days post treatment, the mouse anti-PRL3 antibody concentration was found to be

significantly higher in vitreous humor when administered by IV compared to IVT (Figure 5). This shows that mouse anti-PRL3 antibody could accumulate in the vitreous humor much more when it is administered via IV compared to IVT due to the larger drug dose permitted by IV route. When administered via IV, mouse anti-PRL3 antibody concentration in the blood was significantly higher at 24h, 72h and 168h timepoints compared to when administered via IVT (Figure 6). The concentration of IV-administered mouse anti-PRL3 antibody in vitreous humour was significantly higher in the eyes of the CNV model as compared to normal eyes at 7 days post-treatment (Figure 7). This shows that the compromised Bruch's membrane could allow for the entry of mouse anti-PRL3 antibody into the vitreous humour. The higher drug concentration in the blood and vitreous humour could translate to an enhanced efficacy of anti-PRL3 antibody in CNV mouse model.

The assessment of the safety profile of PRL3-zumab (humanized anti-PRL3 antibody) was performed in cell lines, rabbit model and monkey model. *In vitro* treatment of PRL3-zumab in ocular cell lines (HRMEC, ARPE-19, M10-M1 Müller glial cells) did not show cytotoxicity up to a high dose of 100 µg/ml for 48 hours (Figure 13). IV treatment of PRL3-zumab in monkey model did not show ocular and systemic toxicity up to a high dose of 36mg/kg (Tables 1 and 2). Additionally, IVT and eye drop delivery of PRL3-zumab in rabbit model did not show ocular toxicity up to a high dose of 0.35mg (Figure 14 and Figure 15).

Tables 1 and 2 summarise the results of an ophthalmological toxicology study of PRL3-zumab in monkeys. Biweekly intravenous bolus injection of PRL3-zumab (total 5 doses) were given to 40 cynomolgus monkeys (20 males and 20 females) followed by an 8-week recovery period.

As shown in Table 1, there were ocular abnormalities in 2 individual female cynomolgus monkeys, that is, one developed cataract at 0 mg/kg/dose and the other developed cornea opacity at 4 mg/kg/dose. However, no abnormalities were observed in male counterparts and at higher doses. There was no observed ocular toxicity related to intravenous administration of PRL3-zumab up to a high dose of 36mg/kg/dose in cynomolgus monkeys.

As shown in Table 2, no abnormalities were observed in major body systems in the drug-treated category across all doses except for increased % neutrophil which returned to

baseline levels on Day 51 and during recovery period as well as watery stool which was sporadic.

Table 1. Ocular and systemic safety profile of PRL3-zumab (IV) in monkey toxicology study

	Male cynomolgus monkeys		Female cynomolgus monkeys		
Dose (mg/kg/dose)	Abnormalities detected	No abnormalities detected	Abnormalities detected	No abnormalities detected	Subtotal
0	0	5	1 x Cataract	4	10
4	0	5	1 x Cornea opacity	4	10
12	0	5	0	5	10
36	0	5	0	5	10
Total					40

Table 2. Systemic safety profile of PRL3-zumab (IV) in monkey toxicology study

Abnormalities																	
Dose	Mortality	Infusion reaction	Body weight	Organ weight	Body temperature	ECG†	Blood pressure	Heart rate	Respiratory	Neurological	Necropsy	Histopathology	Serum chemistry	Hematology	Coagulation	Urinalysis	Bowels
0 mg/kg/dose	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
4 mg/kg/dose	No	No	No	No	No	No	No	No	No	No	No	No	No	%Neutrophil increase*; Males: 21%; Females: 38%	No	No	Watery stool; 1X male; 3X females
12 mg/kg/dose	No	No	No	No	No	No	No	No	No	No	No	No	No	%Neutrophil increase*; Males: 28%; Females: 37%	No	No	Watery stool; 3X males; 2X females
36 mg/kg/dose	No	No	No	No	No	No	No	No	No	No	No	No	No	%Neutrophil increase*; Males: 58%; Females: 77%	No	No	Watery stool; 1X female

Differentiating edge

The differentiating edge of PRL3-zumab is essentially that: 1) PRL3 is a novel target in
5 neovascular eye diseases that has not been reported before; 2) PRL3-zumab is a first-in-class
antibody drug; and 3) the feasibility of intravenous (IV) administration of PRL3-zumab due
to its strong safety profile. Our preclinical studies have demonstrated that IV administration
of anti-PRL3 antibody led to a 86% improvement in the recovery of CNV lesions as
10 compared to IVT delivery of the drug. IV delivery allows for a greater amount of drug to be
delivered as compared to IVT which translates to a much-enhanced recovery.

It is worthwhile to note that in the listed clinical trials for wet AMD (clinicaltrials.gov), most
treatments are given via intra-ocular injections. PRL3-zumab could be the first IV-
administered clinically safe anti-angiogenic therapy for the treatment of neovascular eye
15 diseases.

It will be appreciated that many further modifications and permutations of various aspects
of the described embodiments are possible. Accordingly, the described aspects are intended
to embrace all such alterations, modifications, and variations that fall within the spirit and
20 scope of the appended claims.

CLAIMS

1. A method of treating a neovascular eye disease or condition in a subject, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof to the subject.
2. The method of claim 1, wherein the neovascular eye disease or condition is characterised by aberrant blood vessel growth and/or vascular leakage in the eye.
3. The method of claim 1 or 2, wherein the neovascular eye disease or condition is characterised by an increased expression or activity of PRL3 in eye tissue.
4. The method of any one of claims 1 to 3, wherein the subject has been determined to have an increased level of PRL3 in a sample obtained from the subject.
5. The method of any one of claims 1 to 4, wherein the PRL3-binding antibody or antigen-binding fragment thereof is humanised.
6. The method of claim 5, wherein the antibody or antigen-binding fragment thereof binds to an epitope comprising the amino acid sequence KAKFYN (SEQ ID NO: 2) and/or HTHKTR (SEQ ID NO: 3).
7. The method of claim 5 or 6, wherein the antibody or antigen-binding fragment thereof comprises a) a heavy chain variable region (V_H) comprising the VHCDR1 amino acid sequence of GYTFTNYMH (SEQ ID NO: 4), the VHCDR2 amino acid sequence of WIYPGNVNTYYNEKFRG (SEQ ID NO: 5) and the VHCDR3 amino acid sequence of EEKNYPWFAY (SEQ ID NO: 6); and b) a light chain variable region (V_L) comprising the VLCDR1 amino acid sequence of KASQSVEDDGENYMN (SEQ ID NO: 7), the VLCDR2 amino acid sequence of AASNLES (SEQ ID NO: 8) and the VLCDR3 amino acid sequence of QQSNEPFT (SEQ ID NO: 9).
8. The method of claim 7, wherein the antibody or antigen-binding fragment thereof comprises:
 - a) a V_H comprising an amino acid sequence as set forth in any one of SEQ ID NO: 10–19, and a V_L comprising an amino acid sequence as set forth in any one of

SEQ ID NO: 24–32; or

- b) a V_H comprising an amino acid sequence having at least 70% sequence identity to at least one region other than a CDR of the V_H amino acid sequence as set forth in any one of SEQ ID NO: 10–19, and a V_L comprising an amino acid sequence having at least 70% sequence identity to at least one region other than a CDR of the V_L amino acid sequence as set forth in any one of SEQ ID NO: 24–32.
- 9. The method of any one of claims 1 to 8, wherein the antibody or antigen-binding fragment thereof is conjugated to a radioisotope or a cytotoxin.
 - 10. The method of any one of claims 1 to 9, wherein the antibody or antigen-binding fragment thereof is administered intraocularly.
 - 11. The method of claim 10, wherein the antibody or antigen-binding fragment thereof is injected into the vitreous cavity.
 - 12. The method of any one of claims 1 to 9, wherein the antibody or antigen-binding fragment thereof is administered topically to the eye.
 - 13. The method of any one of claims 1 to 9, wherein the antibody or antigen-binding fragment thereof is administered intravenously.
 - 14. The method of any one of claims 1 to 13, wherein the neovascular eye disease is age-related macular degeneration (AMD), diabetic retinopathy (DR), diabetic macular edema (DME), corneal neovascularisation, retinal vein occlusion or retinopathy of prematurity.
 - 15. The method of any one of claims 1 to 14, wherein the subject is undergoing or has undergone an anti-VEGF therapy.
 - 16. The method of claim 15, wherein the subject is non-responsive to the anti-VEGF therapy.
 - 17. The method of any one of claims 1 to 14, wherein the PRL3-binding antibody or

antigen-binding fragment thereof is administered simultaneously or sequentially with an anti-VEGF therapy.

18. A PRL3-binding antibody or antigen-binding fragment thereof, for use in treating a neovascular eye disease or condition in a subject.
19. Use of a PRL3-binding antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating a neovascular eye disease or condition in a subject.
20. A method of inhibiting or reducing intraocular neovascularisation in a subject, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof to the subject.
21. A method of enhancing an anti-VEGF therapy in a subject with a neovascular eye disease or condition, the method comprising administering an effective amount of a PRL3-binding antibody or antigen-binding fragment thereof simultaneously or sequentially with an effective amount of the anti-VEGF therapy to the subject.
22. The method of claim 21, wherein the subject is non-responsive to the anti-VEGF therapy.
23. A method of determining the likelihood of developing a neovascular eye disease or condition in a subject, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to develop a neovascular eye disease or condition.
24. A method of detecting a neovascular eye disease or condition in a subject, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to have a neovascular eye disease or condition.
25. A method of treating a neovascular eye disease or condition in a subject, the method comprising a) detecting the level of PRL3 in a sample from the subject, wherein an

increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to have a neovascular eye disease or condition; and b) treating the subject found likely to have a neovascular eye disease or condition.

26. The method of claim 25, wherein the method comprises treating the subject with a PRL3-binding antibody or antigen-binding fragment thereof.
27. The method of any one of claims 23 to 26, wherein the sample is an ocular tissue sample.
28. A method of identifying a subject with a neovascular eye disease or condition who is likely to be responsive to treatment with a PRL3-binding antibody or antigen-binding fragment thereof, the method comprising detecting the level of PRL3 in a sample from the subject, wherein an increased level of PRL3 in the sample as compared to a reference indicates that the subject is likely to be responsive to treatment with a PRL3-binding antibody or antigen-binding fragment thereof.
29. The method of claim 28, further comprising treating the subject found likely to be responsive to treatment with a PRL3-binding antibody or antigen-binding fragment thereof.
30. An ophthalmic pharmaceutical composition comprising a PRL3 inhibitor.
31. The ophthalmic pharmaceutical composition of claim 30, wherein the PRL3 inhibitor is a PRL3-binding antibody or antigen-binding fragment thereof.
32. The ophthalmic pharmaceutical composition of claim 30 or 31, wherein the composition is formulated for intraocular administration or topical administration to the eye.
33. The ophthalmic pharmaceutical composition of claim 32, wherein the composition is formulated for intravitreal injection.
34. The ophthalmic pharmaceutical composition of claim 32, wherein the composition is

formulated as an eye drop.