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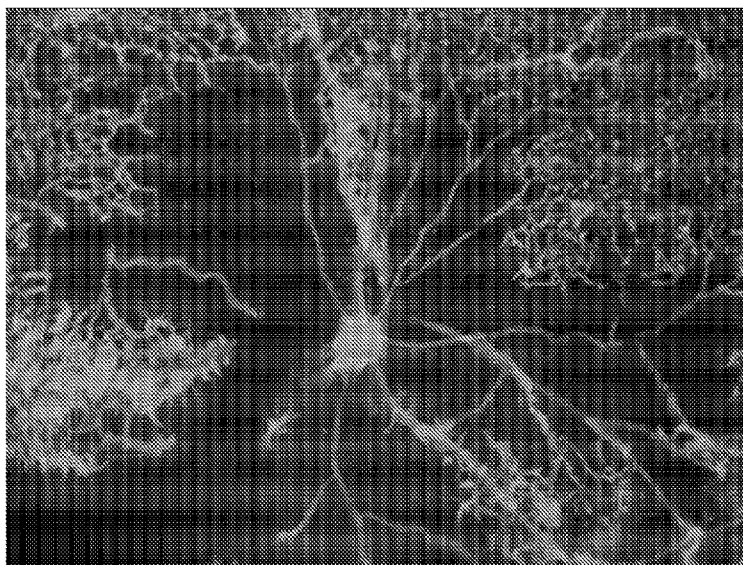


FIG. 1

(57) Abstract: The invention provides methods, compositions, and kits featuring Wnt signaling enhancing compounds for use in preventing or treating retinal disease.



COMPOSITIONS AND METHODS FOR TREATING RETINAL DISEASE

BACKGROUND OF THE INVENTION

The vertebrate retina is a light-sensitive layer of tissue that lines the inner surface of the eye and contains specialized photoreceptor cells referred to as rods and cones. These photoreceptor cells are connected to a network of nerve cells, which processes visual information received in the form of light striking the photoreceptor cells of the retina. This processed visual information is then sent to the brain for decoding into a final visual image.

The retina is susceptible to a variety of diseases, including: diabetic retinopathy, familial exudative vitreoretinopathy (FEVR), retinopathy of prematurity (ROP), Norries disease, and the like. For example, diabetic retinopathy is a retinal disease caused by complications of diabetes, which can eventually lead to blindness. Diabetic retinopathy is a prevalent form of retinal disease, which affects up to 80% of all patients who have had diabetes for 10 or more years. Diabetic retinopathy is the result of changes in the microvascular structure of the retina. The disease is generally considered to have two stages: an initial non-proliferative stage and a subsequent proliferative stage. Non-proliferative diabetic retinopathy is characterized by hyperglycemia-induced intramural pericyte death, as well as a thickening of the basement membrane that leads to incompetence of the vascular walls (e.g., capillary drop out). As the disease progresses, it enters an advanced proliferative stage in which the blood vessels of the retina begin to proliferate along the retina, as well as into the vitreous humor that fills the inside of the eye. Without proper treatment, these proliferating blood vessels can bleed (e.g., causing a macular edema), cloud vision, and ultimately destroy the retina. The primary treatment for diabetic retinopathy is laser treatment (e.g., laser photocoagulation, modified grid laser photocoagulation, pan retinal photocoagulation, etc.). Unfortunately, this type of treatment is not effective in curing diabetic retinopathy. Moreover, it has a significant disadvantage of causing a permanent loss of retinal tissue. In view of the foregoing, there is a clear need to develop compositions and methods for treating retinal diseases.

SUMMARY OF THE INVENTION

As described below, the present invention is based upon the discovery that agents that enhance Wnt signaling have the ability to treat and/or prevent capillary dropout, thereby treating

and/or preventing many forms of retinal disease. The invention features compositions and kits containing Wnt signaling activators. The invention also features methods for using these novel therapeutic molecules to treat a subject having, or at risk of having, a retinal disease associated with capillary dropout such as, for example, diabetic retinopathy, familial exudative vitreoretinopathy (FEVR), retinopathy of prematurity (ROP), or Norries disease.

In one aspect, the invention features a method of treating or preventing capillary dropout in a subject, wherein the method includes administering to the subject an agent that enhances Wnt signaling, thereby treating or preventing capillary dropout. In one embodiment, the subject has or is at risk of developing capillary dropout. In one embodiment, the subject has or is at risk of developing familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), Norries disease, branch retinal vein occlusion, or central retinal vein occlusion. In one embodiment, the subject has or is at risk of developing diabetic retinopathy. In one embodiment, the ocular capillaries of the subject are evaluated by fluorescein angiography (FA). In one embodiment, the agent is administered to the subject when an antecedent lesion is identified. In one embodiment, the antecedent lesion is located in a peripheral capillary. In one embodiment, the antecedent lesion is located in a posterior capillary.

In one aspect, the invention features a method of treating or preventing peripheral capillary dropout in a subject, wherein the method involves administering to the subject an agent that enhances Wnt signaling, thereby treating or preventing capillary dropout. In one embodiment, the subject has or is at risk of familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), Norries disease, branch retinal vein occlusion, or central retinal vein occlusion. In one embodiment, the subject has or is at risk of developing diabetic retinopathy. In one embodiment, the ocular capillaries of the subject are evaluated by fluorescein angiography (FA). In one embodiment, the agent is administered to the subject when an antecedent lesion is identified in a peripheral capillary. In one embodiment, the method reduces capillary loss. In one embodiment, the method reduces capillary occlusion. In one embodiment, the method enhances capillary formation. In one embodiment, In one embodiment, the method enhances retinal function. In one embodiment, the method enhances b-wave response, substantial oscillatory potential, or visual improvement. In one embodiment, the method reduces the symptoms associated with capillary dropout. In one embodiment, the agent enhances the activity of a frizzled-4 transmembrane receptor (Fzd4). In one embodiment, the

agent is an agonist of Fzd4, Low-density lipoprotein receptor-related protein 5 (LRP5), Low-density lipoprotein receptor-related protein 6 (LRP6), or tetraspanin-12 (Tspan12). In one embodiment, the agent is a small molecule, a nucleic acid, a peptide, or a peptide mimetic. In one embodiment, the agent is an antibody or antibody fragment. In one embodiment, the small molecule is 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine. In one embodiment, the antibody is a polyclonal antibody. In one embodiment, the antibody is a monoclonal antibody. In one embodiment, the antibody or antibody fragment is humanized. In one embodiment, the agent is a ligand of Fzd4. In one embodiment, the ligand is Norrin, an active fragment of Norrin, or an active mutant of Norrin. In one embodiment, the agent is a nucleic acid encoding Norrin or a fragment thereof.

In one aspect, the invention features a method of treating or preventing capillary dropout in a subject having or at risk of developing diabetic retinopathy, wherein the method comprises administering Norrin or a fragment thereof to the subject, thereby treating or preventing capillary dropout. In one embodiment, the method further comprises administering at least one additional agent to treat capillary dropout. In one embodiment, the at least one additional agent is an anti-inflammatory, a steroid, anti-VEGF, or a caspase. In one embodiment, the method further comprises treating the patient with laser therapy. In one embodiment, the agent is administered systemically or locally. In one embodiment, the agent is administered orally, parenterally, or topically. In one embodiment, the agent is administered by intravitreal or periocular injection. In one embodiment, the subject is a mammal. In one embodiment, the subject is human.

In one aspect, the invention features a pharmaceutical composition comprising an agent that treats capillary dropout. In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout enhances the activity of a frizzled-4 transmembrane receptor (Fzd4). In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout and is an agonist of Fzd4, Low-density lipoprotein receptor-related protein 5 (LRP5), or tetraspanin-12 (Tspan12). In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout and is a small molecule, a nucleic acid, a peptide, or a peptide mimetic. In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout that is an antibody or antibody fragment. In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout and is a polyclonal antibody. In one embodiment, the pharmaceutical composition includes an agent that treats

capillary dropout and is a monoclonal antibody. In one embodiment, the pharmaceutical composition includes an antibody or antibody fragment that is humanized. In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout and is a ligand of Fzd4. In one embodiment, the pharmaceutical composition includes a ligand that is Norrin or a fragment thereof. In one embodiment, the pharmaceutical composition includes an agent that treats capillary dropout and is a nucleic acid encoding Norrin or a fragment thereof. In one embodiment, the pharmaceutical composition includes at least one additional agent to treat capillary dropout. In one embodiment, the pharmaceutical composition further includes a pharmaceutically acceptable carrier, diluent, or excipient.

In one aspect, the invention features a kit comprising an agent that treats capillary dropout. In one embodiment, the agent that treats capillary dropout enhances the activity of a frizzled-4 transmembrane receptor (Fzd4). In one embodiment, the agent that treats capillary dropout is an agonist of Fzd4, Low-density lipoprotein receptor-related protein 5 (LRP5), or tetraspanin-12 (Tspan12). In one embodiment, the agent that treats capillary dropout is a small molecule, a nucleic acid, a peptide, or a peptide mimetic. In one embodiment, the agent that treats capillary dropout is an antibody or antibody fragment. In one embodiment, the agent that treats capillary dropout is a polyclonal antibody. In one embodiment, the agent that treats capillary dropout is a monoclonal antibody. In one embodiment, the antibody or antibody fragment is humanized. In one embodiment, the agent that treats capillary dropout is a ligand of Fzd4. In one embodiment, the ligand is Norrin or a fragment thereof. In one embodiment, the agent that treats capillary dropout is a nucleic acid encoding Norrin or a fragment thereof. In one embodiment, the kit further includes at least one additional agent to treat capillary dropout. In one embodiment, the kit further comprises instructions for using the agent that treats capillary dropout as described above.

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

Definitions

To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

By “activate” is meant an increase in activity, level, or other measurable parameter relative to a reference (i.e., an untreated control). Such activation can be by about 10%, 25%, 50%, 75% or more.

“Administering” is defined herein as a means of providing an agent or a composition containing the agent to a subject in a manner that results in the agent being inside the subject’s body. Such an administration can be by any route including, without limitation, oral, transdermal (e.g., vagina, rectum, oral mucosa), by injection (e.g., subcutaneous, intravenous, parenterally, intraperitoneally, intrathecal, intraocular), by inhalation (e.g., oral or nasal), or topical (e.g., eyedrops, cream, etc.). Pharmaceutical preparations are, of course, given by forms suitable for each administration route.

By “agent” is meant any small molecule chemical compound, antibody, nucleic acid molecule or polypeptide, or fragments thereof.

By “alteration” is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By “analog” is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

By “antecedent lesion” is meant an implied cellular change due to damage of endothelial cells. For example, an antecedent lesion may be indicated by a retinal vessel that has indistinct margins in an image, and/or in which the vessel diameter is increased with

indistinct margins in a fundus image where the rest of the vessels maintain a crisp architecture.

As used herein, the term “antibody” means not only intact antibody molecules, but also fragments of antibody molecules that retain immunogen-binding ability. Such fragments are also well known in the art and are regularly employed both in vitro and in vivo. Accordingly, as used herein, the term “antibody” means not only intact immunoglobulin molecules but also the well-known active fragments F(ab')₂, and Fab. F(ab')₂, and Fab fragments that lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non specific tissue binding of an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)). The antibodies of the invention comprise whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain V region fragments (scFv), fusion polypeptides, and unconventional antibodies. Unconventional antibodies include, but are not limited to, nanobodies, linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062, 1995), single domain antibodies, single chain antibodies, and antibodies having multiple valencies (e.g., diabodies, tribodies, tetrabodies, and pentabodies). Nanobodies are the smallest fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain. Nanobodies have the affinity and specificity of conventional antibodies although they are only half of the size of a single chain Fv fragment. The consequence of this unique structure, combined with their extreme stability and a high degree of homology with human antibody frameworks, is that nanobodies can bind therapeutic targets not accessible to conventional antibodies. Recombinant antibody fragments with multiple valencies provide high binding avidity and unique targeting specificity to cancer cells. These multimeric scFvs (e.g., diabodies, tetrabodies) offer an improvement over the parent antibody since small molecules of ~60-100kDa in size provide faster blood clearance and rapid tissue uptake. See Power et al., (Generation of recombinant multimeric antibody fragments for tumor diagnosis and therapy. Methods Mol Biol, 207, 335-50, 2003); and Wu et al. (Anti-carcinoembryonic antigen (CEA) diabody for rapid tumor targeting and imaging. Tumor Targeting, 4, 47-58, 1999).

In this disclosure, “comprises,” “comprising,” “containing” and “having” and the like can have the meaning ascribed to them in U.S. Patent law and can mean “includes,” “including,” and the like; “consisting essentially of” or “consists essentially” likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is

recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

As used herein, the term “capillary drop out” means that the small capillary vessels of the retina, which fill the capillary beds of the retina bridging the spaces between arteries and veins, become damaged by intravascular changes that lead to endothelial cell destruction. Consequently, the vessel elements are destroyed by apoptotic destruction and no longer able to nourish the retina. Such vessels are not visualized by angiography. The term “posterior capillary drop out” means that the foveal area of the macula is involved in capillary loss. The term “peripheral capillary drop out” means that areas other than the posterior capillary area are involved in capillary loss.

By “control” is meant a standard or reference condition.

The term “derivative” means a pharmaceutically active compound with equivalent or near equivalent physiological functionality to a given agent (e.g., a Wnt signaling activator or inhibitor). As used herein, the term “derivative” includes any pharmaceutically acceptable salt, ether, ester, prodrug, solvate, stereoisomer including enantiomer, diastereomer or stereoisomerically enriched or racemic mixture, and any other compound which upon administration to the recipient, is capable of providing (directly or indirectly) such a compound or an antivirally active metabolite or residue thereof.

“Detect” refers to identifying the presence, absence or amount of the analyte to be detected.

By “detectable label” is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, or haptens.

By “effective amount” is meant the amount required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject.

Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an “effective” amount.

By “enhances” or “increases” is meant a positive alteration of at least about 10%, 25%, 50%, 75%, or 100% relative to a reference.

By “fragment” is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

By “fuzzy/fuzziness” is meant the angiographic appearance of the vessels in the retina, which are breaking down prior to drop out of the cell wall. As a result of this breakdown, the vessels are irregular in configuration and not able to contain the fluoresceine dye used during a fluoresceine angiogram (FA), which results in the vessels having a fuzzy appearance when visualized by FA.

“Hybridization” means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By “inhibit” is meant a reduction in activity, level, or other measurable parameter relative to a reference (i.e., an untreated control). Such inhibition need not be complete, but can be by about 10%, 25%, 50%, 75% or more.

By “inhibitory nucleic acid” is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (*e.g.*, by 10%, 25%, 50%, 75%, or even 90-100%) in the expression of a target gene. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein.

By “isolated polynucleotide” is meant a nucleic acid (*e.g.*, a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid

molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an “isolated polypeptide” is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By “marker” is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a retinal disease or disorder.

By “Norrin (NDP)” is meant a polypeptide or fragment thereof having at least about 85% amino acid identity to NCBI Accession No. NP_000257.1, as shown below, and having Wnt receptor-binding (e.g., by binding frizzled-4) activity that promotes retinal vascularization.

>gil4557789|reflNP_000257.1| norrin precursor [Homo sapiens]

MRKHVLAASFMSLSLLVIMGDTDSKTDSSFIMDS DPRRCMRHHYVDSISHPLYKCSSKMVLLARCEGHCSQASRS
EPLVSFSTVLKQPFRRSCHCCRPQTSKALKALRLRCSGGMRLTATYRYILSCHCEECS

By “Norrin nucleic acid molecule” is meant a polynucleotide encoding a NDP polypeptide. An exemplary Norrin nucleic acid molecule is provided at NCBI Accession No. NM_000266.3, and is also shown below.

[illegible]

AAAAAAAAAAAAAAAAAAAAAAAAAAAA

As used herein, “obtaining” as in “obtaining an agent” includes synthesizing, purchasing, or otherwise acquiring the agent.

The term “patient” or “subject” refers to an animal which is the object of treatment, observation, or experiment. By way of example only, a subject includes, but is not limited to, a mammal, including, but not limited to, a human or a non-human mammal, such as a non-human primate, bovine, equine, canine, ovine, or feline.

“Pharmaceutically acceptable” refers to approved or approvable by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

“Pharmaceutically acceptable excipient, carrier or diluent” refers to an excipient, carrier or diluent that can be administered to a subject, together with an agent, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

A “pharmaceutically acceptable salt” of a Wnt signaling activator or inhibitor recited herein is an acid or base salt that is generally considered in the art to be suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication. Such salts include mineral and organic acid salts of basic residues such as amines, as well as alkali or organic salts of acidic residues such as carboxylic acids. Specific pharmaceutical salts include, but are not limited to, salts of acids such as hydrochloric, phosphoric, hydrobromic, malic, glycolic, fumaric, sulfuric, sulfamic, sulfanilic, formic, toluenesulfonic, methanesulfonic, benzene sulfonic, ethane disulfonic, 2-hydroxyethylsulfonic, nitric, benzoic, 2-acetoxybenzoic, citric, tartaric, lactic, stearic, salicylic, glutamic, ascorbic, pantoic, succinic, fumaric, maleic, propionic, hydroxymaleic, hydroiodic, phenylacetic, alkanolic such as acetic, $\text{HOOC}-(\text{CH}_2)_n-\text{COOH}$ where n is 0-4, and the like. Similarly, pharmaceutically acceptable cations include, but are not limited to sodium, potassium, calcium, aluminum, lithium and ammonium. Those of ordinary skill in the art will recognize further pharmaceutically acceptable salts for the Wnt signaling activators and inhibitors provided herein, including those listed by *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985). In general, a pharmaceutically acceptable acid or base salt can be synthesized from a parent compound that contains a basic or acidic moiety by any conventional chemical method.

Briefly, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in an appropriate solvent.

As used herein, the terms “prevent,” “preventing,” “prevention,” “prophylactic treatment,” and the like, refer to reducing the probability of developing a disease or condition in a subject, who does not have, but is at risk of or susceptible to developing a disease or condition.

“Primer set” means a set of oligonucleotides that may be used, for example, for PCR. A primer set would consist of at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500, 600, or more primers.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9. With respect to sub-ranges, “nested sub-ranges” that extend from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By “reference” is meant a standard or control condition.

A “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the

reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

By “retinal disease” is meant any condition or disorder that damages or interferes with the normal function of the retina such as, for example, Diabetic Retinopathy, Proliferative Diabetic Retinopathy, Familial Exudative Vitreoretinopathy (FEVR), retinopathy of prematurity (ROP), Norries disease, Incognentia Pigmenti, branch retinal vein occlusion, central retinal vein occlusion, Coats disease, or Persistent Fetal-Vasculature syndrome.

By “siRNA” is meant a double stranded RNA. Optimally, an siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. These dsRNAs can be introduced to an individual cell or to a whole animal; for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity.

By “specifically binds” is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By “hybridize” is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (*e.g.*, a gene described herein), or portions thereof, under various

conditions of stringency. (See, *e.g.*, Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, *e.g.*, formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, *e.g.*, sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25°C, more preferably of at least about 42°C, and even more preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS.

Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel *et al.* (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By “substantially identical” is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

As used herein, the terms “treat,” “treated,” “treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition, or symptoms associated therewith be completely eliminated.

The term “therapeutic effect” refers to some extent of relief of one or more of the symptoms of a disorder or its associated pathology. “Therapeutically effective amount” as used herein refers to an amount of an agent which is effective, upon single or multiple dose

administration to the cell or subject, in prolonging the survivability of the patient with such a disorder, reducing one or more signs or symptoms of the disorder, preventing or delaying, and the like beyond that expected in the absence of such treatment. “Therapeutically effective amount” is intended to qualify the amount required to achieve a therapeutic effect. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the “therapeutically effective amount” (e.g., ED₅₀) of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in a pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

Typically a therapeutically effective dosage should produce a serum concentration of compound of from about 0.1 ng/ml to about 50-100 µg/ml. The pharmaceutical compositions typically should provide a dosage of from about 0.001 mg to about 2000 mg of compound per kilogram of body weight per day. For example, dosages for systemic administration to a human patient can range from 1-10 µg/kg, 20-80 µg/kg, 5-50 µg/kg, 75-150 µg/kg, 100-500 µg/kg, 250-750 µg/kg, 500-1000 µg/kg, 1-10 mg/kg, 5-50 mg/kg, 25-75 mg/kg, 50-100 mg/kg, 100-250 mg/kg, 50-100 mg/kg, 250-500 mg/kg, 500-750 mg/kg, 750-1000 mg/kg, 1000-1500 mg/kg, 1500-2000 mg/kg, 5 mg/kg, 20 mg/kg, 50 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg, or 2000 mg/kg. Pharmaceutical dosage unit forms are prepared to provide from about 1 mg to about 5000 mg, for example from about 100 to about 2500 mg of the compound or a combination of essential ingredients per dosage unit form.

Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms “a,” “an,” and “the” are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The phrase “combination therapy” embraces the administration of a Wnt signaling activator or inhibitor and a second therapeutic agent as part of a specific treatment regimen

intended to provide a beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days, or weeks depending upon the combination selected). "Combination therapy" generally is not intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present invention. "Combination therapy" is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single capsule having a fixed ratio of each therapeutic agent or in multiple, single capsules for each of the therapeutic agents. For example, one combination of the present invention comprises a Wnt signaling activator or inhibitor and at least one additional therapeutic agent (e.g., an anti-viral agent, an immunosuppressive agent, an anti-inflammatory, and the like) at the same or different times or they can be formulated as a single, co-formulated pharmaceutical composition comprising the two compounds. As another example, a combination of the present invention (e.g., a Wnt signaling activator or inhibitor and at least one additional therapeutic agent) is formulated as separate pharmaceutical compositions that can be administered at the same or different time. Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, direct absorption through mucous membrane tissues (e.g., nasal, mouth, vaginal, and rectal), and ocular routes (e.g., intravitreal, intraocular, etc.). The therapeutic agents can be administered by the same route or by different routes. For example, one component of a particular combination may be administered by intravenous injection while the other component(s) of the combination may be administered orally. The components may be administered in any therapeutically effective sequence.

The phrase "combination" embraces groups of compounds or non-drug therapies useful as part of a combination therapy.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The

recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a fluorescein angiogram depicting a posterior capillary dropout phenotype as observed in the oxygen-induced retinopathy (OIR) mouse model.

Figure 2 is a montage of fluorescein angiograms depicting a retinal phenotype as observed in an OIR mouse, which highlights large areas of posterior capillary dropout in the retina.

Figure 3 is a montage of fluorescein angiograms depicting a retinal phenotype as observed in an OIR mouse in which Norrin/Wnt activity is blocked.

Figure 4 shows a fluorescein angiogram depicting posterior capillary sparing in the retina of an OIR mouse treated with intravitreal injection of Norrin.

Figure 5 shows a wide field fluorescein angiogram (Optos system) of a person with Familial Exudative Vitreoretinopathy (FEVR), who has had previous laser treatment of avascular retina (far right of the figure). Healthy vessels are observed in the back of the retina in the vicinity of the optic disc. Adjacent to the treated area, there is an area of fuzziness corresponding to damaged vessels showing leakage of dye from damaged endothelial cells (arrow). These changes precede frank capillary loss, which is what drives reactivation of the acute process of FEVR, retinal destruction, and vision loss.

Figure 6 depicts a higher magnification view of the area of capillary damage shown in FIG. 5. Shown are small areas of microaneurysms and endothelial cell damage as manifested by dye leakage. Left alone this area will result in complete capillary loss.

Figure 7 shows a low magnification wide field fluorescein angiogram (Optos) of a retina of a patient with diabetes prior to the onset of macular diabetic retinopathy (e.g., either background diabetic retinopathy or macular diabetic retinopathy), which reveals numerous small white dots that correspond to microaneurysms surrounding areas of capillary drop out prior to

any change in the macula area. These microaneurism-associated areas are believed to give rise to macular diabetic retinopathy, and according to any exemplary embodiment of the invention, these areas represent target areas for treatment with an activator of Wnt signaling (e.g., Norrin).

Figure 8 shows a high magnification view of the wide field fluorescein angiogram shown in FIG. 7.

Figure 9 shows a regular fluorescein angiogram depicting a normal macula.

Figure 10 shows a wide field fluorescein angiogram depicting a normal macula.

DETAILED DESCRIPTION OF THE INVENTION

The present invention features compositions and methods that are useful in treating retinal diseases. In particular, the invention provides compositions comprising Wnt signaling enhancing compounds for the treatment of retinal diseases such as, for example, familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), Norries disease, branch retinal vein occlusion, and/or central retinal vein occlusion.

The invention is based, at least in part, on the discovery that agents that enhance Wnt signaling (e.g., Norrin) have the ability to treat and/or prevent capillary dropout, thereby treating and/or preventing many forms of retinal disease (e.g., FEVR, diabetic retinopathy, ROP, branch retinal vein occlusion, central retinal vein occlusion, and the like). Additionally, the invention is also based, at least in part, on the discovery that antecedent lesions represent an early prognostic marker for the above-mentioned retinal diseases that may allow treatment prior to a patient's development of symptoms of vision loss. The invention features compositions and kits containing Wnt signaling enhancing compounds. The invention also features methods for using these novel therapeutic molecules to treat a subject having, or at risk of having, a retinal disease such as, for example, familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), Norries disease, branch retinal vein, or central retinal vein occlusion.

Retinal Vascularization

Proper vascular modeling in the retina is essential for ocular development and visual acuity. Abnormal vessel growth during development or in adulthood produces several relatively common diseases such as retinopathy of prematurity, diabetic retinopathy, and age-

related macular degeneration. Normal retinal development occurs through vessels forming at the optic nerve head and spreading over the retina to form a dense network. Connolly, S E, et al, *Microvasc Res*, 1988; 36:275-290; Provis, J M, *Prog Retin Eye Res*, 2001; 20:799-821; Fruttiger, M, *Invest Ophthalmol Vis Sci*, 2002; 43:522-527. Development proceeds through formation of primary vessels along the surface of the developing retina from which divergent vessels begin to extend into the capillary beds that form the outer and inner plexiform layers of the retina. Connolly, 1988; Provis, 2001, Fruttiger, 2002. Vascular development is mediated by a series of growth factors that direct formation and extension of new vessels. Retinal development is unique in the concentration and types of signaling mediators employed to promote angiogenic sprouting from the primary vascular network and the formation of the final capillary architecture. Ohlmann, A, et al, *J Neurosci*, 2005; 25:1701-1710.

Wnt Signaling Pathway

There are three described Wnt pathways, one of which, the canonical β -catenin pathway, plays a role in gene regulation and cell proliferation of both vascular and neural tissue development. The other two pathways, the planar cell polarity and the calcium Ca^{2+} plus pathways, participate in cell migration and cytoskeletal structure and cell adhesion, as well as protein kinase C activity.

In the canonical Wnt pathway, Wnt proteins (i.e., ligands) bind to cell-surface receptors of the Frizzled family, which causes the receptors to activate Dishevelled (DSH) family proteins. Receptors of the Frizzled family may be associated with co-receptors such as LRP5 and LRP6. DSH activation ultimately results in a change in the amount of β -catenin that reaches the nucleus. DSH is a key component of a membrane-associated Wnt receptor complex, which, when activated by Wnt binding, inhibits a second complex of proteins that includes axin, GSK-3, and the protein APC. The axin/GSK-3/APC complex normally promotes the proteolytic degradation of the β -catenin intracellular signaling molecule. However, once the “ β -catenin destruction complex” is inhibited, a stabilized pool of cytoplasmic β -catenin is created, which allows some β -catenin to be able to enter the nucleus and interact with TCF/LEF family transcription factors to promote specific gene expression patterns (see, e.g., R. Baron and M. Kneissel “WNT signaling in bone homeostasis and disease: from human mutations to treatments” *Nature Medicine* 19(2):179-192).

Wnt signaling has been well described in Norrie disease and to a large extent in FEVR. It has also been shown in an animal model that supplementation with Norrin (the disease gene product of Norrie disease) can avoid the phenotypic presentation of Norrie disease—type changes in these animal models by supplementing Norrin to drive the Wnt signaling pathway.

One factor hypothesized to be involved in formation of primary retinal vasculature and retinal capillaries is the protein Norrin. Norrin (NDP), is a 131 amino acid long protein that is secreted into the extracellular space. Meitinger, T, et al, *Nat Genet*, 1993; 5:376-380; Berger, W, et al, *Hum Mol Genet*, 1996; 5:51-59. Two primary domains define the general Norrin protein structure: a signal peptide directs localization of the molecule; and a cysteine-knot motif provides the tertiary confirmation required for receptor binding and activation of signal transduction.

The importance of the cysteine knot-motif is highlighted by computer modeling that demonstrates the requirement of disulfide bonds between the cysteine residues in forming the structural confirmation of Norrin. Mutation(s) of the cysteine residues reduces the affinity of Norrin for its receptor and prevents activation of subsequent signaling pathways. Mutations in these residues also result in severe retinal dysgenesis and Norrie disease. However, mutations in regions other than the cysteine knot-motif produce incomplete protein folding and result in familial exudative vitreoretinopathy (FEVR) and related vitreoretinopathies (Retinopathy of Prematurity, persistent fetal vasculature).

Norrin is a ligand for the Frizzled receptor subtype 4 (Fz4). Norrin binds Fz4 with nanomolar affinity and stimulates a Wnt receptor:β-catenin signal transduction pathway that regulates retinal development and is necessary for regression of hyaloid vessels in the eye. Xu, Q, et al, *Cell*, 2004; 116:883-895; Clevers, H, *Curr Biol*, 2004; 14:R436-437; Nichrs, C, *Dev Cell*, 2004; 6:453-454. Norrin interaction with Fz4 is dependent on the cell surface receptor LRP5. Xu, 2004. Frizzled receptors are coupled to the β-catenin canonical signaling pathway that functions by the activation of Wnt target genes. Wnt protein binding to Frizzled and LRP5 inactivates glycogen synthase kinase (GSK) 3β and Axin. The inactivation of these proteins stabilizes β-catenin, which subsequently accumulates in the cell nucleus and activates the transduction of target genes that are crucial in the G1-S-phase transition, such as cyclin D1 or c-Myc. Willert K, and Nusse R, *Curr Opin Genet Dev*, 1998; 8:95-102. These

pathways promote stimulation and proliferation of retinal stem cells. Inoue, T, et al, Stem Cells, 2006; 24:95-104.

Norrin is encoded by the NDP gene present on chromosome X at position 11.4. The importance of this gene product is highlighted by observations that inactivating mutations lead to Norrie disease, which is characterized by ocular and cochlear vascular defects. Rhem, H L, et al, J Neurosci, 2002; 22:4286-4292; Black, G C, et al, Hum Mol Genet, 1999; 8:2031-2035. Silencing of the NDP gene produces incomplete regression of the primary hyaloid system and abnormal retinal maturation.

Observations that abnormalities in the Fz4 and LRP5/LRP6 receptors that result in the phenotypically similar condition, FEVR, underscore the importance of Norrin signaling. Robitaille, J, et al, Nature Genet, 2002; 32:326-330; Kondo, H, et al, Br J Ophthalmol, 2003; 87:1291-1295; Toomes, C, et al, Am J Hum Genet, 2004; 74:721-730. The close association between the phenotypes produced by Norrin mutations and mutations in the Fz4 and LRP5 receptors bolsters the hypothesis that these molecules form a functional signaling group. Planutis, K, et al, BMC Cell Biology, 2007; 8:12.

Wnt Signaling Enhancing Compounds

In general, the invention features Wnt signaling enhancing compounds that are useful in treating or preventing retinal disease. The Wnt signaling enhancing compounds can be any agent (e.g., a small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide) that enhances Wnt signaling. In embodiments, Wnt signaling enhancing compounds comprise Frizzled (Fz) receptors, Fz co-receptors such as LRP5 and LRP6, axin, GSK-3, APC, DSH, or any other effectors of Wnt signaling downstream of Norrin. In embodiments, the Wnt signaling enhancing compounds are Fz4 specific activators. In an embodiment, the Wnt signaling enhancing compound comprises a small molecule. In preferred embodiments, the Wnt signaling enhancing compound is a polypeptide or polypeptide fragment. In related embodiments, the Wnt signaling enhancing compound is a Norrin polypeptide, or active fragment thereof, that is a Wnt signaling activator. In related embodiments, the Wnt signaling enhancing compound is 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine.

In embodiments, the Wnt signaling enhancing compounds comprises a Norrin polypeptide with the following amino acid sequence:

MRKHVLAASFMSLLVIMGDTDSKTDSSFIMDS DPRRCMRHHYVDSISHPLYKCSSKM
VLLARCEGHCSQASRSEPLVSFSTVLKQPFRSSCHCCRPQTSKLKALRLRCSGGMRLTAT
YRYILSCHCEECNS. In other embodiments, the Wnt signaling enhancing compounds comprise an active fragment of the Norrin polypeptide. Activity of a Norrin polypeptide, or an active fragment thereof, may be assessed by any of a variety of Wnt signaling assays known to one of skill in the art (see, e.g., US Publication No. 2010/0239499, which is hereby incorporated by reference in its entirety for all purposes).

In any of the above aspects and embodiments, the Wnt signaling enhancing compound(s) further contains a targeting moiety. In embodiments, the targeting moiety facilitates delivery of the Wnt signaling enhancing compound(s) to the Wnt receptor (e.g., Fz4). In related embodiments, the targeting moiety contains any of a number of cell penetrating domains known to one of skill in the art such as, e.g., trans-activating transcriptional activator (Tat) from HIV-1 (see Wadia *et al.*, *Nat. Med.* 10:310-315 (2004); and Kameyama *et al.*, *Bioconjugate Chem.* 17:597-602 (2006)) and Pep-1 (see Morris *et al.*, *Nat. Biotechnol.* 19:1173-1176 (2001)).

In any of the above aspects and embodiments, the Wnt signaling enhancing compound(s) further contains a detectable moiety. Detectable moieties are well known in the art and can be detected by spectroscopic, photochemical, biochemical, immunochemical, physical, or chemical means. Exemplary moieties include, but are not limited to, enzymes, fluorescent molecules, particle labels, electron-dense reagents, radiolabels, biotin, digoxigenin, or a hapten or a protein that has been made detectable.

The Wnt signaling enhancing compound(s) can be covalently or non-covalently linked to a moiety (e.g., targeting moiety and/or detectable moiety). In embodiments, the Wnt signaling enhancing compound(s) are covalently linked to the moiety. In related embodiments, the covalent linkage of the moiety is N-terminal to the polypeptide fragment. In related embodiments, the covalent linkage of the moiety is C-terminal to the peptide fragment.

The invention further embraces variants and equivalents which are substantially homologous to the Wnt signaling enhancing compound(s) described herein. These can contain, for example, conservative substitution mutations, i.e., the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another

basic amino acid, or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

The invention also provides isolated polypeptides of the activators of the invention, as well as isolated polynucleotides encoding the polypeptides. In addition, the invention further provides expression vectors comprising the isolated polynucleotides, as well as host cells containing the expression vectors.

The term “polynucleotide encoding a polypeptide” encompasses a polynucleotide which includes only coding sequences for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequences. The polynucleotides of the invention can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

The present invention further relates to variants of the polynucleotides, for example, fragments, analogs, and derivatives. The variant of the polynucleotide can be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide. In certain embodiments, the polynucleotide can have a coding sequence which is a naturally occurring allelic variant of the coding sequence of the disclosed polypeptides. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence that have, for example, a substitution, deletion, or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

In embodiments, the polynucleotides can comprise the coding sequence for the mature polypeptide fused in the same reading frame to a polynucleotide which aids, for example, in expression and secretion of a polypeptide from a host cell (e.g., a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell). The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides can also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

In embodiments, the polynucleotides can comprise the coding sequence for the mature polypeptide fused in the same reading frame to a marker sequence that allows, for example, for purification of the encoded polypeptide. For example, the marker sequence can be a

hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or the marker sequence can be a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (e.g., COS-7 cells) is used. Additional tags include, but are not limited to, Calmodulin tags, FLAG tags, Myc tags, S tags, SBP tags, Softag 1, Softag 3, V5 tag, Xpress tag, Isopeptag, SpyTag, Biotin Carboxyl Carrier Protein (BCCP) tags, GST tags, fluorescent protein tags (e.g., green fluorescent protein tags), maltose binding protein tags, Nus tags, Strep-tag, thioredoxin tag, TC tag, Ty tag, and the like.

In embodiments, the present invention provides isolated nucleic acid molecules having a nucleotide sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, or at least 96%, 97%, 98% or 99% identical to a polynucleotide encoding a polypeptide comprising Wnt signaling enhancing compound(s) of the present invention.

By a polynucleotide having a nucleotide sequence at least, for example, 95% “identical” to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the amino- or carboxy-terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 80% identical, at least 85% identical, at least 90% identical, and in some embodiments, at least 95%, 96%, 97%, 98%, or 99% identical to a reference sequence can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other

sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, the polynucleotide variants contain alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. In some embodiments, nucleotide variants are produced by silent substitutions due to the degeneracy of the genetic code. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

The polypeptides of the present invention can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides comprising a Wnt signaling enhancing compound(s) as described herein. It will be recognized in the art that some amino acid sequences of the invention can be varied without significant effect of the structure or function of the protein. Thus, the invention further includes variations of the polypeptides which show substantial activity or which include regions of the Wnt signaling enhancing compound(s) of the present invention. Such mutants include deletions, insertions, inversions, repeats, and type substitutions.

The polypeptides and analogs can be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half-life, absorption of the protein, or binding affinity. The moieties can also reduce or eliminate any desirable side effects of the proteins and the like. An overview for those moieties can be found in Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing Co., Easton, PA (2000).

The isolated polypeptides described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable transformed host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis

to provide functional analogs thereof. *See, e.g.* Zoeller et al., *Proc. Nat'l. Acad. Sci. USA* 81:5662-5066 (1984) and U.S. Pat. No. 4,588,585.

In embodiments, a DNA sequence encoding a polypeptide of interest would be constructed by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

Once assembled (e.g., by synthesis, site-directed mutagenesis, or another method), the polynucleotide sequences encoding a particular isolated polypeptide of interest will be inserted into an expression vector and optionally operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene can be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

Recombinant expression vectors are used to amplify and express DNA encoding the Wnt signaling enhancing compound(s). Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a Wnt signaling enhancing compound(s) or a bioequivalent analog operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred

by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are operatively linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. Generally, operatively linked means contiguous, and in the case of secretory leaders, means contiguous and in reading frame. Structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *Escherichia coli*, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

Suitable host cells for expression of a polypeptide include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *bacilli*. Higher eukaryotic cells include established cell lines of mammalian origin. Cell-free translation systems could also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are well known in the art (see Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual*, Elsevier, N.Y., 1985).

Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells can be performed because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell

lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing column chromatography, and the like), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence, glutathione-S-transferase, and the like can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography.

For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a cancer stem cell protein-Fc composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. High performance liquid

chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Methods of Treatment

The invention includes methods for treating or preventing retinal disease with the Wnt signaling enhancing compound(s) described herein.

In aspects, the invention provides methods for enhancing Wnt signaling in a cell. In embodiments, the methods involve enhancing Wnt signaling with a Norrin polypeptide. In related embodiments, the methods involve contacting the cell with a Norrin polypeptide, or a fragment thereof. In related embodiments, the methods involve contacting the cell with 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine.

In embodiments, the cell is in the retina of a subject. In related embodiments, contacting occurs by therapeutic administration of the inhibitor to the retina of the subject in the form of a pharmaceutical composition.

In any of the above aspects and embodiments, the methods may further involve contacting the retinal cell with, or administering to the retina of a subject, an anti-viral agent, an immunosuppressive agent, or an anti-inflammatory.

In any of the above aspects and embodiments, the subject is a mammal (e.g., human) or the cell is from a mammal (e.g., human).

Methods for evaluating the therapeutic efficacy of the methods of the invention are standard in the art. For example, efficacy of treatment can be evaluated by assessing retinal vascularization (e.g., by regular or wide field FA), patient symptoms, visual acuity, and the like.

Pharmaceutical Compositions/Methods of Delivery

The present invention is also directed to pharmaceutical compositions comprising an effective amount of one or more compounds according to the present invention (including a pharmaceutically acceptable salt, thereof), optionally in combination with a pharmaceutically acceptable carrier, excipient or additive.

A “pharmaceutically acceptable derivative or prodrug” means any pharmaceutically acceptable salt, ester, salt of an ester, or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally or ocularly administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the retina) relative to the parent species.

While the Wnt signaling enhancing compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more compounds of the invention or other agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

The Wnt signaling enhancing compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, vaginally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, infusion techniques, intraperitoneally, eye or ocular, intravitreal, intrabuccal, transdermal, intranasal, into the brain, including intracranial and intradural, into the joints, including ankles, knees, hips, shoulders, elbows, wrists, directly into tumors, and the like, and in suppository form.

The pharmaceutically active compounds of this invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals.

Modifications of the active compound can affect the solubility, bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species. Further, the modifications can affect the anti-angiogenesis activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the derivative and testing its activity according to known methods well within the routine practitioner's skill in the art.

Pharmaceutical compositions based upon these chemical compounds comprise the above-described Wnt signaling enhancing compounds in a therapeutically effective amount for treating diseases and conditions which have been described herein, optionally in combination with a pharmaceutically acceptable additive, carrier and/or excipient. One of ordinary skill in the art will recognize that a therapeutically effective amount of one of more compounds according to the present invention will vary with the infection or condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient (animal or human) treated.

To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of one or more of the compounds according to the present invention is preferably intimately admixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., ocular, oral, topical or parenteral, including gels, creams ointments, lotions and time released implantable preparations, among numerous others. In preparing pharmaceutical compositions in oral dosage form, any of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavouring agents, preservatives, colouring agents and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carriers, such as dextrose, mannitol, lactose and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount for the desired indication, without causing serious toxic effects in the patient treated.

Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound or its prodrug derivative can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically

compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a dispersing agent such as alginic acid or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material-of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or enteric agents.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets optionally may be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Methods of formulating such slow or controlled release compositions of pharmaceutically active ingredients, are known in the art and described in several issued US Patents, some of which include, but are not limited to, US Patent Nos. 3,870,790; 4,226,859; 4,369,172; 4,842,866 and 5,705,190, the disclosures of which are incorporated herein by reference in their entireties. Coatings can be used for delivery of compounds to the intestine (see, e.g., U.S. Patent Nos. 6,638,534, 5,541,171, 5,217,720, and 6,569,457, and references cited therein).

The active compound or pharmaceutically acceptable salt thereof may also be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose or fructose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

Solutions or suspensions used for ocular, parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

A skilled artisan will recognize that in addition to tablets, other dosage forms can be formulated to provide slow or controlled release of the active ingredient. Such dosage forms include, but are not limited to, capsules, granulations and gel-caps.

Liposomal suspensions may also be pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposomal formulations may be prepared by dissolving appropriate lipid(s) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension. Other methods of preparation well known by those of ordinary skill may also be used in this aspect of the present invention.

The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of

bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations and compositions suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. If administered intravenously, preferred carriers include, for example, physiological saline or phosphate buffered saline (PBS).

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients including those which aid dispersion may

be included. Of course, where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Administration of the active compound may range from continuous (intravenous drip) to several oral administrations per day (for example, Q.I.D.) and may include oral, topical, eye or ocular, parenteral, intramuscular, intravenous, sub-cutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration, among other routes of administration, including through an eye or ocular route.

Application of the subject therapeutics may be local, so as to be administered at the site of interest. Various techniques can be used for providing the subject compositions at the site of interest, such as injection, use of catheters, trocars, projectiles, pluronic gel, stents, sustained drug release polymers or other device which provides for internal access. Where an organ or tissue is accessible because of removal from the patient, such organ or tissue may be bathed in a medium containing the subject compositions, the subject compositions may be painted onto the organ, or may be applied in any convenient way.

The Wnt signaling enhancing compounds may be administered through a device suitable for the controlled and sustained release of a composition effective in obtaining a desired local or systemic physiological or pharmacological effect. The method includes positioning the sustained released drug delivery system at an area wherein release of the agent is desired and allowing the agent to pass through the device to the desired area of treatment.

More specifically, the Wnt signaling enhancing compound is administered through an ocular device suitable for direct implantation into the vitreous of the eye. Such devices of the present invention are surprisingly found to provide sustained controlled release of various compositions to treat the eye without risk of detrimental local and systemic side effects. An object of the present ocular method of delivery is to maximize the amount of drug contained in an intraocular device while minimizing its size in order to prolong the duration of the implant. See, e.g., U.S. Patents 5,378,475; 5,773,019; 6,001,386; 6,217,895, 6,375,972, and 6,756,058 and U.S. Publications 20050096290 and 200501269448.

Other methods of delivery of Wnt signaling enhancing compounds include: an ocular delivery system that could be applied to an intra-ocular lens to prevent inflammation or posterior capsular opacification, an ocular delivery system that could be inserted directly into the vitreous, under the retina, or onto the sclera, and wherein inserting can be achieved by injecting the system or surgically implanting the system, a sustained release drug delivery system, and a method for providing controlled and sustained administration of an agent effective in obtaining a desired local or systemic physiological or pharmacological effect comprising surgically implanting a sustained release drug delivery system at a desired location.

Examples include, but are not limited to the following: a sustained release drug delivery system comprising an inner reservoir comprising an effective amount of an agent effective in obtaining a desired local or systemic physiological or pharmacological effect, an inner tube impermeable to the passage of said agent, said inner tube having first and second ends and covering at least a portion of said inner reservoir, said inner tube sized and formed of a material so that said inner tube is capable of supporting its own weight, an impermeable member positioned at said inner tube first end, said impermeable member preventing passage of said agent out of said reservoir through said inner tube first end, and a permeable member positioned at said inner tube second end, said permeable member allowing diffusion of said agent out of said reservoir through said inner tube second end; a method for administering a compound of the invention to a segment of an eye, the method comprising the step of implanting a sustained release device to deliver the compound of the invention to the vitreous of the eye or an implantable, sustained release device for administering a compound of the invention to a segment of an eye; a sustained release drug delivery device comprising: a) a drug core comprising a therapeutically effective amount of at least one first agent effective in

obtaining a diagnostic effect or effective in obtaining a desired local or systemic physiological or pharmacological effect; b) at least one unitary cup essentially impermeable to the passage of said agent that surrounds and defines an internal compartment to accept said drug core, said unitary cup comprising an open top end with at least one recessed groove around at least some portion of said open top end of said unitary cup; c) a permeable plug which is permeable to the passage of said agent, said permeable plug is positioned at said open top end of said unitary cup wherein said groove interacts with said permeable plug holding it in position and closing said open top end, said permeable plug allowing passage of said agent out of said drug core, through said permeable plug, and out said open top end of said unitary cup; and d) at least one second agent effective in obtaining a diagnostic effect or effective in obtaining a desired local or systemic physiological or pharmacological effect; or a sustained release drug delivery device comprising: an inner core comprising an effective amount of an agent having a desired solubility and a polymer coating layer, the polymer layer being permeable to the agent, wherein the polymer coating layer completely covers the inner core.

The Wnt signaling enhancing compounds may be administered as microspheres. For example, Norrin may be purchased from R&D Systems, Minneapolis, Minn., or cloned, expressed and purified is loaded into biodegradable microspheres substantially as described by Jiang, C, et al, Mol Vis, 2007; 13:1783-92 using the spontaneous emulsification technique of Fu, K, et al, J Pharm Sci, 2003; 92:1582-91. Microspheres are synthesized and loaded by dissolving 200 mg of 50:50 PLGA (DURECT Corp., Birmingham, Ala.) in 5 ml of 4:1 volume ratio trifluoroethanol:dichloromethane supplemented with 8 mg magnesium hydroxide to minimize protein aggregation during encapsulation. 10 µg Norrin may be reconstituted in 300 µl 7 mg bovine serum albumin (BSA) and 100 mg docusate sodium (Sigma-Aldrich, St. Louis, Mo.) dissolved in 3 ml PBS. The solution may be vortexed and poured into 200 ml of 1% (w/v) polyvinyl alcohol (PVA, 88% hydrolyzed) with gentle stirring. Microspheres may be hardened by stirring for three hours, collected by centrifugation, and washed three times to remove residual PVA. If the microspheres are not to be immediately injected they are rapidly frozen in liquid nitrogen, lyophilized for 72 h, and stored in a dessicator at -20° C. Norrin containing microspheres exhibit average diameters of 8 µm as determined by a particle size.

The Wnt signaling enhancing compounds may also be administered by intravitreal injection. For example, Norrin in solution, may be packaged into microspheres as described above, or expressed in cells, or in purified form in solution may be exposed to the retina by intravitreal injection substantially as described by Jiang, 2007. Intravitreal injection may be performed under general anesthesia using an ophthalmic operating microscope (Möller-Wedel GmbH, Wedel, Germany) using beveled glass micro-needles with an outer diameter of approximately 100 μm . Microsphere suspensions are prepared in PBS at 2 and 10% (w/v) and briefly vortexed immediately before injection to ensure a uniform dispersion. A 30-gauge hypodermic needle may be used to perforate the sclera 1.5 mm behind the limbus. Five μl of test sample is optionally injected by way of this passage into the vitreous using a 50 μl Hamilton Syringe (Hamilton Co, Reno, Nev.). To ensure adequate delivery and prevent shock the needle is held in place for one min after the injection is completed and subsequently withdrawn slowly. In addition, paracentesis may be simultaneously performed to relieve pressure and thereby prevent reflux.

The Wnt signaling enhancing compounds may also be administered by delivery to the retina by a controlled release delivery system. An implantable controlled release delivery system is described in U.S. Patent Application Publication 2005/0281861 A1 which is incorporated herein by reference for its entire disclosure, figures, examples, and methods. Norrin is packaged into such as system at 100 μg per final formulated capsule. For example, a Norrin containing drug delivery systems may be placed in the eye using forceps or a trocar after making a 2-3 mm incision in the sclera. Alternatively, no incision may be made and the system placed in an eye by inserting a trocar or other delivery device directly through the eye. The removal of the device after the placement of the system in the eye can result in a self-sealing opening. One example of a device that is used to insert the implants into an eye is disclosed in U.S. Patent Application Publication No. 2004/0054374 which is incorporated herein by reference. The location of the system may influence the concentration gradients of therapeutic component or drug surrounding the element, and thus influence the release rates (e.g., an element placed closer to the edge of the vitreous may result in a slower release rate). Thus, it is preferred if the system is placed near the retinal surface or in the posterior portion of the vitreous.

The above methods are particularly suitable for treating ocular conditions such as familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), and/or Norries disease.

The Wnt signaling enhancing compound may be utilized in combination with at least one known other therapeutic agent, or a pharmaceutically acceptable salt of said agent. Examples of known therapeutic agents which can be used for combination therapy include, but are not limited to, corticosteroids (e.g., cortisone, prednisone, dexamethasone), non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., ibuprofen, celecoxib, aspirin, indomethacin, naproxen), alkylating agents such as busulfan, cis-platin, mitomycin C, and carboplatin; antimitotic agents such as colchicine, vinblastine, paclitaxel, and docetaxel; topo I inhibitors such as camptothecin and topotecan; topo II inhibitors such as doxorubicin and etoposide; and/or RNA/DNA antimetabolites such as 5-azacytidine, 5-fluorouracil and methotrexate; DNA antimetabolites such as 5-fluoro-2'-deoxy-uridine, ara-C, hydroxyurea and thioguanine; antibodies such as Herceptin® and Rituxan®.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of the present invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

In certain pharmaceutical dosage forms, the pro-drug form of the compounds may be preferred. One of ordinary skill in the art will recognize how to readily modify the present compounds to pro-drug forms to facilitate delivery of active compounds to a targeted site within the host organism or patient. The routine practitioner also will take advantage of favorable pharmacokinetic parameters of the pro-drug forms, where applicable, in delivering the present compounds to a targeted site within the host organism or patient to maximize the intended effect of the compound.

Preferred prodrugs include derivatives where a group which enhances aqueous solubility or active transport through the gut membrane is appended to the structure of formulae described herein. See, e.g., Alexander, J. et al. *Journal of Medicinal Chemistry* **1988**, *31*, 318-322; Bundgaard, H. *Design of Prodrugs*; Elsevier: Amsterdam, 1985; pp 1-92; Bundgaard, H.; Nielsen, N. M. *Journal of Medicinal Chemistry* **1987**, *30*, 451-454; Bundgaard, H. *A Textbook of Drug Design and Development*; Harwood Academic Publ.:

Switzerland, 1991; pp 113-191; Digenis, G. A. et al. *Handbook of Experimental Pharmacology* **1975**, 28, 86-112; Friis, G. J.; Bundgaard, H. *A Textbook of Drug Design and Development*; 2 ed.; Overseas Publ.: Amsterdam, 1996; pp 351-385; Pitman, I. H. *Medicinal Research Reviews* **1981**, 1, 189-214. The prodrug forms may be active themselves, or may be those such that when metabolized after administration provide the active therapeutic agent *in vivo*.

Pharmaceutically acceptable salt forms may be the preferred chemical form of compounds according to the present invention for inclusion in pharmaceutical compositions according to the present invention.

Certain of the compounds, in pharmaceutical dosage form, may be used as agents for preventing a disease or condition from manifesting itself. In certain pharmaceutical dosage forms, the pro-drug form of the compounds according to the present invention may be preferred. In particular, prodrug forms which rely on C₁ to C₂₀ ester groups or amide groups (preferably a hydroxyl, free amine or substituted nitrogen group) which may be transformed into, for example, an amide or other group may be particularly useful in this context.

The present compounds or their derivatives, including prodrug forms of these agents, can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to appropriate salts or complexes of the active compounds according to the present invention which retain the desired biological activity of the parent compound and exhibit limited toxicological effects to normal cells. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, and polyglutamic acid, among others; (b) base addition salts formed with metal cations such as zinc, calcium, sodium, potassium, and the like, among numerous others.

The compounds herein are commercially available or can be synthesized. As can be appreciated by the skilled artisan, further methods of synthesizing the compounds of the formulae herein will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. Synthetic chemistry transformations and protecting group methodologies

(protection and deprotection) useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, 2nd. Ed., Wiley-VCH Publishers (1999); T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 3rd. Ed., John Wiley and Sons (1999); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1999); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and subsequent editions thereof.

The compounds of this invention may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention. The compounds of this invention may also be represented in multiple tautomeric forms, in such instances, the invention expressly includes all tautomeric forms of the compounds described herein (e.g., alkylation of a ring system may result in alkylation at multiple sites, the invention expressly includes all such reaction products). All such isomeric forms of such compounds are expressly included in the present invention. All crystal forms of the compounds described herein are expressly included in the present invention.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as hereinabove recited, or an appropriate fraction thereof, of the administered ingredient.

The dosage regimen for treating a disorder or a disease with the Wnt signaling enhancing compounds of this invention and/or compositions of this invention is based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods.

The amounts and dosage regimens administered to a subject will depend on a number of factors, such as the mode of administration, the nature of the condition being treated, the body weight of the subject being treated and the judgment of the prescribing physician.

The amount of compound included within therapeutically active formulations according to the present invention is an effective amount for treating the disease or condition.

In general, a therapeutically effective amount of the present preferred compound in dosage form usually ranges from slightly less than about 0.025 mg/kg/day to about 2.5 g/kg/day, preferably about 0.1 mg/kg/day to about 100 mg/kg/day of the patient or considerably more, depending upon the compound used, the condition or infection treated and the route of administration, although exceptions to this dosage range may be contemplated by the present invention. In its most preferred form, compounds according to the present invention are administered in amounts ranging from about 1 mg/kg/day to about 100 mg/kg/day. The dosage of the compound will depend on the condition being treated, the particular compound, and other clinical factors such as weight and condition of the patient and the route of administration of the compound. It is to be understood that the present invention has application for both human and veterinary use.

For oral administration to humans, a dosage of between approximately 0.1 to 100 mg/kg/day, preferably between approximately 1 and 100 mg/kg/day, is generally sufficient.

Where drug delivery is systemic rather than topical, this dosage range generally produces effective blood level concentrations of active compound ranging from less than about 0.04 to about 400 micrograms/cc or more of blood in the patient.

The compound is conveniently administered in any suitable unit dosage form, including but not limited to one containing 1 to 3000 mg, preferably 5 to 500 mg of active ingredient per unit dosage form. An oral dosage of 10-250 mg is usually convenient.

The concentration of active compound in the drug composition will depend on absorption, distribution, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

In certain embodiments, the compound is administered once daily; in other embodiments, the compound is administered twice daily; in yet other embodiments, the

compound is administered once every two days, once every three days, once every four days, once every five days, once every six days, once every seven days, once every two weeks, once every three weeks, once every four weeks, once every two months, once every six months, or once per year. The dosing interval can be adjusted according to the needs of individual patients. For longer intervals of administration, extended release or depot formulations can be used.

The compounds of the invention can be used to treat diseases and disease conditions that are acute, and may also be used for treatment of chronic conditions. In certain embodiments, the compounds of the invention are administered for time periods exceeding two weeks, three weeks, one month, two months, three months, four months, five months, six months, one year, two years, three years, four years, or five years, ten years, or fifteen years; or for example, any time period range in days, months or years in which the low end of the range is any time period between 14 days and 15 years and the upper end of the range is between 15 days and 20 years (e.g., 4 weeks and 15 years, 6 months and 20 years). In some cases, it may be advantageous for the compounds of the invention to be administered for the remainder of the patient's life. In preferred embodiments, the patient is monitored to check the progression of the disease or disorder, and the dose is adjusted accordingly. In preferred embodiments, treatment according to the invention is effective for at least two weeks, three weeks, one month, two months, three months, four months, five months, six months, one year, two years, three years, four years, or five years, ten years, fifteen years, twenty years, or for the remainder of the subject's life.

Still other objects, features, and attendant advantages of the present invention will become apparent to those skilled in the art from a reading of the preceding detailed description of embodiments constructed in accordance therewith, taken in conjunction with the accompanying drawings.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptide of the present invention may be employed on conjunction with other therapeutic compounds.

The invention provides for pharmaceutical compositions containing at least one Wnt signaling activator described herein. In embodiments, the pharmaceutical compositions contain a pharmaceutically acceptable carrier, excipient, or diluent, which includes any pharmaceutical agent that does not itself induce the production of an immune response harmful to a subject receiving the composition, and which may be administered without undue toxicity. As used herein, the term “pharmaceutically acceptable” means being approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia, European Pharmacopoeia or other generally recognized pharmacopoeia for use in mammals, and more particularly in humans. These compositions can be useful for treating and/or preventing viral infection and/or autoimmune disease.

A thorough discussion of pharmaceutically acceptable carriers, diluents, and other excipients is presented in *Remington's Pharmaceutical Sciences* (17th ed., Mack Publishing Company) and *Remington: The Science and Practice of Pharmacy* (21st ed., Lippincott Williams & Wilkins), which are hereby incorporated by reference. The formulation of the pharmaceutical composition should suit the mode of administration. In embodiments, the pharmaceutical composition is suitable for administration to humans, and can be sterile, non-particulate and/or non-pyrogenic.

Pharmaceutically acceptable carriers, excipients, or diluents include, but are not limited, to saline, buffered saline, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffer, and combinations thereof.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives, and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include, but are not limited to: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

In embodiments, the pharmaceutical composition is provided in a solid form, such as a lyophilized powder suitable for reconstitution, a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder.

In embodiments, the pharmaceutical composition is supplied in liquid form, for example, in a sealed container indicating the quantity and concentration of the active ingredient in the pharmaceutical composition. In related embodiments, the liquid form of the pharmaceutical composition is supplied in a hermetically sealed container.

Methods for formulating the pharmaceutical compositions of the present invention are conventional and well known in the art (see Remington and Remington's). One of skill in the art can readily formulate a pharmaceutical composition having the desired characteristics (e.g., route of administration, biosafety, and release profile).

Methods for preparing the pharmaceutical compositions include the step of bringing into association the active ingredient with a pharmaceutically acceptable carrier and, optionally, one or more accessory ingredients. The pharmaceutical compositions can be prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product. Additional methodology for preparing the pharmaceutical compositions, including the preparation of multilayer dosage forms, are described in *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems* (9th ed., Lippincott Williams & Wilkins), which is hereby incorporated by reference.

Pharmaceutical compositions suitable for oral administration can be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound(s) described herein, a derivative thereof, or a pharmaceutically acceptable salt or prodrug thereof as the active ingredient(s). The active ingredient can also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (e.g., capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more

pharmaceutically acceptable carriers, excipients, or diluents, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets, and pills, the pharmaceutical compositions can also comprise buffering agents. Solid compositions of a similar type can also be prepared using fillers in soft and hard-filled gelatin capsules, and excipients such as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet can be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets can be prepared using binders (for example, gelatin or hydroxypropylmethyl cellulose), lubricants, inert diluents, preservatives, disintegrants (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-actives, and/or dispersing agents. Molded tablets can be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent.

The tablets and other solid dosage forms, such as dragees, capsules, pills, and granules, can optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the art.

In some embodiments, in order to prolong the effect of an active ingredient, it is desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the active ingredient then depends upon its rate of dissolution which, in turn, can depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered active ingredient is accomplished by dissolving or suspending the compound in an oil

vehicle. In addition, prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

Controlled release parenteral compositions can be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, emulsions, or the active ingredient can be incorporated in biocompatible carrier(s), liposomes, nanoparticles, implants or infusion devices.

Materials for use in the preparation of microspheres and/or microcapsules include biodegradable/bioerodible polymers such as polyglactin, poly-(isobutyl cyanoacrylate), poly(2-hydroxyethyl-L-glutamine) and poly(lactic acid).

Biocompatible carriers which can be used when formulating a controlled release parenteral formulation include carbohydrates such as dextrans, proteins such as albumin, lipoproteins or antibodies.

Materials for use in implants can be non-biodegradable, e.g., polydimethylsiloxane, or biodegradable such as, e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(ortho esters).

In embodiments, the active ingredient(s) are administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation, or solid particles containing the compound. A nonaqueous (*e.g.*, fluorocarbon propellant) suspension can be used. The pharmaceutical composition can also be administered using a sonic nebulizer, which would minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the active ingredient(s) together with conventional pharmaceutically-acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

Dosage forms for topical or transdermal administration of an active ingredient(s) includes powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active ingredient(s) can be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants as appropriate.

Transdermal patches suitable for use in the present invention are disclosed in *Transdermal Drug Delivery: Developmental Issues and Research Initiatives* (Marcel Dekker Inc., 1989) and U.S. Pat. Nos. 4,743,249, 4,906,169, 5,198,223, 4,816,540, 5,422,119, 5,023,084, which are hereby incorporated by reference. The transdermal patch can also be any transdermal patch well known in the art, including transscrotal patches. Pharmaceutical compositions in such transdermal patches can contain one or more absorption enhancers or skin permeation enhancers well known in the art (*see, e.g.*, U.S. Pat. Nos. 4,379,454 and 4,973,468, which are hereby incorporated by reference). Transdermal therapeutic systems for use in the present invention can be based on iontophoresis, diffusion, or a combination of these two effects.

Transdermal patches have the added advantage of providing controlled delivery of active ingredient(s) to the body. Such dosage forms can be made by dissolving or dispersing the active ingredient(s) in a proper medium. Absorption enhancers can also be used to increase the flux of the active ingredient across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active ingredient(s) in a polymer matrix or gel.

Such pharmaceutical compositions can be in the form of creams, ointments, lotions, liniments, gels, hydrogels, solutions, suspensions, sticks, sprays, pastes, plasters and other kinds of transdermal drug delivery systems. The compositions can also include pharmaceutically acceptable carriers or excipients such as emulsifying agents, antioxidants, buffering agents, preservatives, humectants, penetration enhancers, chelating agents, gel-forming agents, ointment bases, perfumes, and skin protective agents.

Examples of emulsifying agents include, but are not limited to, naturally occurring gums, e.g. gum acacia or gum tragacanth, naturally occurring phosphatides, e.g. soybean lecithin and sorbitan monooleate derivatives.

Examples of antioxidants include, but are not limited to, butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, and cysteine.

Examples of preservatives include, but are not limited to, parabens, such as methyl or propyl p-hydroxybenzoate and benzalkonium chloride.

Examples of humectants include, but are not limited to, glycerin, propylene glycol, sorbitol and urea.

Examples of penetration enhancers include, but are not limited to, propylene glycol, DMSO, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol, propylene glycol, diethylene glycol monoethyl or monomethyl ether with propylene glycol monolaurate or methyl laurate, eucalyptol, lecithin, Transcutol[®], and Azone[®].

Examples of chelating agents include, but are not limited to, sodium EDTA, citric acid and phosphoric acid.

Examples of gel forming agents include, but are not limited to, Carbopol, cellulose derivatives, bentonite, alginates, gelatin and polyvinylpyrrolidone.

In addition to the active ingredient(s), the ointments, pastes, creams, and gels of the present invention can contain excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons, and volatile unsubstituted hydrocarbons, such as butane and propane.

Injectable depot forms are made by forming microencapsule matrices of compound(s) of the invention in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of compound to polymer, and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include

poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

Subcutaneous implants are well known in the art and are suitable for use in the present invention. Subcutaneous implantation methods are preferably non-irritating and mechanically resilient. The implants can be of matrix type, of reservoir type, or hybrids thereof. In matrix type devices, the carrier material can be porous or non-porous, solid or semi-solid, and permeable or impermeable to the active compound or compounds. The carrier material can be biodegradable or may slowly erode after administration. In some instances, the matrix is non-degradable but instead relies on the diffusion of the active compound through the matrix for the carrier material to degrade. Alternative subcutaneous implant methods utilize reservoir devices where the active compound or compounds are surrounded by a rate controlling membrane, e.g., a membrane independent of component concentration (possessing zero-order kinetics). Devices consisting of a matrix surrounded by a rate controlling membrane also suitable for use.

Both reservoir and matrix type devices can contain materials such as polydimethylsiloxane, such as Silastic™, or other silicone rubbers. Matrix materials can be insoluble polypropylene, polyethylene, polyvinyl chloride, ethylvinyl acetate, polystyrene and polymethacrylate, as well as glycerol esters of the glycerol palmitostearate, glycerol stearate, and glycerol behenate type. Materials can be hydrophobic or hydrophilic polymers and optionally contain solubilising agents.

Subcutaneous implant devices can be slow-release capsules made with any suitable polymer, e.g., as described in U.S. Pat. Nos. 5,035,891 and 4,210,644, which are hereby incorporated by reference.

In general, at least four different approaches are applicable in order to provide rate control over the release and transdermal permeation of a drug compound. These approaches are: membrane-moderated systems, adhesive diffusion-controlled systems, matrix dispersion-type systems and microreservoir systems. It is appreciated that a controlled release percutaneous and/or topical composition can be obtained by using a suitable mixture of these approaches.

In a membrane-moderated system, the active ingredient is present in a reservoir which is totally encapsulated in a shallow compartment molded from a drug-impermeable laminate,

such as a metallic plastic laminate, and a rate-controlling polymeric membrane such as a microporous or a non-porous polymeric membrane, e.g., ethylene-vinyl acetate copolymer. The active ingredient is released through the rate controlling polymeric membrane. In the drug reservoir, the active ingredient can either be dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium such as silicone fluid. On the external surface of the polymeric membrane, a thin layer of an adhesive polymer is applied to achieve an intimate contact of the transdermal system with the skin surface. The adhesive polymer is preferably a polymer which is hypoallergenic and compatible with the active drug substance.

In an adhesive diffusion-controlled system, a reservoir of the active ingredient is formed by directly dispersing the active ingredient in an adhesive polymer and then by, e.g., solvent casting, spreading the adhesive containing the active ingredient onto a flat sheet of substantially drug-impermeable metallic plastic backing to form a thin drug reservoir layer.

A matrix dispersion-type system is characterized in that a reservoir of the active ingredient is formed by substantially homogeneously dispersing the active ingredient in a hydrophilic or lipophilic polymer matrix. The drug-containing polymer is then molded into disc with a substantially well-defined surface area and controlled thickness. The adhesive polymer is spread along the circumference to form a strip of adhesive around the disc.

A microreservoir system can be considered as a combination of the reservoir and matrix dispersion type systems. In this case, the reservoir of the active substance is formed by first suspending the drug solids in an aqueous solution of water-soluble polymer and then dispersing the drug suspension in a lipophilic polymer to form a multiplicity of unleachable, microscopic spheres of drug reservoirs.

Any of the above-described controlled release, extended release, and sustained release compositions can be formulated to release the active ingredient in about 30 minutes to about 1 week, in about 30 minutes to about 72 hours, in about 30 minutes to 24 hours, in about 30 minutes to 12 hours, in about 30 minutes to 6 hours, in about 30 minutes to 4 hours, and in about 3 hours to 10 hours. In embodiments, an effective concentration of the active ingredient(s) is sustained in a subject for 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, or more after administration of the pharmaceutical compositions to the subject.

Dosages

When the agents described herein are administered as pharmaceuticals to humans or animals, they can be given per se or as a pharmaceutical composition containing active ingredient in combination with a pharmaceutically acceptable carrier, excipient, or diluent.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of the invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. Generally, agents or pharmaceutical compositions of the invention are administered in an amount sufficient to reduce or eliminate symptoms associated with viral infection and/or autoimmune disease.

Exemplary dose ranges include 0.01 mg to 250 mg per day, 0.01 mg to 100 mg per day, 1 mg to 100 mg per day, 10 mg to 100 mg per day, 1 mg to 10 mg per day, and 0.01 mg to 10 mg per day. A preferred dose of an agent is the maximum that a patient can tolerate and not develop serious or unacceptable side effects. In embodiments, the agent is administered at a concentration of about 10 micrograms to about 100 mg per kilogram of body weight per day, about 0.1 to about 10 mg/kg per day, or about 1.0 mg to about 10 mg/kg of body weight per day.

In embodiments, the pharmaceutical composition comprises an agent in an amount ranging between 1 and 10 mg, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg.

In embodiments, the therapeutically effective dosage produces a serum concentration of an agent of from about 0.1 ng/ml to about 50-100 µg/ml. The pharmaceutical compositions typically should provide a dosage of from about 0.001 mg to about 2000 mg of compound per kilogram of body weight per day. For example, dosages for systemic administration to a human patient can range from 1-10 µg/kg, 20-80 µg/kg, 5-50 µg/kg, 75-150 µg/kg, 100-500 µg/kg, 250-750 µg/kg, 500-1000 µg/kg, 1-10 mg/kg, 5-50 mg/kg, 25-75 mg/kg, 50-100 mg/kg, 100-250 mg/kg, 50-100 mg/kg, 250-500 mg/kg, 500-750 mg/kg, 750-1000 mg/kg, 1000-1500 mg/kg, 1500-2000 mg/kg, 5 mg/kg, 20 mg/kg, 50 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg, 1500 mg/kg, or 2000 mg/kg. Pharmaceutical dosage unit forms are prepared to provide from about 1 mg to about 5000 mg, for example from about 100 to about 2500 mg of the compound or a combination of essential ingredients per dosage unit form.

In embodiments, about 50 nM to about 1 μ M of an agent is administered to a subject. In related embodiments, about 50-100 nM, 50-250 nM, 100-500 nM, 250-500 nM, 250-750 nM, 500-750 nM, 500 nM to 1 μ M, or 750 nM to 1 μ M of an agent is administered to a subject.

Determination of an effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Generally, an efficacious or effective amount of an agent is determined by first administering a low dose of the agent(s) and then incrementally increasing the administered dose or dosages until a desired effect (e.g., reduce or eliminate symptoms associated with viral infection or autoimmune disease) is observed in the treated subject, with minimal or acceptable toxic side effects. Applicable methods for determining an appropriate dose and dosing schedule for administration of a pharmaceutical composition of the present invention are described, for example, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Goodman *et al.*, eds., 11th Edition, McGraw-Hill 2005, and *Remington: The Science and Practice of Pharmacy*, 20th and 21st Editions, Gennaro and University of the Sciences in Philadelphia, Eds., Lippencott Williams & Wilkins (2003 and 2005), each of which is hereby incorporated by reference.

Combination Therapies

The agents and pharmaceutical compositions described herein can also be administered in combination with another therapeutic molecule. The therapeutic molecule can be any compound used to retinal disease, or symptoms thereof. Examples of such compounds include, but are not limited to, anti-viral agents, immunosuppressants, anti-inflammatories, and the like.

The Wnt signaling enhancing compounds can be administered before, during, or after administration of the additional therapeutic agent. In embodiments, the Wnt signaling enhancing compounds is administered before the first administration of the additional therapeutic agent. In embodiments, the Wnt signaling enhancing compounds is administered after the first administration of the additional therapeutic agent (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more). In embodiments, the Wnt signaling enhancing compounds is administered simultaneously with the first administration of the additional therapeutic agent.

The amount of therapeutic agent administered to a subject can readily be determined by the attending physician or veterinarian. Generally, an efficacious or effective amount of an Wnt signaling enhancing compound and an additional therapeutic is determined by first administering a low dose of one or both active agents and then incrementally increasing the administered dose or dosages until a desired effect is observed (e.g., reduced symptoms associated with viral infection or autoimmune disease), with minimal or no toxic side effects. Applicable methods for determining an appropriate dose and dosing schedule for administration of a combination of the present invention are described, for example, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 11th Edition., supra, and in *Remington: The Science and Practice of Pharmacy*, 20th and 21st Editions, supra.

Kits

The invention provides for kits containing at least one Wnt signaling enhancing compound as described herein. The kits are suitable for use in preventing or treating capillary dropout associated with retinal disease. In embodiments, the Wnt signaling enhancing compounds is provided as a pharmaceutical composition. In embodiments, the kit provides instructions for use. The instructions for use can pertain to any of the methods described herein.

Kits according to this aspect of the invention may comprise a carrier means, such as a box, carton, tube or the like, having in close confinement therein one or more container means, such as vials, tubes, ampules, bottles and the like. In embodiments, the kit provides a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale of the kit and the components therein for human administration.

EXAMPLES

It should be appreciated that the invention should not be construed to be limited to the examples that are now described; rather, the invention should be construed to include any and all applications provided herein and all equivalent variations within the skill of the ordinary artisan.

Example 1: Identification of optimal time to initiate treatment of capillary dropout.

Capillary drop out is a very serious problem for many retinal vascular diseases. It has been previously suggested that norrin can be used to treat capillary dropout. *See Trese, M. T. et*

al., *Retinal Physician*, April 2011. For example, in a murine capillary dropout model (*see* Figures 1 and 2; *see also* Figure 3), capillary dropout is reduced or eliminated by norrin treatment (*see* Figure 4).

It has now been discovered that there is a peripheral vascular change on wide field angiography that proceeds capillary loss. This antecedent lesion is characterized by the presence of vessels having fuzzy margins during fluorescein angiograms. *See* Figures 5, 6, and 7. Specifically, Figure 5 is a wide field fluorescein angiogram (Optos system) of a person with Familial Exudative Vitreoretinopathy (FEVR). This patient previously received laser therapy to treat avascular retina (as shown by the retinal damage present on the far right of the image). Healthy optical nerve is present throughout the left half of the image. Between the healthy optical nerve and the retinal damage from the laser therapy is a region of “fuzzy” vessels (i.e., where vessel diameter is increased with indistinct margins in a fundus image as compared to the remaining vessels, which maintain a crisp architecture). These fuzzy vessels are an indication of cellular change due to damage of endothelial cells. These changes precede frank capillary loss, which is what drives the pathogenesis of many retinal disorders. Illustratively, Figure 6 depicts a higher magnification view of the area of capillary damage shown in FIG. 5. Shown are small areas of microaneurysms (i.e., punctate white spots) and endothelial cell damage as manifested by dye leakage. Left alone this area will ultimately result in complete capillary loss.

Additionally, Figure 7 shows a low magnification wide field fluorescein angiogram (Optos) of a retina of a patient with diabetes prior to the onset of macular diabetic retinopathy (e.g., either background diabetic retinopathy or macular diabetic retinopathy), which reveals numerous small white dots that correspond to microaneurysms surrounding areas of capillary drop out prior to any change in the macula area. These microaneurysm-associated areas are believed to give rise to macular diabetic retinopathy, and according to an exemplary embodiment of the invention, these areas represent target areas for treatment with an activator of Wnt signaling (e.g., Norrin). Figure 8 shows a high magnification view of the wide field fluorescein angiogram shown in FIG. 7. In comparison, Figures 9 and 10 show regular and wide field, respectively, fluorescein angiogram depicting normal macula.

This finding is of great clinical significance because it will assist practitioners in accurately identifying and effectively treating early stage retinal disease. Early detection and early treatment of capillary loss will result in preservation and/or reformation of the altered vessels.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Incorporation by Reference

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

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What is claimed is:

1. A method of treating or preventing capillary dropout in a subject, wherein the method comprises administering to the subject an agent that enhances Wnt signaling, thereby treating or preventing capillary dropout.
2. The method of claim 1, wherein the subject has or is at risk of developing capillary dropout.
3. The method of claim 2, wherein the subject has or is at risk of developing familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), Norries disease, branch retinal vein occlusion, or central retinal vein occlusion.
4. The method of claim 2, wherein the subject has or is at risk of developing diabetic retinopathy.
5. The method of any one of claims 1 to 3, wherein the ocular capillaries of the subject are evaluated by fluorescein angiography (FA).
6. The method of claim 5, wherein the agent is administered to the subject when an antecedent lesion is identified.
7. The method of claim 6, wherein the antecedent lesion is located in a peripheral capillary.
8. The method of claim 6, wherein the antecedent lesion is located in a posterior capillary.
9. A method of treating or preventing peripheral capillary dropout in a subject, wherein the method comprises administering to the subject an agent that enhances Wnt signaling, thereby treating or preventing capillary dropout.
10. The method of claim 9, wherein the subject has or is at risk of familial exudative vitreoretinopathy (FEVR), diabetic retinopathy, retinopathy of prematurity (ROP), Norries disease, branch retinal vein occlusion, or central retinal vein occlusion.
11. The method of claim 9, wherein the subject has or is at risk of developing diabetic retinopathy.

12. The method of any one of claims 9 to 11, wherein the ocular capillaries of the subject are evaluated by fluorescein angiography (FA).

13. The method of claim 12, wherein the agent is administered to the subject when an antecedent lesion is identified in a peripheral capillary.

14. The method of any one of claims 1 to 13, wherein the method reduces capillary loss.

15. The method of any one of claims 1 to 14, wherein the method reduces capillary occlusion.

16. The method of any one of claims 1 to 15, wherein the method enhances capillary formation.

17. The method of any one of claims 1 to 16, wherein the method enhances retinal function.

18. The method of any one of claims 1 to 17, wherein the method enhances b-wave response, substantial oscillatory potential, or visual improvement.

19. The method of any one of claims 1 to 18, wherein the method reduces the symptoms associated with capillary dropout.

20. The method of any one of claims 1 to 19, wherein the agent enhances the activity of a frizzled-4 transmembrane receptor (Fzd4).

21. The method of any one of claims 1 to 20, wherein the agent is an agonist of Fzd4, Low-density lipoprotein receptor-related protein 5 (LRP5), Low-density lipoprotein receptor-related protein 6 (LRP6), or tetraspanin-12 (Tspan12).

22. The method of any one of claims 1 to 21, wherein the agent is a small molecule, a nucleic acid, a peptide, or a peptide mimetic.

23. The method of claim 22, wherein the agent is an antibody or antibody fragment.

24. The method of claim 22, wherein the small molecule is 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine.

25. The method of claim 23, wherein the antibody is a polyclonal antibody.

26. The method of claim 23, wherein the antibody is a monoclonal antibody.
27. The method of any one of claims 23 to 26, wherein the antibody or antibody fragment is humanized.
28. The method of any one of claims 1 to 22, wherein the agent is a ligand of Fzd4.
29. The method of claim 28, wherein the ligand is Norrin, an active fragment of Norrin, or an active mutant of Norrin.
30. The method of any one of claims 1 to 22, wherein the agent is a nucleic acid encoding Norrin or a fragment thereof.
31. A method of treating or preventing capillary dropout in a subject having or at risk of developing diabetic retinopathy, wherein the method comprises administering Norrin or a fragment thereof to the subject, thereby treating or preventing capillary dropout.
32. The method of any one of claims 1 to 31, wherein the method further comprises administering at least one additional agent to treat capillary dropout.
33. The method of claim 31, wherein the at least one additional agent is an anti-inflammatory, a steroid, anti-VEGF, or a caspase.
34. The method of any one of claims 1 to 33, wherein the method further comprises treating the patient with laser therapy.
35. The method of any one of claims 1 to 34, wherein the agent is administered systemically or locally.
36. The method of claim 35, wherein the agent is administered orally, parenterally, or topically.
37. The method of claim 35 or 36, wherein the agent is administered by intravitreal or periocular injection.
38. The method of any one of claims 1 to 37, wherein the subject is a mammal.
39. The method of claim 38, wherein the subject is human.

40. A pharmaceutical composition comprising an agent that treats capillary dropout for use in any one of the methods of claims 1 to 39.
41. The pharmaceutical composition of claim 40, wherein the agent that treats capillary dropout enhances the activity of a frizzled-4 transmembrane receptor (Fzd4).
42. The pharmaceutical composition of claim 40 or 41, wherein the agent that treats capillary dropout is an agonist of Fzd4, Low-density lipoprotein receptor-related protein 5 (LRP5), or tetraspanin-12 (Tspan12).
43. The pharmaceutical composition of any one of claims 40 to 42, wherein the agent that treats capillary dropout is a small molecule, a nucleic acid, a peptide, or a peptide mimetic.
44. The pharmaceutical composition of claim 43, wherein the agent that treats capillary dropout is an antibody or antibody fragment.
45. The pharmaceutical composition of claim 44, wherein the agent that treats capillary dropout is a polyclonal antibody.
46. The pharmaceutical composition of claim 44, wherein the agent that treats capillary dropout is a monoclonal antibody.
47. The pharmaceutical composition of any one of claims 44 to 46, wherein the antibody or antibody fragment is humanized.
48. The pharmaceutical composition of any one of claims 40 to 43, wherein the agent that treats capillary dropout is a ligand of Fzd4.
49. The pharmaceutical composition of claim 48, wherein the ligand is Norrin or a fragment thereof.
50. The pharmaceutical composition of any one of claims 40 to 43, wherein the agent that treats capillary dropout is a nucleic acid encoding Norrin or a fragment thereof.
51. The pharmaceutical composition of claim any one of claims 40 to 50, wherein the pharmaceutical composition further comprises at least one additional agent to treat capillary dropout.

52. The pharmaceutical composition of claim any one of claims 40 to 51, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent, or excipient.
53. A kit comprising an agent that treats capillary dropout for use in any one of the methods of claims 1 to 39.
54. The kit of claim 53, wherein the agent that treats capillary dropout enhances the activity of a frizzled-4 transmembrane receptor (Fzd4).
55. The kit of claim 53 or 54, wherein the agent that treats capillary dropout is an agonist of Fzd4, Low-density lipoprotein receptor-related protein 5 (LRP5), or tetraspanin-12 (Tspan12).
56. The kit of any one of claims 53 to 55, wherein the agent that treats capillary dropout is a small molecule, a nucleic acid, a peptide, or a peptide mimetic.
57. The kit of claim 56, wherein the agent that treats capillary dropout is an antibody or antibody fragment.
58. The kit of claim 57, wherein the agent that treats capillary dropout is a polyclonal antibody.
59. The kit of claim 57, wherein the agent that treats capillary dropout is a monoclonal antibody.
60. The kit of any one of claims 57 to 59, wherein the antibody or antibody fragment is humanized.
61. The kit of any one of claims 53 to 56, wherein the agent that treats capillary dropout is a ligand of Fzd4.
62. The kit of claim 61, wherein the ligand is Norrin or a fragment thereof.
63. The kit of any one of claims 53 to 56, wherein the agent that treats capillary dropout is a nucleic acid encoding Norrin or a fragment thereof.
64. The kit of any one of claims 53 to 63, wherein the kit further comprises at least one additional agent to treat capillary dropout.

65. The kit of any one of claims 53 to 64, wherein the kit further comprises instructions for using the agent that treats capillary dropout in any one of the methods of claims 1 to 39.

1/7

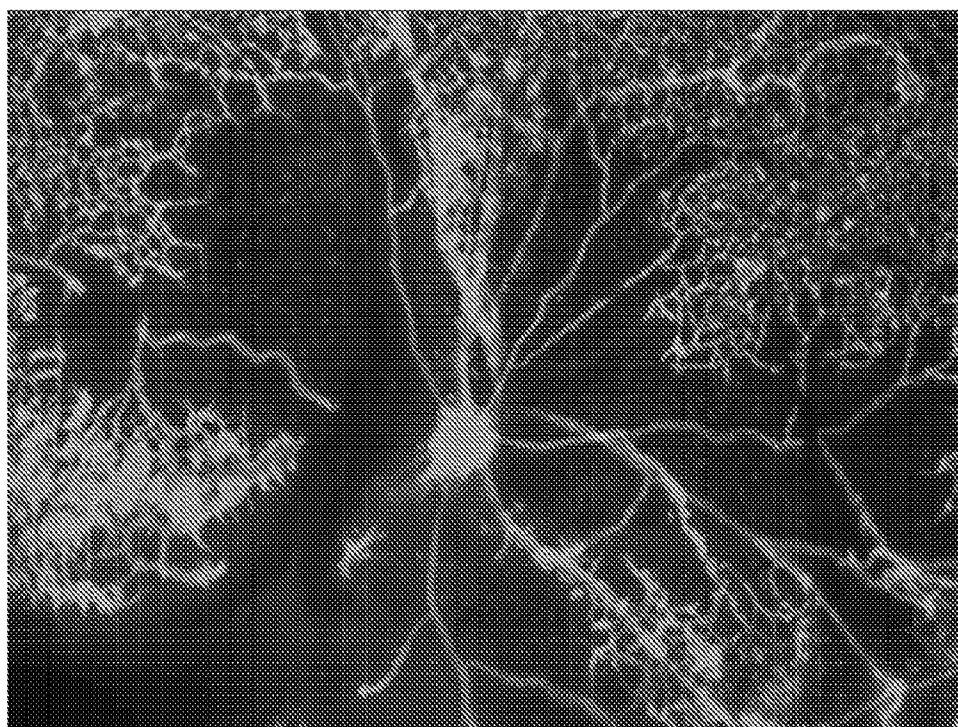


FIG. 1

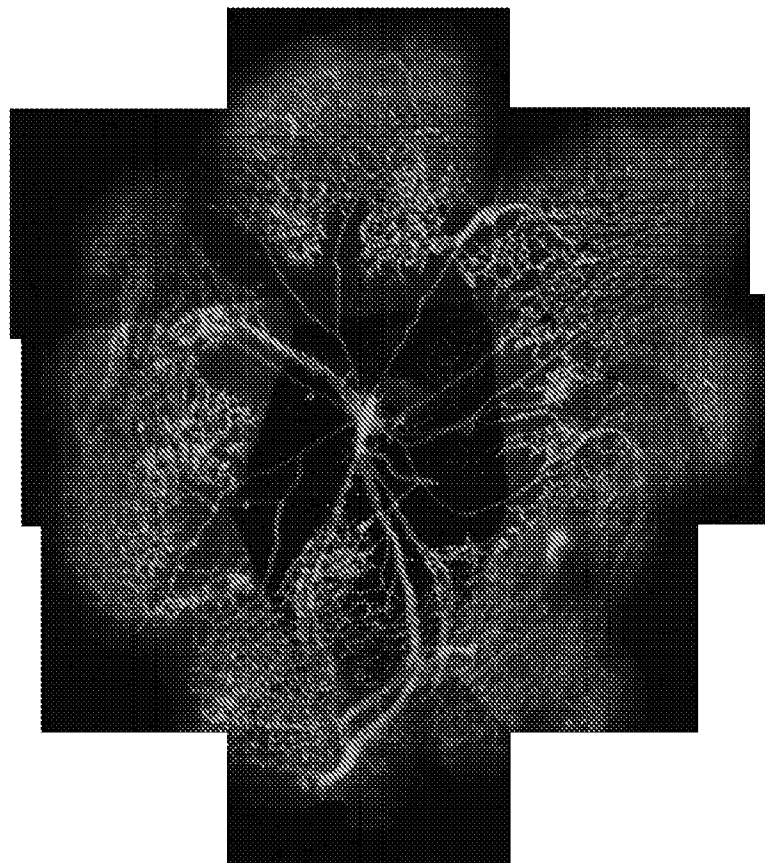


FIG. 2

2/7

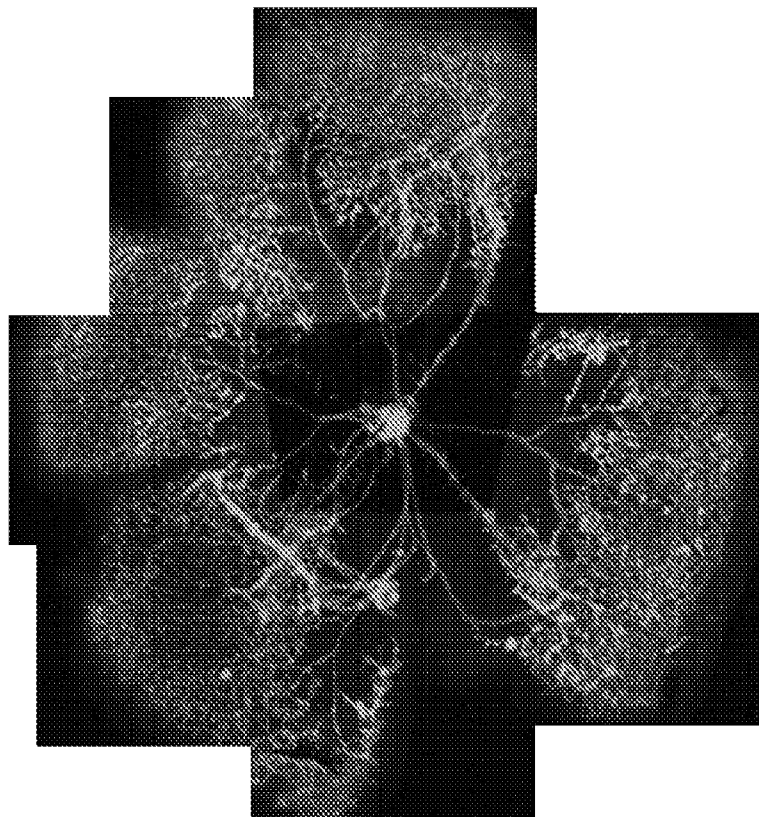


FIG. 3

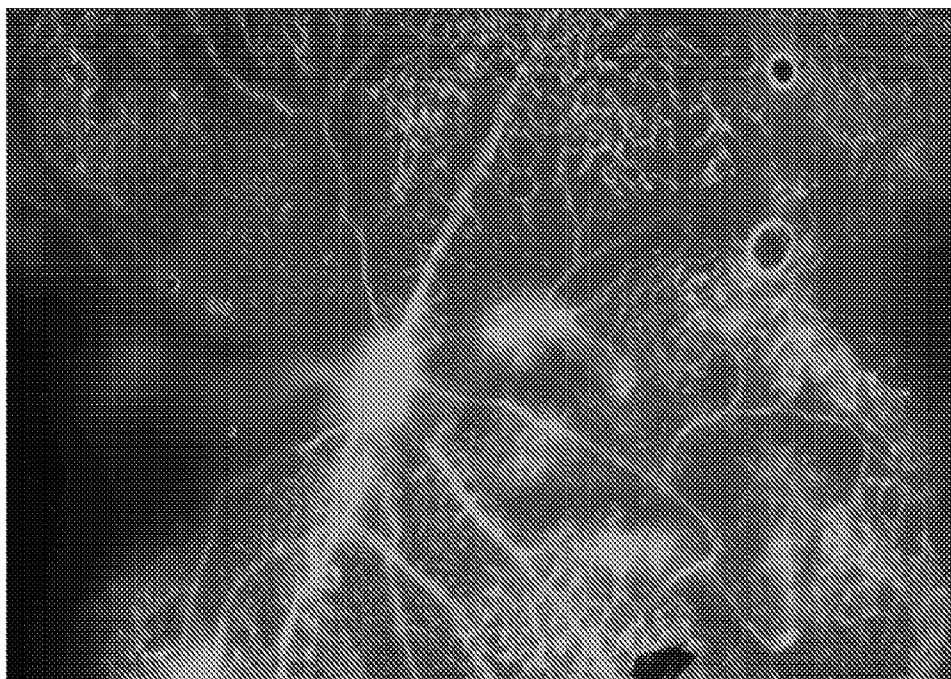


FIG. 4

3/7



FIG. 5

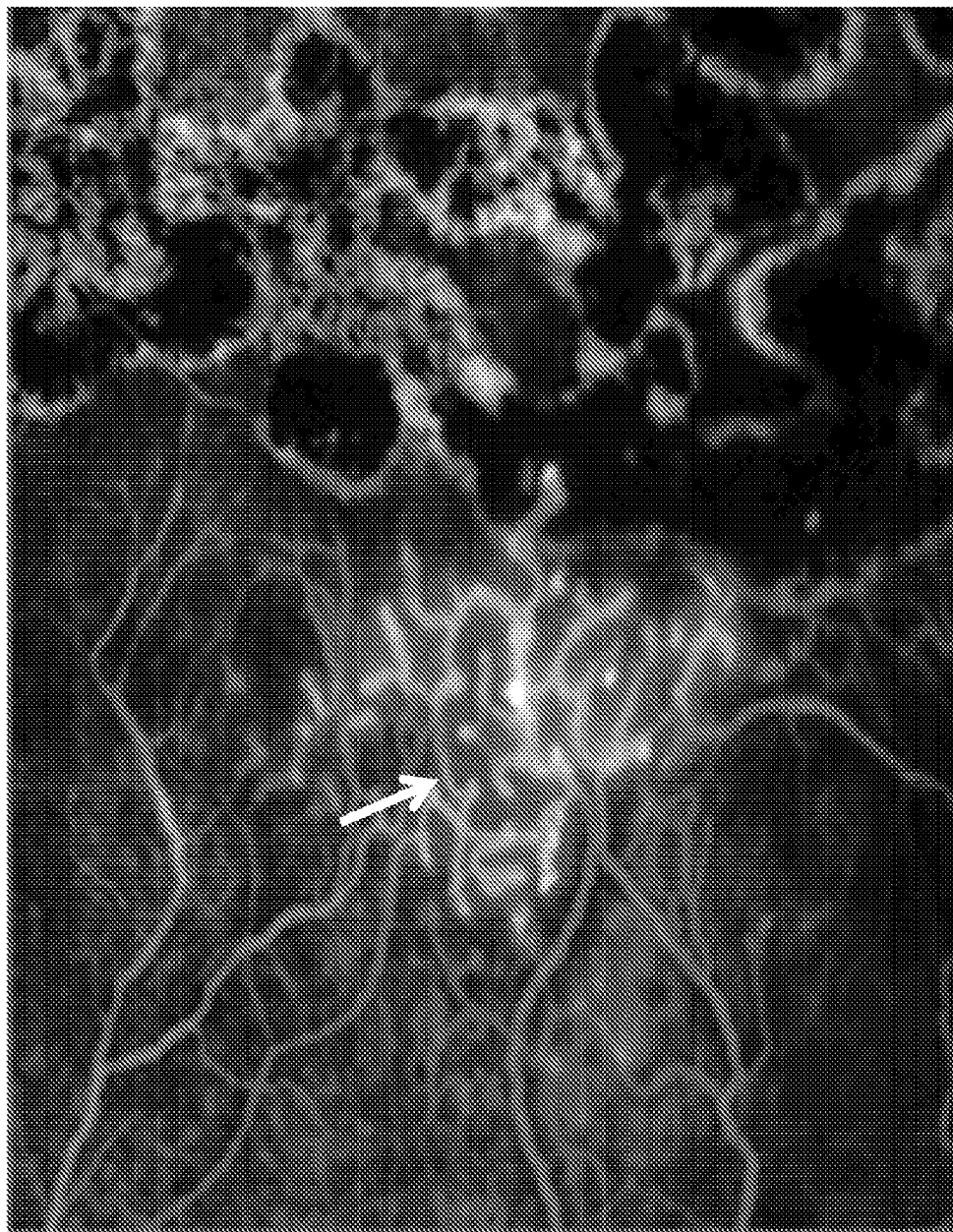


FIG. 6

5/7

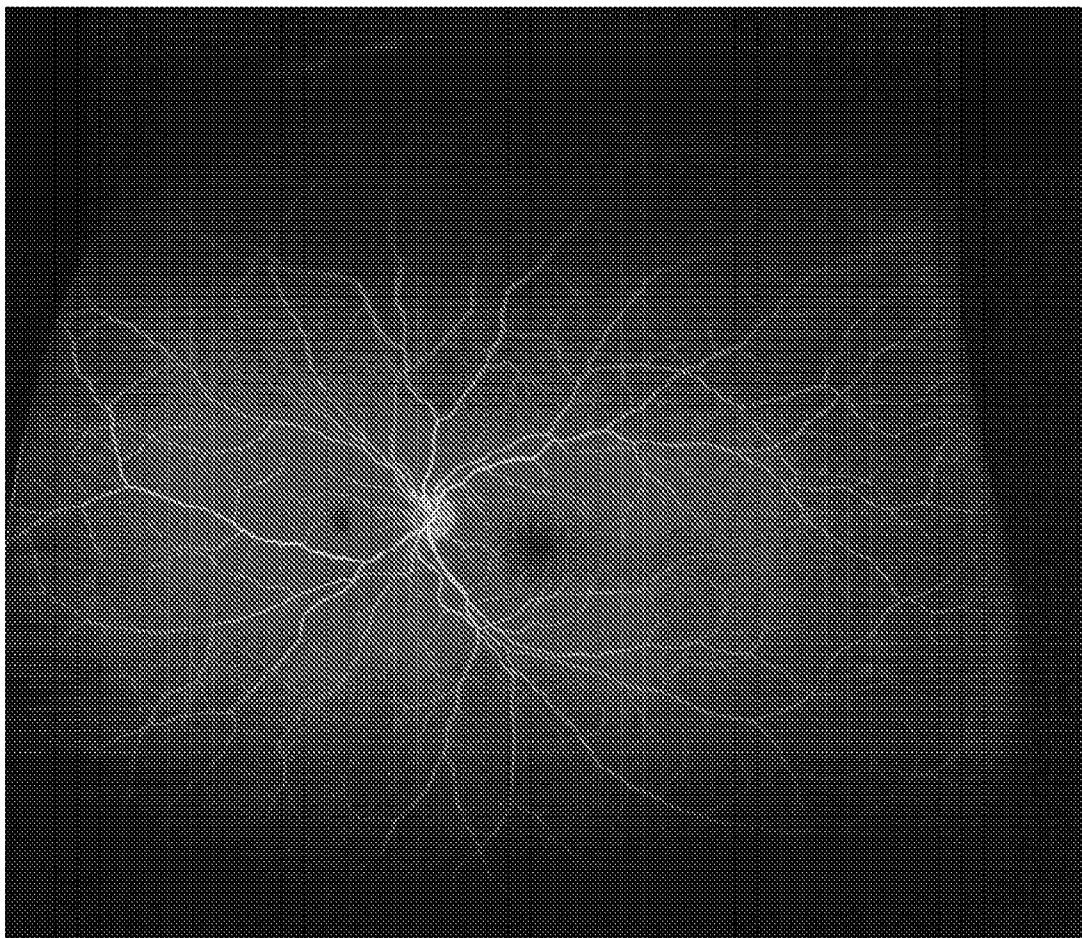


FIG. 7

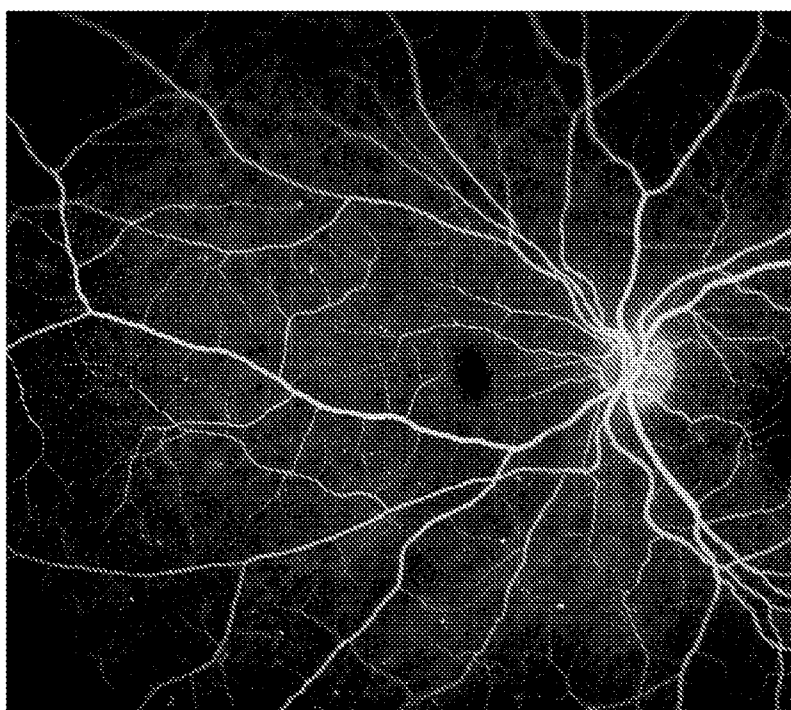
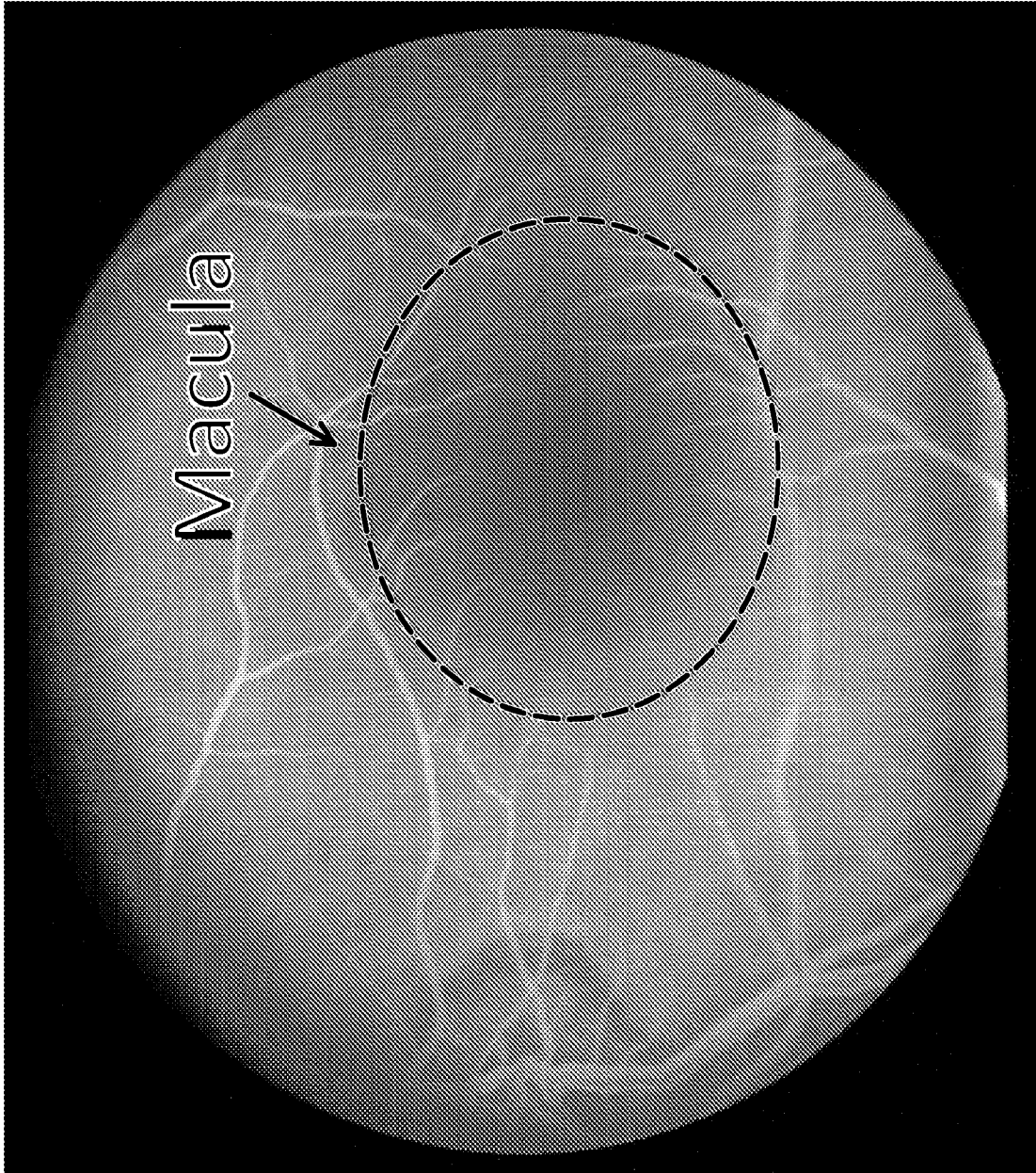


FIG. 8

FIG. 9



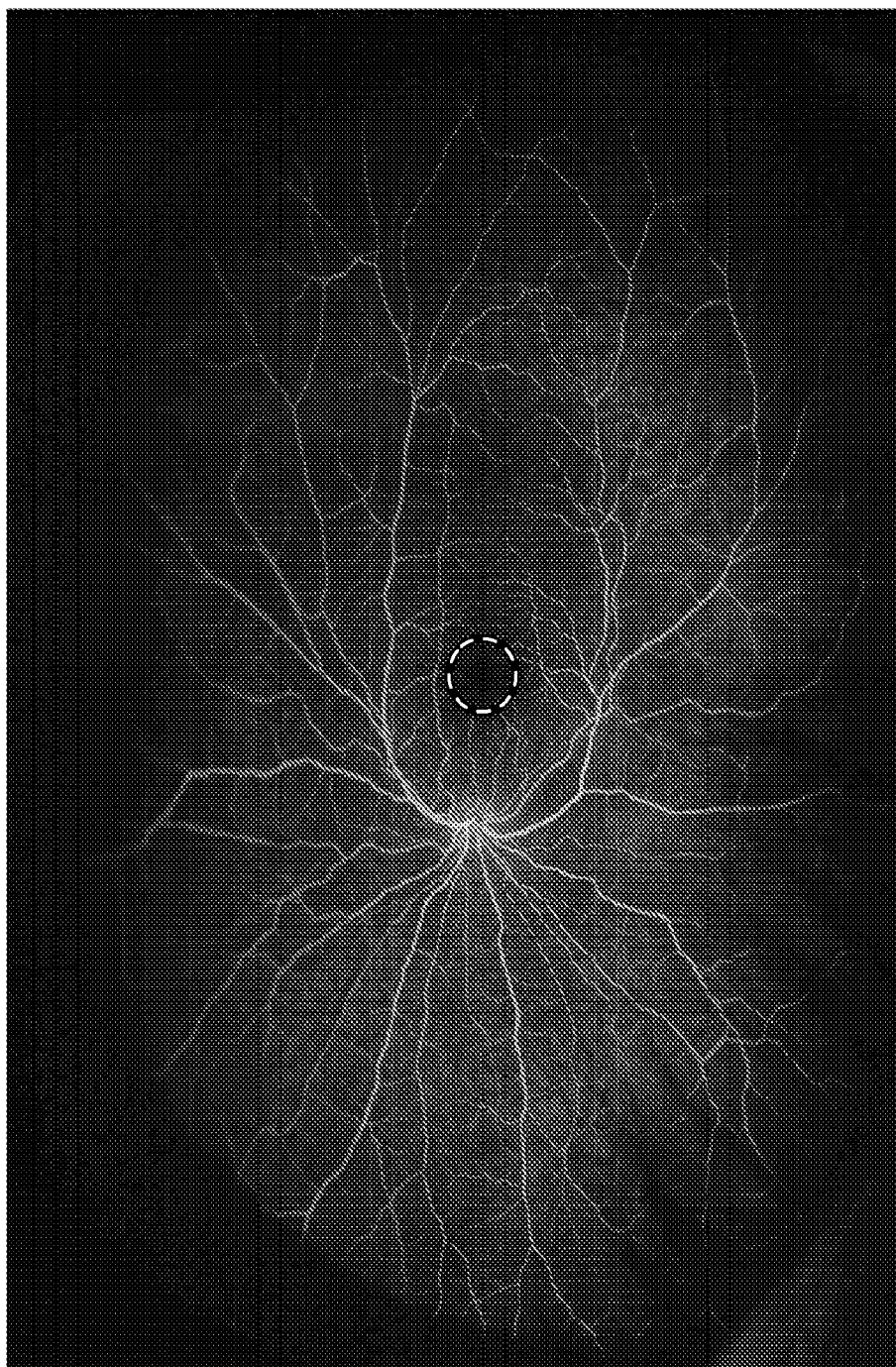


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/032411**A. CLASSIFICATION OF SUBJECT MATTER****A61K 38/17(2006.01)i, A61K 38/16(2006.01)i, A61K 48/00(2006.01)i, A61P 27/02(2006.01)i, A61P 27/00(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 38/17; A61B 10/00; A61K 48/00; A61K 38/16; A61P 27/02; A61P 27/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: capillary dropout, Wnt, diabetic retinopathy, norrin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010-0239499 A1 (DRENSER) 23 September 2010 See abstract; paragraphs [0001]-[0007]; and claims 1-23.	1-4, 9-11, 31
A	SEITZ et al., `Norrin mediates neuroprotective effects on retinal ganglion cells via activation of the Wnt/ $\beta$ -catenin signaling pathway and the induction of neuroprotective growth factors in Muller cells` The Journal of Neuroscience, Vol.30, No.17, pp.5998-6010 (2010) See the whole document.	1-4, 9-11, 31
A	OHLMANN et al., `Norrin promotes vascular regrowth after oxygen-induced retinal vessel loss and suppresses retinopathy in mice` The Journal of Neuroscience, Vol.30, No.1, pp.183-193 (2010) See the whole document.	1-4, 9-11, 31
A	CHEN et al., `Wnt signaling mediates pathological vascular growth in proliferative retinopathy clinical perspective` Circulation, Vol.124, No.17, pp.1871-1881 (2011) See the whole document.	1-4, 9-11, 31



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 October 2013 (22.10.2013)

Date of mailing of the international search report

22 October 2013 (22.10.2013)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/032411

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MASCKAUCHAN et al., `Wnt/Frizzled signaling in the vasculature: new angiogenic factors in sight` Physiology, Vol.21, No.3, pp.181-188 (2006) See the whole document.	1-4,9-11,31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/032411

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: See extra sheet
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
See extra sheet
3. ☒ Claims Nos.: See extra sheet
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

(Continuation of: Box No. II)

2. Claims 5-8, 12-13, 23-26, 29, 33, 36, 39, 41, 44-46, 49, 54, 57-59, 62

Claims 5 and 12 are worded in reference to the ocular capillaries of any one of claims 1-3 and any one of claims 9-11, respectively. However, said ocular capillaries have not been worded in any one of claims 1-3 and 9-11. Therefore, claims 5 and 12 do not meet the requirement of PCT Article 6. Claims 6-8 and 13 do not comply with PCT Article 6 because they are referring to unsearchable claims 5 and 12, respectively.

Claims 23-26, 29, 36, 39, 41, 44-46, 49, 54, 57-59 and 62 are unclear since they are referring to multiple dependent claims which do not comply with PCT Rule 6.4(a).

Claim 33 is worded in reference to at least one additional agent of claim 31. However, said at least one additional agent has not been worded in claim 31. Therefore, claim 33 does not meet the requirement of PCT Article 6.

3. Claims 14-22, 27-28, 30, 32, 34-35, 37-38, 40, 42-43, 47-48, 50-53, 55-56, 60-61, 63-65

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/032411

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010-0239499 A1	23/09/2010	None	