

**TREATMENT OF OCULAR DISEASES USING
1-(4-{[4-(DIMETHYLAMINO)PIPERIDIN-1-YL]CARBONYL}PHENYL)-3-[4-(4,6-DIMORPHOLIN-4-YL-1,3,5-TRIAZIN-2-YL)PHENYL]UREA**

Related Application

5 This application claims priority to U.S. Provisional Application No. 63/609,230, filed December 12, 2023, the entire contents of which is hereby incorporated by reference.

FIELD OF THE INVENTION

10 The present invention relates to methods for treating ocular diseases, such as neovascular age-related macular degeneration (wet AMD), or age-related macular degeneration (dry AMD), diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central
15 retinal vein occlusion, cystoid macular edema, neovascular glaucoma, polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa, in a patient by administering 1-(4-{[4-(Dimethylamino)piperidin-1-yl]carbonyl}phenyl)-3-[4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl]urea (gedatolisib).

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BACKGROUND

 According to research by the World Health Organization (WHO) in 2015, almost 217 million people aged 18 years or older globally are suffering from various ocular disorders that could lead to vision impairment and finally cause permanent blindness (see, *e.g.*, Afarid, M., *et al.*, *J. Nanobiotechnol.* 20, 361 (2022). Visual impairment is a public health problem that affects
25 patient functional status and quality of life (see, *e.g.*, Zhang Y, *et al.*, *Invest Ophthalmol Vis Sci.* 2017 Nov 1;58(13):5616-5627). Blindness or vision impairment is among the top 10 disabilities in the United States according to Centers for Disease Control and Prevention (see, *e.g.*, Zhang Y, *et al.*, 2017).

30 Age-related macular degeneration (AMD), in particular, affects millions of people worldwide and is the leading cause of irreversible blindness in the developed world in

individuals over the age of 60 (see, Klein R, *et al.*, *Ophthalmology* 2007; 114(2):253–62); and Wong WL, *et al.*, *Lancet Glob Heal* 2014;2:c106–16). AMD presents a major global health concern with a significant impact on quality of life for the elderly population (see, Thomas *et al.*, *Med Clin North Am.* 2021 May;105(3):473-491). Loss of central vision impacts the ability to read, drive, recognize faces, and perform basic living tasks.

There are two main types of AMD, neovascular and nonneovascular AMD, which can be further classified based on specific features of the disease. Nonneovascular AMD (“dry” AMD) accounts for almost 80% to 85% of all cases and generally carries a more favorable visual prognosis. Neovascular AMD (“wet” AMD) affects the remaining 15% to 20% and accounts for approximately 80% of severe vision loss as a result of AMD (see, Ferris FL III, *et al.*, *Arch. Ophthalmol.* 1984;102:1640–2).

As there is no cure for AMD, preventative and proactive measures are crucial (see, Thomas *et al.*, 2021). Disease progression can be slowed by addressing certain modifiable risk factors, such as smoking, diet, and cardiovascular disease (see U. Chakravarthy, T.Y., *et al.*, *BMC Ophthalmol*, 10 (2010), p. 31). The Age-Related Eye Disease Study (AREDS) led to the development of a specific combination of vitamin supplementation for the prevention of disease progression for patients with specific characteristics of AMD (see M.D. Davis, *et al.*, *Arch Ophthalmol*, 123 (11) (2005), pp. 1484-1498). Additionally, because a primary etiology of many ocular diseases is highly correlated with a dysfunctional ocular vascular system, inhibiting the activity of vascular endothelial growth factor (VEGF) using anti-VEGF or VEGFR inhibiting agents, such as anti-VEGF antibodies, has shown benefit and has become standard of care (see, *e.g.*, Kovach JL, *et al.*, *J. Ophthalmol.* 2012;2012:786870). However, not all types of AMD or AMD disease processes respond to anti-VEGF therapy or meet criteria for recommending AREDS vitamins. Moreover, the prognosis is variable depending on the stage of the disease and VEGF therapies can affect other cell types (*e.g.*, dendritic cells) or normal vascularity in other parts of the eye (*e.g.*, retina, venous extra macular regions), which can counteract and/or reduce the therapeutic benefit. Further, there are often safety issues with VEGF therapies (*e.g.*, hypertension, artery clots, complications in wound healing, and/or cardiotox (see, *e.g.*, Gardner, V., *et al.* 2017, “Anti-VEGF Therapy in Cancer: A Double-Edged Sword” in D. Simionescu (ed.), *Physiologic and Pathologic Angiogenesis: Signaling Mechanisms and Targeted Therapy*).

In the last ten years, numerous preclinical and clinical studies have been performed to develop therapeutics for different ophthalmic disorders (see, *e.g.*, Gorantla S, *et al.*, RSC Adv. 2020;10(46):27835–55). Considerable accomplishments have been made in the innovation of ophthalmic pathological mechanisms and eye disease management. Notwithstanding, given the
5 unique anatomical and physiological features of the eye, detection and treatment of these diseases face many challenges (see, *e.g.*, Afarid, M., *et al.*, *J. Nanobiotechnol.* 20, 361 (2022). Accordingly, there exists a need for improved therapies for treating ocular diseases, including AMD and neovascular AMD, in particular.

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SUMMARY

Provided herein are methods for treating ocular diseases by administering a therapeutically effective amount of 1-(4-[[4-(Dimethylamino)piperidin-1-yl]carbonyl]phenyl)-3-[4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl]urea, hereinafter “gedatolisib”, or a pharmaceutically acceptable salt, solvate, or ester thereof, either alone or in combination with
15 other therapies or agents to a patient in need thereof. As demonstrated herein, gedatolisib treatment inhibited neovascularization in a choroidal neovascularization model at a level better than standard of care anti-VEGF treatment. Moreover, systemic treatment with gedatolisib (*e.g.*, by intravenous, topical, or intramuscular administration) resulted in ocular accumulation sufficient to inhibit neovascularization. Furthermore, gedatolisib treatment was shown to regress
20 established neovascularization. Accordingly, gedatolisib can be used in the treatment of ocular diseases, in particular those associated with neovascularization. Exemplary ocular diseases include, but are not limited to, neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic
25 degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, neovascular glaucoma, polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.

In one embodiment, a method of treating an ocular disease in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of an ocular disease; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.

5 In another embodiment, a method for treating a human patient who has been determined to have an ocular disease is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the ocular disease.

Gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, can be
10 administered to a patient by any suitable means. In one embodiment, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is formulated for intravenous administration. In one embodiment, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is formulated for topical administration (e.g., an eye drop to be applied onto the eye). In one embodiment, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester
15 thereof, is formulated for intraocular administration. In other embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is formulated for intravitreal, suprachoroidal or intravascular administration.

In some embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient in a therapeutically active amount. In embodiments,
20 gedatolisib is administered at a concentration in a range of, for example, 0.01-20 mg/ml, 0.01-10 mg/ml or 0.01-4 mg/ml. Other concentration ranges and concentrations are disclosed herein. In some embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient at a dose of between 0.001mg/kg and 10mg/mk. In other embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is
25 administered to the patient at a dose of about, for example, 0.01-5mg/kg, 0.1-5mg/kg, 1-10mg/kg, 1-5mg/kg or at 5 mg/kg. In embodiments, a topical dosage to the eye comprises about 5-500 ug (e.g., 6-400 ug) of gedatolisib in 1-2 drops. In other embodiments, gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered once a day, twice a day, three times a day, four times a day, or for 1 day, 5 days, 1 week, 1-3months, 4-6 months, 7-9

months, 10-12 months, 1-5 years, or more than 5 years running consecutively or intermittently on a daily, or two to four times daily, weekly or monthly administration schedule.

In one embodiment, a method of treating neovascular age-related macular degeneration in a human subject is provided, wherein the method comprises: (a) selecting a human subject in
5 need of treatment of neovascular age-related macular degeneration; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have neovascular age-related macular degeneration is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically
10 acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the neovascular age-related macular degeneration.

The efficacy of the treatment methods provided herein can be assessed using any suitable means, including but not limited to electronic eye charts, fundus photography, optical coherent tomography, electroretinography, and other commonly available eye clinic exam instruments.
15 For example, the treatment can be assessed by one or more recognized clinical scales for evaluating the particular ocular disease, *e.g.*, neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis,
20 pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, neovascular glaucoma, polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or
25 cessation) of one or more symptoms of neovascular age-related macular degeneration. Exemplary symptoms of neovascular age-related macular degeneration include, but are not limited to, visual distortions, reduced central vision in one or both eyes, the need for brighter light when reading or doing close-up work, difficulty adjusting to low light levels, increased blurriness of printed words, difficulty recognizing faces, and a well-defined blurry spot or blind
30 spot in the field of vision, compared to baseline.

In some embodiments, the patient maintains vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the patient maintains vision, as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “maintaining vision” is defined as losing 15 letters of visual acuity (*e.g.*, at 12 months compared to baseline).

In some embodiments, the treatment results in an improvement in vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS or (ETDRS-DRSS) test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more letters (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 6, 9, or 12 months compared to baseline). In some embodiments, the treatment results in an improvement, as assessed by a “Best-Corrected Visual Acuity” test. In some embodiments, the treatment results in an improvement, according to the “Best-Corrected Visual Acuity” test, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more letters (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 6, 9, or 12 months compared to baseline).

In some embodiments, the treatment results in a “doubling of the visual angle” in best corrected distance visual acuity at, for example, 6, 9, or 12 months after the start of treatment.

In some embodiments, the treatment results in a “halving of the visual angle” in best corrected distance visual acuity at, for example, 6, 9, or 12 months after the start of treatment.

In some embodiments, the treatment results in an improvement in one or more symptoms of neovascular age-related macular degeneration compared to baseline as assessed by, for example, eye dilation and visual examination, optical coherence tomography (OCT), an Amsler

grid, indocyanine green angiography, and/or fluorescein angiography. In some embodiments, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement compared to baseline, as assessed by eye dilation and visual examination, optical coherence tomography (OCT), an Amsler grid,

5 indocyanine green angiography, and/or fluorescein angiography. In some embodiments, the treatment results in a reduction in fluid, blood, and/or drusen compared to baseline. In some embodiments, the treatment results in a reduction in fluid, blood, and/or drusen by at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% compared to baseline, as assessed by eye dilation and visual examination, optical coherence

10 tomography (OCT), an Amsler grid, indocyanine green angiography, and/or fluorescein angiography.

In some embodiments, a method of treating glaucoma in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of glaucoma; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a

15 pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have glaucoma is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the glaucoma.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or

20 cessation) of one or more symptoms of glaucoma. Exemplary symptoms of glaucoma, include but are not limited to, eye pain, eye pressure or intraocular pressure, headaches, rainbow-colored halos around lights, low vision, blurred vision, narrowed vision (tunnel vision), blind spots, nausea, vomiting, and/or red eyes. In one embodiment, the treatment results in an improvement in one or more symptoms of glaucoma compared to baseline, as assessed by, for example, a

25 visual acuity test, eye dilation and visual examination, a visual field test to measure peripheral vision, tonometry, optic nerve imaging, gonioscopy and/or pachymetry. In one embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement in one or more symptoms of glaucoma compared to baseline, as assessed by a visual acuity test, eye dilation and visual examination, a visual field

30 test to measure peripheral vision, tonometry, optic nerve imaging, gonioscopy and/or

pachymetry. In one embodiment, the glaucoma is open-angle glaucoma. In another embodiment, the glaucoma is angle-closure glaucoma. In another embodiment, the glaucoma is low-tension or normal-tension glaucoma. In another embodiment, the glaucoma is congenital glaucoma. In another embodiment, the glaucoma is uveitic (inflammatory) glaucoma. In another embodiment, the glaucoma is neovascular glaucoma.

In one embodiment, a method of treating diabetic retinopathy in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of diabetic retinopathy; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have diabetic retinopathy is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the diabetic retinopathy.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of diabetic retinopathy. Exemplary symptoms of diabetic retinopathy include, but are not limited to, floaters, blurry vision, vision that changes sometimes from blurry to clear, seeing blank or dark areas in the field of vision, poor night vision, distortion of color vision, and/or loss of vision. In one embodiment, the treatment results in an improvement in one or more symptoms of diabetic retinopathy compared to baseline, as assessed by, for example, eye dilation and visual examination, optical coherence tomography (OCT), and/or fluorescein angiography. In one embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of diabetic retinopathy compared to baseline, as assessed by eye dilation and visual examination, optical coherence tomography (OCT), and/or fluorescein angiography. In one embodiment, the diabetic retinopathy is non-proliferative diabetic retinopathy (NPDR). In another embodiment, the diabetic retinopathy is proliferative diabetic retinopathy (PDR).

In one embodiment, a method of treating diabetic macular edema in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of diabetic macular edema; and (b) administering to the human subject a therapeutically effective

amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have diabetic macular edema is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency
5 sufficient to treat the diabetic macular edema.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of diabetic macular edema. Exemplary symptoms of diabetic macular edema include, but are not limited to, decreased visual acuity (VA), blurred vision, metamorphopsia, changes in color perception, and/or difficulty reading.

10 In some embodiments, the treatment results in an improvement in one or more symptoms of diabetic macular edema compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of diabetic macular edema compared to
15 baseline, *e.g.*, as assessed by as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In one embodiment, a method of treating retinitis pigmentosa in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of retinitis pigmentosa; and (b) administering to the human subject a therapeutically effective
20 amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have retinitis pigmentosa is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the retinitis pigmentosa.

25 In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of retinitis pigmentosa. Exemplary symptoms of retinitis pigmentosa include, but are not limited to, loss of night vision, loss of peripheral (side) vision, and/or difficulty seeing different colors. In one embodiment, the treatment results in an improvement in one or more symptoms of retinitis pigmentosa compared to baseline, as assessed
30 by, for example, electroretinography, visual field teasing, and/or optical coherence tomography.

In one embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of retinitis pigmentosa compared to baseline, as assessed by electroretinography, visual field teasing, and/or optical coherence tomography.

5 In one embodiment, a method of treating choroidal neovascularization in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of choroidal neovascularization; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.

10 In another embodiment, a method for treating a human patient who has been determined to have choroidal neovascularization is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the choroidal neovascularization.

15 In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma). Exemplary symptoms of choroidal neovascularization include, but are not limited to, an acute decrease in visual acuity, relative scotoma, and/or metamorphopsia.

20 In some embodiments, the treatment results in an improvement in one or more symptoms of choroidal neovascularization compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of choroidal neovascularization compared
25 to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

30 In some embodiments, the patient having choroidal neovascularization maintains vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the

patient maintains vision, as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “maintaining vision” is defined as losing 15 letters of visual acuity (*e.g.*, at 12 months compared to baseline).

5 In some embodiments, the treatment results in an improvement in vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method) in a patient having choroidal neovascularization. In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more
10 letters (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 12 months compared to baseline).

In one embodiment, a method of treating central retinal vein occlusion in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of
15 central retinal vein occlusion; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have central retinal vein occlusion is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with
20 a frequency sufficient to treat the central retinal vein occlusion.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of central retinal vein occlusion. Exemplary symptoms of central retinal vein occlusion include, but are not limited to blurry or distorted vision, transient visual obscurations, pain, redness, irritation and/or other problems.

25 In some embodiments, the treatment results in an improvement in one or more symptoms of central retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of central retinal vein occlusion compared

to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In one embodiment, a method of treating macular edema following retinal vein occlusion in a human subject is provided, wherein the method comprises: (a) selecting a human subject in
5 need of treatment of macular edema following retinal vein occlusion; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have macular edema following retinal vein occlusion is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically
10 acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the macular edema following retinal vein occlusion.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of macular edema following retinal vein occlusion. Exemplary symptoms of macular edema following retinal vein occlusion include, but are not
15 limited to, the sudden appearance of floaters or severe loss of vision.

In some embodiments, the treatment results in an improvement in one or more symptoms of macular edema following retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%,
20 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of macular edema following retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In one embodiment, a method of treating cystoid macular edema in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of
25 cystoid macular edema; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have cystoid macular edema is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency
30 sufficient to treat the cystoid macular edema.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of cystoid macular edema include. Exemplary symptoms of cystoid macular edema include, but are not limited to, a decrease in visual acuity that is associated with retinal edema, loss of contrast sensitivity and color vision, metamorphopsia (*e.g.*,
5 that can be demonstrated on Amsler grid), micropsia, and central scotoma.

In some embodiments, the treatment results in an improvement in one or more symptoms of cystoid macular edema compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
10 95%, or 100% improvement of one or more symptoms of cystoid macular edema compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In one embodiment, a method of treating polypoidal choroidal vasculopathy in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need of
15 treatment of polypoidal choroidal vasculopathy; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have polypoidal choroidal vasculopathy is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester
20 thereof, in an amount and with a frequency sufficient to treat the polypoidal choroidal vasculopathy.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of polypoidal choroidal vasculopathy. Exemplary symptoms of polypoidal choroidal vasculopathy include, but are not limited to blurred vision or a
25 blind spot in (or near) the center of vision in one or both eyes. These symptoms may appear suddenly and tend not to vary throughout the day.

In some embodiments, the treatment results in an improvement in one or more symptoms of polypoidal choroidal vasculopathy compared to baseline, *e.g.*, as assessed by indocyanine green angiography (ICGA). In some embodiment, the treatment results in an at least 25%, 30%,
30 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%

improvement of one or more symptoms of polypoidal choroidal vasculopathy compared to baseline, *e.g.*, ICGA.

In one embodiment, a method of treating ocular von Hippel Lindau Disease lesions in a human subject is provided, wherein the method comprises: (a) selecting a human subject in need
5 of treatment of ocular von Hippel Lindau Disease lesions; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient who has been determined to have ocular von Hippel Lindau Disease lesions is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt,
10 solvate, or ester thereof, in an amount and with a frequency sufficient to treat the ocular von Hippel Lindau Disease lesions.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of Ocular von Hippel Lindau Disease.

In one embodiment, a method of treating retinopathy of prematurity in a human subject
15 (*e.g.*, a pediatric subject) is provided, wherein the method comprises: (a) selecting a human subject in need of treatment of retinopathy of prematurity; and (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof. In another embodiment, a method for treating a human patient (*e.g.*, a pediatric subject) who has been determined to have retinopathy of prematurity is provided, the
20 method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the retinopathy of prematurity.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of retinopathy of prematurity. Exemplary symptoms of
25 retinopathy of prematurity include, but are not limited to abnormal blood vessels grow in the retina in babies who are premature or who weigh less than 3 pounds at birth.

In some embodiments, the treatment results in an improvement in one or more symptoms of retinopathy of prematurity compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results
30 in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,

95%, or 100% improvement of one or more symptoms of retinopathy of prematurity compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

Gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, can be administered (1) alone or (2) in combination with one or more therapies or therapeutic agents. For example, gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, can be administered prior to, after, or concurrently with one or more therapies or therapeutic agents suitable for treatment of an ocular disease or disorder, non-limiting examples of which are provided herein.

Further provided are kits that include a dose of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, adapted for use in the methods described herein, *e.g.*, for effective treatment of an ocular disease, such as diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, neovascular glaucoma, polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa. In one embodiment, the kit comprises: (a) a dose of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, and (b) instructions for using gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in the methods described herein.

Also provided are uses of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, for treatment of an ocular disease wherein gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient in an amount and with a frequency to treat the ocular disease, such as neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, neovascular glaucoma, polypoidal choroidal

vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.

BRIEF DESCRIPTION OF THE DRAWINGS

5 **FIGS. 1A-1J** demonstrate the analysis process with two images utilized in the laser choroid neovascularization (LCVN) image intensity analysis. Specifically, **FIG. 1A** is the original vehicle-treated image. **FIG. 1B** is the original 5 mg/kg dose administered two times gedatolisib-treated image. **FIG. 1C** is the green channel (8 bit 0 to 256) threshold analysis for the vehicle-treated image. **FIG. 1D** is the green channel (8 bit 0 to 256) threshold analysis for
10 the 5 mg/kg gedatolisib-treated image. **FIG. 1E** is the green channel (8 bit 0 to 125) visual inspection only for the vehicle-treated image. **FIG. 1F** is the green channel (8 bit 0 to 125) visual inspection only for the 5 mg/kg gedatolisib-treated image. **FIG. 1G** is the triangle thresholded image for the vehicle-treated image. **FIG. 1H** is the triangle thresholded image for the 5 mg/kg gedatolisib-treated image. **FIG. 1I** is the particle analysis (500 infinity pixels –
15 intensity taken from largest blob) for the vehicle-treated image. **FIG. 1J** is the particle analysis (500 infinity pixels – intensity taken from largest blob) for the 5 mg/kg gedatolisib-treated image.

FIG. 2 is a graph which plots choroid neovascularization (CNV) area data in square microns for each image at different concentrations of gedatolisib for each lesion in the test.

20 **FIGS. 3A-3D** depict representative single LCNV lesion images at intravenously administered dosages of vehicle (**FIG. 3A**), gedatolisib at a 1.5 mg/kg dose administered two times (**FIG. 3B**), gedatolisib at a 5 mg/kg dose administered two times (**FIG. 3C**) or gedatolisib at a 15 mg/kg dose administered two times (**FIG. 3D**).

FIG. 4 is a graph which depicts CNV lesion area for the vehicle, 1.5 mg/kg dose x2, a 5
25 mg/kg dose administered two times, and 15 mg/kg dose administered two times. Statistical p-values are listed above the brackets at the top of the bars for demonstrating the differences between doses.

FIG. 5 is a graph which depicts VEGF concentration for the vehicle, 1.5 mg/kg dose administered two times, a 5 mg/kg dose administered two times, and 15 mg/kg dose administered
30 two times, as assessed by ELISA.

FIG. 6 is a graph which depicts VEGF concentration adjusted for total protein for the vehicle, 1.5 mg/kg dose administered two times, a 5 mg/kg dose administered two times, and 15 mg/kg dose administered two times, as assessed by ELISA.

FIG. 7 provides summary data for quantification of gedatolisib concentration found in total Brown Norway rat whole eye following two doses injected intravenously at the indicated concentrations (mg/Kg), at each timepoint noted on the x-axis, as measured by mass spectroscopy.

FIGS. 8A-8B is a graph that depicts the linearity of dosing for three concentrations measured on Days 0, 4 and 8 post intravenous injection of two doses at the concentrations indicated in Brown Norway Rats (BNR).

FIG. 9 shows a comparison of Day 4 versus Day 8 dose versus measured concentration graphs for two dose intravenous administration of gedatolisib.

FIGS. 10A-10B show the distribution to ocular compartments and tissue pharmacokinetic data with ^{14}C -labeled gedatolisib following a single intravenous administered gedatolisib 5mg/Kg dose in Long Evans rats in the whole eye (**FIG. 10A**) and uveal tract (**FIG. 10B**).

FIG. 11 depicts the results of an impedance test of vascular endothelial cell response to added cytokines.

FIG. 12 depicts the results of an impedance test of retinal endothelial cells response to VEGF and gedatolisib.

FIG. 13 is a schematic diagram of the anatomy of the eye.

FIG. 14 depicts the results for lesion size in an L-CNV suppression model treated with 5 mg/kg of gedatolisib twice prior (4 days prior and 3 days prior) to laser treatment or once before (4 hours) and once after (20 hours) laser treatment.

FIGS. 15A-15B depicts the results for lesion size in an established NV regression model treated with 2 mg/kg or 5 mg/kg of gedatolisib administered 6 days after laser treatment, when NV was near its peak. **FIG. 15A** shows results for lesion size (in μm^2) as measured by qFA over the 15-day experimental study. **FIG. 15B** shows results for lesion size (in μm^2) as measured by IB4 retinal flatmounts as measured on day 15.

FIG. 16A depicts results showing that at a dose of 2 mg/kg and 12 mg/kg, a statistically significant reduction in lesion area as detected by IB4 flat mount staining is demonstrated by the administration of gedatolisib. Statistical p-values demonstrating differences between doses are indicated by the brackets above the bars in the chart.

5 **FIG. 16B** depicts results showing that a typical sigmoid dose response is demonstrated for intravenous administration of gedatolisib for reduction in lesion area as detected by IB4 flat mount staining. An IC_{50} for total acute administration of 8 mg/kg gedatolisib and IC_{80} of 10 mg/kg is estimated from the sigmoid dose response data.

10 **FIG. 17** depicts results showing that at a dose of 12 mg/kg, gedatolisib administered intramuscularly employing depot method demonstrates reduction of LCNV area as detected by IB4 flat mount staining.

15 **FIG. 18** depicts results of an analysis of LCNV area of individual raw data points (laser holes) from rat eyes treated with topically administered gedatolisib, showing a ~40% reduction in mean LCNV area (vehicle arm = 0.026657 mm³; gedatolisib arm = 0.016118 mm³; p -value = 0.001166), when outlier data points were excluded.

DETAILED DESCRIPTION

20 The following detailed description is provided to aid those skilled in the art in practicing the present invention. Exemplary embodiments will hereinafter be described in detail. However, these embodiments are only exemplary, and the present disclosure is not limited thereto but rather is defined by the scope of the appended claims. Those of ordinary skill in the art may make modifications and variations in the embodiments described herein without departing from the spirit or scope of the present disclosure.

25 Disclosed herein are methods for treating ocular diseases (*e.g.*, neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, neovascular glaucoma,
30 polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease

lesions, diabetic retinopathy, and retinitis pigmentosa) by administering a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, either alone or in combination with other therapies or agents to a patient in need thereof.

5 In order that the present description may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

10 As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. The use of "or" or "and" means "and/or" unless stated otherwise.

The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration and the like, encompasses variations of up to $\pm 10\%$ from the specified value. Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, etc., used herein are to be understood as being modified by the term "about".

15 As used herein, the term "subject" includes any human or non-human animal. For example, the methods and compositions described herein can be used to treat a subject (*e.g.*, a human patient) having an ocular disease.

20 The term "intraocular" as used herein refers to inside or within the eye.

Various aspects described herein are described in further detail in the following subsections.

I. Ocular Diseases

25 A schematic diagram of the anatomy of the eye is shown in **FIG. 13** A non-limiting list of exemplary ocular diseases, primary eye location and main dysfunction are summarized below in Table 1.

30

Table 1: Exemplary Ocular Diseases

<u>Disease</u>	<u>Eye Location</u>	<u>Primary Dysfunction</u>
Wet AMD	Macula	Neovascularization of macula
Dry AMD	Macula	Deposition of drusen, leading to retinal dysfunction
Diabetic macular edema	Retina in macular area	Accumulation of excess fluid, leading to pressure that reduces vision
Diabetic retinopathy	Retina	Retinal neovascularization of macula
Retinitis pigmentosa	Retina	Loss of vision
Glaucoma (e.g., neovascular)	Optic nerve	Intraocular pressure buildup
Choroidal neovascularization	Choroidal vasculature	Neovascularization
Central retinal vein occlusion	Central retinal vein	Distorted vision
Macular edema following retinal vein occlusion	Macula	Accumulation of excess fluid, leading to pressure that reduces vision
Cystoid macular edema	Retina in macular area	Accumulation of excess fluid, leading to pressure that reduces vision
Polypoidal choroidal vasculopathy	Vascular layers in choroid	Distorted vision
Retinopathy of prematurity	Retina	Neovascularization
Ocular von Hippel Lindau disease lesions	Retina	Retinal hemangioblastoma

Non-limiting examples of ocular diseases are described in further detail below.

A. Neovascular Age-Related Macular Degeneration and Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is an eye disease of the macula that blurs the central vision. Loss of central vision occurs when aging causes damage to the cells within the macula, *i.e.*, in the part of the eye containing rods and cones that enables sharp vision directly out the front of the eye (*i.e.* not peripheral vision). The macula is described by an area within the central portion of vision (the light- and color-sensitive tissue at the back of the eye) where the retina and choroid describe layers of cells in front of (anterior) and behind the Bruch’s membrane (posterior), respectively, that extends around the back of the eye beyond the macula area (see **FIG. 13**).

There are two types of AMD, the “wet” (neovascular) and “dry” forms. Both forms have an effect on the macula (or central area of vision). The substantive difference between neovascular AMD and dry AMD is that the neovascular form has vascularization of the macula that arises from choroidal layer vasculature growing into an area of the retina that is generally less vascularized than surrounding regions of the eye.

Dry AMD accounts for 85-90% of existing AMD cases in the USA. Dry AMD is characterized most frequently by the deposition of acellular, polymorphous debris called “drusen,” consisting of esterified cholesterol, phosphatidylcholine, and proteins, between the retinal pigment epithelium (RPE) and Bruch’s membrane (BM). Excessive drusen deposition and other metabolic dysfunction may damage the RPE, BM, photoreceptors, retina (comprehensively also known as geographic atrophy which can lead to loss of vision) and even affect the choroid vasculature. Dry AMD is characterized by thinning of the macula and loss of function of RPE, photoreceptor (rods and cones) (so called Geographic Atrophy or GA) and other cell types critical to functional vision. Dry AMD has been characterized by hypoxia, increased expression of stress-related cytokines such as those driving angiogenesis (eg. increase in VEGF, ANG2, PDGF in the central portion of the macula and loss of PEDF from the central portion of the macula), dysfunctional glucose metabolism, and consequent decrease in pH. These factors contribute to an environment that is stressful to the RPE, rods and cones, leading to their deterioration. Dry AMD is often described as a precursor to wet AMD (though this is not firmly established) and cycles of neovascular growth, scarring cycles with “dry” presentations are described.

Dry AMD can progress to the less prevalent and more severe neovascular form of AMD, characterized by growth of abnormal choroidal blood vessels within the sub-RPE and sub-retinal space. The dysfunctional choroidal vessels are comprised of aberrant endothelial cells, fragile, and prone to leaking or bursting, which can lead to fluid buildup (edema) and retinal fibrotic scarring from iterative neovascularization and regression. A variant of neovascular AMD involves choroidal neovascularization (CNV), which is responsible for eventual severe vision loss in 80-90% of AMD patients. About 200,000 new cases of wet AMD are diagnosed each year in North America. Due to the aging baby boomer population and dramatically increasing “blue

light” damage, the National Eye Institute estimates that the prevalence of wet AMD will continue to grow.

Neovascular AMD is typically diagnosed by eye dilation and visual examination, optical coherence tomography (OCT), or fluorescein angiography. Contemporary methods do not always require eye dilation. Symptoms of neovascular AMD include, but are not limited to: visual distortions (such as straight lines seeming bent), reduced central vision in one or both eyes, the need for brighter light when reading or doing close-up work, difficulty adjusting to low light levels (such as when entering a dimly lit restaurant or theater), increased blurriness of printed words, difficulty recognizing faces, and/or a well-defined blurry spot or blind spot in the field of vision.

Available treatments for neovascular AMD include anti-VEGF drugs, which helps reduce the number of abnormal blood vessels in the retina and slows any leaking from blood vessels. This medicine is typically delivered to the eye through a very slender needle. Laser surgery can also be used to treat neovascular AMD.

As of 2023, there is only one approved treatment for GA or dry AMD, pegcetacoplan, which is designed to bind to and inhibit convertases, a component of the complement system. This treatment reportedly only slows the eventual progression of lesion growth. This medicine is typically delivered to the eye through a very slender needle.

B. Diabetic Retinopathy

People with diabetes can have an eye disease called diabetic retinopathy. This occurs when high blood sugar levels cause damage to blood vessels in the retina. These blood vessels can swell and leak or they can close, stopping blood from passing through. Sometimes abnormal, new blood vessels grow on the retina. All of these changes adversely impact vision.

There are two types of diabetic retinopathy. The first one is non-proliferative diabetic retinopathy (NPDR) and is the early stage of diabetic eye disease. With NPDR, tiny blood vessels leak, making the retina swell. When the macula swells, it is called macular edema. In addition, with NPDR, blood vessels in the retina can close off and prevent blood from reaching the macula. This is called macular ischemia. Sometimes tiny particles called exudates can form in the retina.

The second type of diabetic retinopathy is proliferative diabetic retinopathy (PDR). PDR is the more advanced stage of diabetic eye disease and occurs when the retina starts growing new blood vessels. This is called neovascularization. These fragile new vessels often bleed into the vitreous. A little bleeding can cause a few dark floaters, whereas a lot of bleeding can block all vision. These new blood vessels can form scar tissue, which can cause problems with the macula or lead to a detached retina. PDR can result in the loss of central and peripheral (side) vision.

Symptoms of diabetic retinopathy include, but are not limited to seeing floaters, having blurry vision, having vision that changes sometimes from blurry to clear, seeing blank or dark areas in the field of vision, having poor night vision, and noticing colors appear faded or washed out, and losing vision altogether. Diabetic retinopathy is typically diagnosed by eye dilation and visual examination, optical coherence tomography (OCT), or fluorescein angiography.

Available treatments for diabetic retinopathy including medications injected into the eye (e.g., VABYSMO® (faricimab-svoa), LUCENTIS® (ranibizumab), EYLEA® (aflibercept), or AVASTIN® (bevacizumab). These drugs are injected using topical anesthesia. The injections can cause mild discomfort, such as burning, tearing or pain, for 24 hours after the injection. Possible side effects include a buildup of pressure in the eye and infection. These injections need to be repeated. In some cases, the medication is used with photocoagulation. Photocoagulation (*i.e.*, focal laser treatment), can also be used to stop or slow the leakage of blood and fluid in the eye. Panretinal photocoagulation (*i.e.*, scatter laser treatment) can also be used to shrink the abnormal blood vessels. During this procedure, the areas of the retina away from the macula are treated with scattered laser burns. The burns cause the abnormal new blood vessels to shrink and scar. Another treatment option is a vitrectomy, wherein a tiny incision in the eye is made to remove blood from the middle of the eye (vitreous), as well as scar tissue that's tugging on the retina.

C. Diabetic Macular Edema

Diabetic macular edema (DME) refers the accumulation of excess fluid in the extracellular space within the retina in the macular area, typically in the inner nuclear, outer plexiform, Henle's fiber layer, and subretinal space (see, *e.g.*, Otani T, *et al.*, *American Journal of Ophthalmology*. 1999;127(6):688-693; and Yanoff M, *et al.*, *Survey of Ophthalmology*. 1984;28:505-511). DME can present with decreased visual acuity (VA), metamorphopsia,

changes in color perception, and difficulty reading, although it can also present asymptotically. For example, mild to extensive DME can be present without symptoms evident to the patient. Risk factors for DME include a longer duration of diabetes mellitus (DM), poor control of DM with elevated hemoglobin A1c (HbA1c), hypertension, hyperlipidemia, impaired renal function and the use of thiazolidinediones.

DME is suspected in patients with any level of diabetic retinopathy who present with blurred vision or metamorphopsias. A detailed history, including the approximate date of onset of diabetes, the use of insulin versus oral antihyperglycemic agents, and the quality of metabolic control (e.g., HbA1c level) should be elicited. Any associated medical problems (e.g., hypertension, hypercholesterolemia, renal disease, and thyroid disease) should be identified, along with a thorough review of medications.

Patients undergo a detailed biomicroscopic examination using the slit lamp biomicroscope and indirect ophthalmoscope. Historically, DME classifications were based on the ETDRS definitions of clinically significant macular edema (CSME). The specific criteria for diagnosing CSME were:

- (1) Retinal thickening at or within 500 μm of the center of the fovea;
- (2) Hard exudates at or within 500 μm of the center of the fovea if adjacent to an area of retinal thickening; and
- (3) Retinal thickening of at least 1 disc area any portion of which is within 1500 μm (approximately 1 disc diameter) from the center of the fovea.

CSME as defined by the Early Treatment of Diabetic Retinopathy Study (ETDRS) in the 1980s was a clinical diagnosis made by slit-lamp examination using a contact lens. While clinical examinations remain essential for the full evaluation of DME, Optical Coherence Tomography (OCT) is now routinely used to complement physical examination in the diagnosis of DME.

DME is typically treated in two ways. First, the underlying cause (e.g., is high blood sugar or high blood pressure) is addressed. In addition, therapeutic measures are employed to heal the retina. Such measures include, but are not limited to anti-VEGF medicines, focal-grid macular laser surgery, corticosteroids, and nonsteroidal anti-inflammatory drugs (NSAIDs).

D. Retinitis Pigmentosa

Retinitis Pigmentosa is an eye disease in which the back wall of the eye (retina) is damaged. It is a rare, inherited degenerative eye disease that causes severe vision impairment. Symptoms often begin in childhood. Symptoms include, but are not limited to, loss of night vision (photoreceptor rods), gradual loss of peripheral (side) vision (often referred to as “tunnel vision”), and difficulty seeing different colors (photoreceptor cones).

Retinitis Pigmentosa can be diagnosed and measured by genetic testing, electroretinography (which measures the electrical activity of the rods and cones in the retina, or how well the retina responds to controlled light patterns), visual field teasing, and/or optical coherence tomography.

There is currently no specific treatment for retinitis pigmentosa. However, protecting the eye’s retina by using UV sunglasses can help delay the start of symptoms. A retinal prosthesis (artificial retina) has been developed for individuals with very advanced disease and severe vision loss.

E. Glaucoma

Glaucoma is a chronic, progressive eye disease caused by damage to the optic nerve, which leads to visual field loss. One of the major risk factors is eye pressure or intraocular pressure (IOP). An abnormality in the eye’s drainage system can cause fluid to build up related to vascular disease and furthermore leading to excessive pressure that causes damage to the optic nerve (*i.e.*, the bundle of nerve fibers that connects the retina with the brain). This damage leads to loss of eyesight. An increase in the IOP physically distorts the delicate eye geometry that permits normal vision. The vision loss starts out in the edges of the visual field and slowly impacts the central vision. Once vision is lost, through physical destruction of different cell components that work together to provide normal vision, generally at advanced stages reports clearly suggest that vision cannot be recovered.

There are many different types of glaucoma. Open-angle glaucoma is the most common form of glaucoma and is caused by damage to the vasculature and more specifically to block the filter in the eye’s drainage canals or trabecular network. Angle-closure glaucoma is a type of glaucoma that is caused by a rapid blockage of the eye’s drainage canals due to a closed or narrow angle between the iris and cornea where the filter is located. Low-tension or normal-tension glaucoma is a type of glaucoma in which damage occurs to the optic nerve without eye

pressure exceeding its normal range. Congenital glaucoma is glaucoma that occurs in infants when there are incorrect or underdeveloped drainage canals in the eye during the prenatal period. Uveitic (inflammatory) glaucoma is glaucoma caused by autoimmune and inflammatory disorders. Neovascular glaucoma is glaucoma associated with poorly controlled diabetes and other conditions that damage the blood vessels in the body, including vasculature of the eye.

Symptoms of glaucoma include, but are not limited to: eye pain, eye pressure, headaches, rainbow-colored halos around lights, low vision, blurred vision, narrowed vision (tunnel vision), blind spots, nausea, vomiting, and red eyes.

Glaucoma can be diagnosed by a variety of different tests, including, but not limited to, a visual acuity test (*i.e.*, to measure how well a person can see at various distances), pupil dilation via eye drops that allow a close-up exam of the eye's optic nerve and retina, a visual field test to measure a person's side or peripheral vision, tonometry (*i.e.*, to determine the fluid pressure inside the eye), optic nerve imaging (*i.e.*, photographs of the optic nerve are taken to indicate areas of damage), gonioscopy (*i.e.*, wherein a lens is placed on the eye to look at the area called the drainage angle to determine whether the drain is open or closed, and if any damage has occurred), and/or pachymetry (*i.e.*, measurement of corneal thickness).

Glaucoma is typically treated with medications, laser therapy, and/or surgery. With respect to medications, there are many types of prescription eye drops that can be used to treat glaucoma. Some decrease fluids and increase drainage to improve eye pressure. However, since glaucoma is a lifelong condition, daily eye drops are required for life. You may have to apply them more than once a day.

F. Choroidal Neovascularization

Choroidal neovascularization ("CNV") is part of the spectrum of exudative age-related macular degeneration (AMD) that consists of an abnormal growth of vessels from the choroidal vasculature and can extend to the neurosensory retina through ruptures in the Bruch's membrane. CNV can also develop in a number of other conditions, such as myopic degeneration, chronic central serous chorioretinopathy, macular telangiectasia type 2, various white dot syndromes and other uveitic processes, some choroidal tumors, myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma.

Leakage of retinal edema and hemorrhage from CNV threatens visual acuity. Histologically, neovascular membranes of the eye are classified into three types. Type 1 ("occult"), when the neovascular membrane is located below the RPE. Type 1 CNV demonstrates occult leakage on fluorescein angiography. Polypoidal choroidal vasculopathy (PCV) is a subtype of Type 1 CNV that is characterized by the presence of polyp-like aneurysmal dilations of the branching vascular network. Type 2 ("classic"), passes through the retinal pigment epithelium (RPE) and is located above the RPE in the subretinal space. This is related to the angiographic classification of a classic CNV. Type 3 is defined as Retinal Angiomatous Proliferation (RAP), which corresponds to neovascularization that develops within the neurosensory retina and progresses posteriorly into the subretinal space. Patients with CNV experience an acute decrease in visual acuity, relative scotoma, and/or metamorphopsia. The retinal examination shows a grayish macular lesion associated with subretinal fluid, cystoid macular edema, exudation, and/or hemorrhages. Antiangiogenic therapy shows the best result both histologically with the regression of the neovascular lesion and functionally with improvement of the visual acuity.

15 G. Central Retinal Vein Occlusion

Central retinal vein occlusion (CRVO), is a condition in which the main vein that drains blood from the retina closes off partially or completely. Many patients with CRVO have symptom, such as blurry or distorted vision due to swelling of the center part of the retina, known as the macula. Some patients have mild symptoms that wax and wane, called transient visual obscurations. Patients with severe CRVO and secondary complications, such as glaucoma, often have pain, redness, irritation and other problems. CRVO is typically diagnosed based on medical signs (*e.g.*, a characteristic pattern of retinal hemorrhages) and patient reported symptoms.

In patients with CRVO, vascular endothelial growth factor (VEGF) is elevated, which leads to swelling as well as new vessels that are prone to bleeding. The most common treatment involves periodic injections into the eye of an anti-VEGF drug to reduce the new blood vessel growth and swelling. Although anti-VEGF drugs reduce the swelling, they are not a cure. As the drug leaves the eye and moves into the bloodstream, the effect in the eye wears off, so re-injection is often needed. Another treatment for CRVO is injections of intraocular steroid (*e.g.*, a liquid steroid called triamcinolone or a small steroid pellet called dexamethasone implant

(Ozurdex(R)). Such steroid injections typically last several months, but can cause elevated intraocular pressure requiring eye drops or increased rate of cataract formation.

There are two types of CRVO. Non-ischemic CRVO is the milder form that is characterized by leaky retinal vessels with macular edema. Ischemic CRVO is a more severe type with closed-off small retinal blood vessels. Patients with ischemic CRVO have worse vision with less chance for improvement. They have a tendency for the eye to cause new blood vessels to grow and in the front of the eye, these new vessels can clog the outflow of normal eye fluids. The eye pressure goes up and glaucoma can develop. In the back of the eye, new blood vessels can cause bleeding.

10 *H. Macular Edema Following Retinal Vein Occlusion*

Retinal vein occlusion is a blockage of the veins carrying blood away from the retina. This can lead to swelling and fluid leakage, which is typically associated with the sudden appearance of floaters or severe loss of vision. The result is an unwanted buildup of liquid at the back of the eye known as macular edema (“MEfRVO”). If left untreated, the condition can blur vision and eventually lead to blindness. The blood vessel leakage and swelling in the retina is caused by vascular endothelial growth factor (VEGF), which is released when blood flow is disrupted. Risk factors for developing MEfRVO include age (over 50), cardiovascular (heart) disease, being overweight or obese, diabetes, glaucoma, high blood pressure (particularly when uncontrolled), and smoking.

20 MEfRVO patients can receive injections into the eye with anti-VEGF drugs, which inhibit the abnormal growth of blood vessels and decrease leakage in the eye. Sometimes called Intravitreal therapy, these drugs have been proven effective to reduce swelling which improves vision, but they are not a cure. Laser therapy is another approach for managing macular edema, but it is used less often but can have a more permanent effect. A third, less common approach is intraocular injections of steroids. These are sometimes used in people where anti-VEGF drugs have been ineffective.

25 *I. Cystoid Macular Edema*

Cystoid Macular Edema (“CME”) refers to a retinal thickening of the macula due to a disruption of the normal blood-retinal barrier, which causes leakage from the perifoveal retinal capillaries and accumulation of fluid within the intracellular spaces of the retina, primarily

in the outer plexiform layer (see, *e.g.*, Gass JD, Norton EW *et al.*, *Trans Am Acad Ophthalmol Otolaryngol.* 1969;73:665-682). Visual loss occurs from retinal thickening and fluid collection that distorts the architecture of the photoreceptors. CME is a leading cause of central vision loss in the developed world (see, *e.g.*, Hogan P, Dall T, *et al.*, *Diabetes Care.* 2003;26:917-932).

- 5 Symptoms include a decrease in visual acuity that is associated with retinal edema, loss of contrast sensitivity and color vision, metamorphopsia that can be demonstrated on Amsler grid, micropsia, and central scotoma.

CME is diagnosed using a slit lamp or direct/indirect ophthalmoscopy. Clinically significant foveal edema and retinal thickening of more than 300 μm is viewed as a loss of foveal
10 reflex. This can be better visualized using green light to outline the cystic spaces. Subclinical foveal edema is described as edema less than 300 μm and is better seen through retinal imaging (see, *e.g.*, Staurengi G, *et al. Dev Ophthalmol.* 2010;47:27-48).

CME is typically treated by medical therapy (*e.g.*, NSAIDS, Corticosteroids, Carbonic anhydrase inhibitors (CAIs), anti-VEGF agents, pharmacologic vitreolysis agents (*e.g.*,
15 chondroitinase, dispase, hyaluronidase, plasmin, and microplasmin) or surgery.

J. Polypoidal Choroidal Vasculopathy

Polypoidal choroidal vasculopathy (PCV) is a disease primarily affecting the vascular layer of blood vessels in the choroid, resulting in damage to the overlying retina where the photoreceptor cells responsible for vision reside. Patients with PCV often experience blurred
20 vision or a blind spot in (or near) the center of their vision in one or both eyes. These symptoms may appear suddenly and tend not to vary throughout the day. PCV is typically diagnosed by indocyanine green angiography (ICGA).

PCV is characterized by abnormally shaped vessels in the choroid, but the precise causes of PCV is unknown. PCV tends to occur in individuals over the age of 60 (but may occur much
25 younger). It affects those of Asian and African descent more than Caucasians. PCV shares some clinical features with wet age-related macular degeneration. The abnormal vessels in PCV cause vision loss when they leak fluid or blood into or under the retina. The abnormal vessels in PCV may also cause scarring or loss (sometimes called atrophy) of retinal tissue. Although PCV appears to affect only one eye in some patients, it often goes on to affect both eyes over time, so
30 frequent monitoring is important.

Some patients with PCV experience irreversible central vision loss in one or both eyes. Early diagnosis and treatment, however, can restore vision and prevent further vision loss in some patients. The most common treatments for PCV are intravitreal (in-the-eye) injections of an anti-VEGF medication and photodynamic therapy (PDT). On rare occasions, surgical
5 vitrectomy (a procedure to remove the eye's vitreous gel) can be used to remove or displace a large hemorrhage caused by PCV.

K. Retinopathy of Prematurity

Retinopathy of prematurity ("ROP") is an eye disease that can happen in babies who are premature (born early) or who weigh less than 3 pounds at birth. ROP occurs when abnormal
10 blood vessels grow in the retina (the light-sensitive layer of tissue in the back of the eye). Some babies with ROP have mild cases and get better without treatment. In other cases, some babies need treatment to protect their vision and prevent blindness.

There are 5 different stages of ROP that physicians use to categorize and monitor the severity of the disease. The stages range from stage 1 (mild) to stage 5 (severe). Babies in Stages
15 1 and 2 usually get better without treatment and go on to have healthy vision. Some babies who develop Stage 3 get better with no treatment and go on to have healthy vision. However, other babies with Stage 3 need treatment to stop abnormal blood vessels from damaging the retina and causing retinal detachment (an eye problem that can cause vision loss). Doctors usually start treatment in Stage 3. Babies in stage 4 have partially detached retinas and need treatment. In
20 stage 5, the retina detaches completely. Even with treatment, babies in Stage 5 may have vision loss or blindness. Both stages 4 and 5 are very serious. Babies in these stages often need surgery. However, even with treatment, they may have vision loss.

There are no obvious symptoms of ROP that you can see. In advanced cases of ROP, the retina may partially or completely pull away from its normal position at the back of the eye.
25 This is called retinal detachment and it can cause vision loss and blindness. If a baby had ROP that caused damage, later on it may be evident that their eyes wander, shake, or make other unusual movements, their eyes don't follow objects, their pupils look white, and/or they have trouble recognizing faces. Babies who had ROP are also more likely to have other eye problems as they get older, including: retinal detachment, nearsightedness, amblyopia (lazy eye), crossed

eyes. Treatment options include laser treatment, injections (*e.g.*, of anti-VEGF drugs), or eye surgery.

L. Ocular von Hippel Lindau Disease Lesions

5 Von Hippel-Lindau Syndrome (“VHL”) is a rare, autosomal dominant, familial
neoplastic disease that affects the central nervous system and multiple organs such as the
kidneys, pancreas, adrenals, and reproductive organs. Mutations in the tumor suppressor gene
VHL cause the disease, which commonly manifests as a variety of tumors, such as
hemangioblastomas of the retina and brain as well as renal cell carcinoma. The disease affects
10 one in every 36,000 live births and shows near- complete penetrance (90%) by 65 years of age
(see *e.g.*, Benjamin C, *et al.* Clinical Features and Natural History of von Hippel-Lindau Disease.
Qjm. 2012;77(2):1151-1163). Diagnosis is confirmed with a positive familial history and at least
one VHL-related tumor (see, *e.g.*, Coppin L, *et al.*, Optimization of next-generation sequencing
technologies for von Hippel Lindau (VHL) mosaic mutation detection and development of
15 confirmation methods, The Journal of Molecular Diagnostics (2019)). However, 20% of
mutations are de novo, and diagnosis for patients with a negative family history is confirmed
with the occurrence of two VHL-related tumors and at least one retinal hemangioblastoma.
Retinal hemangioblastomas present the earliest around age 20, but are not present in every
diagnosed case of VHL (see, *e.g.*, Lonser RR, *et al.* von Hippel-Lindau disease. Lancet.
20 2003;361:2059-2067).

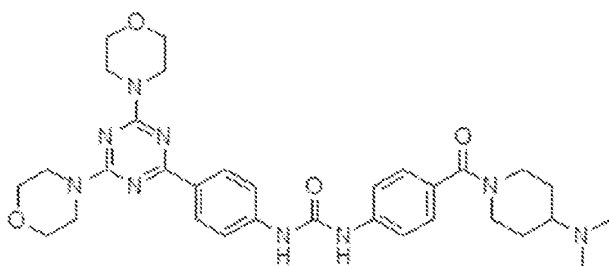
Retinal hemangioblastoma is best diagnosed with pharmacological dilation of pupil and
examination using an ophthalmoscope (see, *e.g.*, Lonser RR, *et al.* von Hippel-Lindau disease.
Lancet. 2003;361:2059-2067). Retinal hemangioblastomas treatment is not always necessary.
When it compromises vision, treatment options involve diathermy and cryocoagulation to reduce
25 the size of retinal angiomas. Laser photocoagulation has shown efficacy when tumors are
<1.5mm (see, *e.g.*, Singh AD, *et al.*, *Ophthalmology*. 2002;109(10):1799-1806). External beam
radiotherapy can be used for larger tumors (diameter >4mm) which tend to be resistant against
standard treatment. Tumors found near the optic disc can be treated with intravitreal anti-VEGF
therapy if necessary.

30

II. Gedatolisib

1-(4-{[4-(Dimethylamino)piperidin-1-yl]carbonyl}phenyl)-3-[4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl]urea (also known as gedatolisib) is a pan-class I isoform PI3K/mTOR inhibitor with high potency (NCT02626507, April 24, 2020). The chemical synthesis of gedatolisib is disclosed in U.S. Patent Nos. 8,039,469; 8,217,036; 8,445,486; 8,575,159; 8,748,421; 8,859,542; 9,174,963; 10,022,381, which are hereby incorporated by reference in their entirety. Gedatolisib may be prepared in crystalline form and is chemically and physically stable at 25°C and 60% Relative Humidity (RH) for up to 3 years in this form. As a free base gedatolisib is insufficiently water soluble. To allow the preparation of an aqueous solution formulation suitable for intravenous, topical, injectable intraocular, or other parenteral administration at the therapeutic dosage levels small amounts of acid are required. Accordingly, formulations that allow for therapeutic dosage levels have been developed.

1-(4-{[4-(Dimethylamino)piperidin-1-yl]carbonyl}phenyl)-3-[4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl]urea, has the chemical structure:



1-(4-{[4-(dimethylamino)piperidin-1-yl]
carbonyl}phenyl)-3-[4-(4,6-dimorpholin-4-yl-
1,3,5-triazin-2-yl)phenyl]urea

Gedatolisib is a small molecule, which inhibits Phosphatidylinositol-3 kinase and Mammalian Target of Rapamycin. Phosphatidylinositol-3 kinase (PI3K) is an enzyme that phosphorylates the 3-position of the inositol ring of phosphatidylinositol (D. Whitman et al., (1988)) to make a critical signaling phospholipid, PIP3. Pluralities of PI3K subtypes exist, with three major subtypes of PI3Ks having now been identified based on their *in vitro* substrate specificity. These three are designated class I (a & b), class II, and class III (B. Vanhaesebroeck, (1997)).

1-(4-{[4-(Dimethylamino)piperidin-1-yl]carbonyl}phenyl)-3-[4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl]urea is an inhibitor of PI3 kinase and mTOR that is useful for the treatment of cancer. Mammalian Target of Rapamycin (mTOR) is a cell-signaling protein that regulates the response of tumor cells to nutrients and growth factors, as well as controlling tumor blood supply through effects on Vascular Endothelial Growth Factor (VEGF). Some mTOR inhibitors like gedatolisib bind directly to the mTOR kinase. This has at least two important effects. First, mTOR is a downstream mediator of the PI3K/Akt pathway, having both forward signaling properties and feedback properties on the pathway. The PI3K/Akt pathway is thought to be over activated in numerous cancers and may account for the widespread response from various cancers to mTOR inhibitors. The PI3K/Akt pathway is demonstrated to be over activated in numerous metabolic diseases as well, consuming increased levels of oxygen and glucose chronically and furthermore creating a state of metabolic dysfunction within the disease tissue such as exemplified by increased cytokine production. The over-activation of signaling nodes upstream in the PI3K/Akt pathway would normally cause mTOR kinase to be over activated as well. However, in the presence of mTOR inhibitors, this process is blocked. The blocking effect prevents mTOR from signaling to downstream pathways that control critical cell functions such as reducing protein translation, glucose consumption, and ultimately affect diseased cell growth and proliferation. One of the major effects of PI3K/Akt pathway inhibition by reducing protein translation is reducing cytokine production and anti-angiogenesis, via the lowering of the production of VEGF and other angiogenic protein production levels (e.g., Ang2, FGF, PDGF).

As used herein the terms “gedatolisib” and “1-(4-{[4-(Dimethylamino)piperidin-1-yl]carbonyl}phenyl)-3-[4-(4,6-dimorpholin-4-yl-1,3,5-triazin-2-yl)phenyl]urea” refer to the same compound and may be used interchangeably. In some embodiment of the present invention pharmaceutically acceptable salts, solvates or esters of gedatolisib, as would be known to those of skill in the art, may be used in the methods described herein.

Representative “pharmaceutically acceptable salts” include but are not limited to, e.g., water-soluble and water-insoluble salts or their acid forms, such as the acetate, aluminum, amsonate (4,4-diaminostilbene-2,2-disulfonate), benzathine (N,N'-dibenzylethylenediamine), benzenesulfonate, benzoate, bicarbonate, bismuth, bisulfate, bitartrate, borate, bromide, butyrate, calcium, calcium edetate, camsylate (camphorsulfonate), carbonate, chloride, choline, citrate,

clavulariate, diethanolamine, dihydrochloride, diphosphate, edetate, edisylate (camphorsulfonate), esylate (ethanesulfonate), ethylenediamine, fumarate, gluceptate (glucoheptonate), gluconate, glucuronate, glutamate, hexafluorophosphate, hexylresorcinate, hydrabamine(N,N'-bis(dehydroabietyl)ethylenediamine), hydrobromide, hydrochloride, 5 hydroxynaphthoate, 1-hydroxy-2-naphthoate, 3-hydroxy-2-naphthoate, iodide, isothionate (2-hydroxyethanesulfonate), lactate, lactobionate, laurate, lauryl sulfate, lithium, magnesium, malate, maleate, mandelate, meglumine (1-deoxy-1-(methylamino)-D-glucitol), mesylate, methyl bromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, palmitate, pamoate (4,4'-methylenebis-3-hydroxy-2-naphthoate, 10 or embonate), pantothenate, phosphate, picrate, polygalacturonate, potassium, propionate, p-toluenesulfonate, salicylate, sodium, stearate, subacetate, succinate, sulfate, sulfosalicylate, suramate, tannate, tartrate, teoclate (8-chloro-3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione), trieth iodide, tromethamine(2-amino-2-(hydroxymethyl)-1,3-propanediol), valerate, and zinc salts.

15 Pharmaceutically acceptable esters include, but are not limited to, alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl and heterocyclyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids, and boronic acids.

20 Pharmaceutical acceptable solvates and hydrates are complexes of a compound with one or more solvent or water molecules, or 1 to about 100, or 1 to about 10, or one to about 2, 3 or 4, solvent or water molecules.

Pharmaceutical formulations comprising therapeutic dosage levels of gedatolisib are known in the art and include aqueous intravenous formulations, as well as nanoparticle formulations.

25 PCT application publication WO2016/097949 discloses aqueous intravenous formulations of gedatolisib with lactic acid and/or orthophosphoric acid, which form clear, particulate free solutions. The formulations include gedatolisib, lactic acid, and water. The gedatolisib has a concentration in the solution less than 6mg/ml (preferably about 5mg/ml), and there is sufficient lactic acid present to provide a clear solution (preferably at least 2.5 mole 30 equivalents). The gedatolisib forms a 1:1 (mole equivalent) lactate salt with lactic acid.

Therefore, the formulations can be prepared using the gedatolisib free base or using a lactic acid salt of gedatolisib.

The formulations with orthophosphoric acid include gedatolisib, orthophosphoric acid, and water. The gedatolisib is present at a solution concentration of less than 4 mg/ml (preferably from 3.0 to 3.5mg/ml) and sufficient orthophosphoric acid is present to provide a clear solution (preferably at least 5 mole equivalents).

Formulations including gedatolisib and cyclodextrins are disclosed in PCT Application Publication WO 2019/234632. The pharmaceutical aqueous formulations include gedatolisib, or a pharmaceutically acceptable organic or inorganic acid salt thereof, a pharmaceutically acceptable organic or inorganic acid, which is not a sulphonic acid, a pharmaceutically acceptable beta- or gamma-cyclodextrin and water. In an embodiment, the beta-cyclodextrin is (2-Hydroxypropyl)- β -cyclodextrin (CAS number 128446-35-5, EC Number 420-920-1, also sometimes known as Cavitron). The gedatolisib is present at a solution concentration of at least 6 mg/ml and the solutions are clear.

The pharmaceutically acceptable organic acid used (including for a salt thereof) are lactic acid, tartaric acid, malic acid, citric acid, succinic acid, acetic acid or maleic acid. The acid may be used in its racemic form, or as a single stereoisomeric form (or mixtures thereof), where applicable. Examples of a pharmaceutically acceptable beta-cyclodextrin are 2-hydroxypropyl-beta-cyclodextrin and sulphobutylether- β -cyclodextrin (SBECD). Examples of such a pharmaceutically acceptable gamma-cyclodextrin are gamma-cyclodextrin and 2-hydroxypropyl-gamma-cyclodextrin. The non-limiting exemplary amount of pharmaceutically acceptable beta- or gamma-cyclodextrin for use in the formulations is from 2 to 30% w/v.

Formulations including gedatolisib and methanesulphonic acid and/or ethanesulphonic acid are disclosed in PCT application publication WO2019/038657. The formulations include gedatolisib, or a methanesulphonate salt thereof, methanesulphonic acid, and water. The gedatolisib is present at a solution concentration of less than 35mg/ml or up to 30mg/ml (preferably from 6 to 30mg/ml) and sufficient methanesulphonic acid is present to provide a clear solution. Another formulation disclosed is gedatolisib, or an ethanesulphonate salt thereof, ethanesulphonic acid and water. The gedatolisib is present at a solution concentration of less than

35mg/ml or up to 30mg/ml (preferably from 6 to 30mg/ml) and sufficient ethanesulphonic acid is present to provide a clear solution.

The use of methanesulphonic acid and ethanesulphonic acid enables a solution concentration of up to 30mg/ml of gedatolisib to be achieved for a pharmaceutical aqueous solution formulation that is suitable for intravenous or parenteral administration to a patient, i.e. a clear, essentially particle-free solution.

Any of the above-mentioned formulations may be freeze-dried to provide a lyophilized solid composition, a bulking agent may be added to the formulation prior to the freeze-drying process commencing. A bulking agent may not be present if the formulation of the invention contains a pharmaceutically acceptable beta- or gamma-cyclodextrin. The primary function of the bulking agent is to provide the freeze-dried solid with a non-collapsible, structural integrity that will allow rapid reconstitution on constitution of the aqueous formulation prior to administration, and it should also facilitate efficient lyophilization. Bulking agents are typically used when the total mass of solutes in the formulation is less than 2g/100ml. Bulking agents may also be added to achieve isotonicity with blood. The bulking agent may be selected from a saccharide, sugar alcohol, amino acid or polymer, or be a mixture of two or more of any thereof. Preferably, the bulking agent is a sugar or sugar alcohol, or a mixture thereof. Preferably, the sugar is sucrose. Preferably, the sugar alcohol is mannitol. Constitution of the lyophilized solid composition may be achieved using an appropriate quantity of water and/or an aqueous solution of a suitable tonicity modifier in order to ensure that a clear solution is obtained.

Therapeutic agents containing at least one basic nitrogen atom (*i.e.*, protonatable nitrogen-containing therapeutic agents), such as gedatolisib, represent an important group of therapeutic agents. However, nanoparticle formulations of this class of drugs are often hindered by undesirable properties, e.g., unfavorable burst release profiles and poor drug loading. PCT application publication WO2015/138835 discloses therapeutic nanoparticles of gedatolisib which have a controlled release rate of the therapeutic agent.

The therapeutic nanoparticles include gedatolisib (preferably in an amount of about 1 to 20 weight percent), a substantially hydrophobic acid, and a polymer selected from diblock poly(lactic) acid-poly(ethylene)glycol copolymer or a diblock poly(lactic acid-co-glycolic acid)-poly(ethylene)glycol copolymer, and combination thereof. The molar ratio of the substantially

hydrophobic acid to the gedatolisib ranges from about 0.25:1 to about 2:1 and the pK_a of the protonated gedatolisib is at least about 1.0 pK_a units greater than the pK_a of the hydrophobic acid. The hydrophobic acid and the gedatolisib form a hydrophobic ion pair in the therapeutic nanoparticle. Additionally, the nanoparticles can include a targeting ligand, which may increase target binding (cell binding/target uptake), making the nanoparticle target specific.

In general, a “nanoparticle” refers to any particle having a diameter of less than 1000 nm. Preferably, the therapeutic nanoparticles may have a diameter ranging from 60 to 120 nm. For example, the nanoparticle may have a diameter ranging from about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, or about 110 nm, up to about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 110 nm, or about 120 nm.

As used herein, the “substantially hydrophobic acid” is an acid which has a pK_a in water of about -1.0 to about 5.0. Preferably, the substantially hydrophobic acid has a pK_a in water of about 2.0 to about 5.0. Exemplary substantially hydrophobic acids include, but are not limited to, fatty acids. For example, the fatty acid may be a saturated fatty acid, including, but not limited to, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecanoic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, nonadecylic acid, arachidic acid, heneicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, heptacosylic acid, montanic acid, nonacosylic acid, melissic acid, henatriacontylic acid, lacceroic acid, psyllic acid, geddic acid, ceroplastic acid, hexatriacontylic acid, or combinations thereof. Additionally, the fatty acid may be a omega-3 fatty acid, including, but not limited to, hexadecatrienoic acid, alpha-linolenic acid, stearidonic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid, heneicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, tetracosapentaenoic acid, tetracosahexaenoic acid, or combinations thereof. The fatty acid may also be an omega-6 fatty acid, including, but not limited to, linoleic acid, gamma-linolenic acid, eicosadienoic acid, dihomo-gamma-linolenic acid, arachidonic acid, docosadienoic acid, adrenic acid, docosapentaenoic acid, tetracosatetraenoic acid, tetracosapentaenoic acid, or combinations thereof. The fatty acid may also be an omega-9 fatty acid, including, but not limited to, oleic acid, eicosenoic acid, mead acid, erucic acid, nervonic acid, or combinations thereof. The fatty acid may also be a polyunsaturated fatty acid, including, but not limited, rumenic acid, a-calendic

acid, β -calendic acid, jacaric acid, α -eleostearic acid, β -eleostearic acid, catalpic acid, punicic acid, rumelenic acid, α -parinaric acid, β -parinaric acid, bosscopentacnoic acid, pinolenic acid, podocarpic acid, or combinations thereof.

Alternatively, the hydrophobic acid can be a bile acid. For example, in some
5 embodiments, the bile acid includes but is not limited to, chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid, hycholic acid, beta-muricholic acid, cholic acid, lithocholic acid, an amino acid-conjugated bile acid, or combinations thereof.

Alternatively, the hydrophobic acid may include but is not limited to, dioctyl
sulfosuccinic acid, 1-hydroxy-2-naphthoic acid, dodecylsulfuric acid, naphthalene- 1,5-
10 disulfonic acid, naphthalene-2-sulfonic acid, pamoic acid, undecanoic acid, or combinations thereof.

The nanoparticles may be combined with pharmaceutically acceptable carriers to form a pharmaceutical composition. As would be appreciated by one of skill in this art, the carriers may be chosen based on the route of administration, the location of the target issue, the time course of
15 delivery of the drug, etc.

The pharmaceutical nanoparticle compositions can be administered to a patient or subject by any means known in the art including oral and parenteral routes. The nanoparticle compositions may be administered by injection (*e.g.*, intravenous, subcutaneous or intramuscular, intraperitoneal injection), rectally, vaginally, topically (as by powders, creams,
20 ointments, or drops), or by inhalation (as by sprays).

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension, or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as
25 a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables. In one embodiment, the
30 inventive conjugate is suspended in a carrier fluid comprising 1 % (w/v) sodium carboxymethyl

cellulose and 0.1% (v/v) TweenTM 80. The injectable formulations can be sterilized, for example, by filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

5 Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the encapsulated or unencapsulated conjugate is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders such as, for example, carboxymethylcellulose, alginates,
10 gelatin, polyvinylpyrrolidinone, sucrose, and acacia, (c) humectants such as glycerol, (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, (e) solution retarding agents such as paraffin, (f) absorption accelerators such as quaternary ammonium compounds, (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, (h) absorbents such as kaolin and
15 bentonite clay, and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets, and pills, the dosage form may also comprise buffering agents.

Aqueous pharmaceutical formulations of gedatolisib, such as those described above, that are suitable for intravenous administration generally have a pH of from 3 to 9. However, lower
20 pH values are tolerated in certain settings. The pH may range from about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or 8 up to about 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, or 9. Preferably, the pH is from 3 to 8 or from 4 to 8.

III. Formulations

25 In some embodiments, the gedatolisib used in the method of the present application can be formulated with one or more pharmaceutically acceptable excipients to form pharmaceutical compositions, such as intravenous formulations. Non-limiting examples of gedatolisib formulations suitable for intravenous delivery include those described in Section II, such as those described in PCT Publications WO 2016/097949, WO 2019/234632 and WO 2019/038657.

The pharmaceutical compositions used in the methods disclosed herein may be specially formulated in solid, semisolid, or liquid form, including those adapted for parenteral administration, for example, by intravenous, topical, intraocular injection, subcutaneous, intratumoral or intramuscular injection or infusion as, for example, a sterile solution or
5 suspension.

In embodiments, the formulation is a topical formulation, i.e., suitable for topical administration. Topical formulations for ocular delivery include eye drops, eye gels and eye creams.

Gedatolisib is incorporated into the formulation (e.g., topical formulation) at a
10 concentration effective to deliver the desired amount to the eye. In embodiments, gedatolisib is incorporated into the formulation at a concentration of at least 0.1mg/mL or at least 0.5mg/mL, or at least 1mg/mL or at least 4 mg/ml, or at least 6 mg/ml or at least 10 mg/ml. In
embodiments, gedatolisib is incorporated into the formulation at a concentration range of 0.1-35
mg/ml, 0.01-10 mg/ml, 0.01-5 mg/ml, 0.01-4 mg/ml, 0.01-2 mg/ml, 0.01-1 mg/ml, 1-10 mg/ml,
15 1-5 mg/ml, 1-4 mg/ml, 1-2 mg/ml, 2-30 mg/ml, 2-25 mg/ml, 2-20 mg/ml, 2-15 mg/ml, 3-35
mg/ml, 3-30 mg/ml, 3-25 mg/ml, 3-20 mg/ml, 3-15 mg/ml, 4-35 mg/ml, 4-30 mg/ml, 4-25
mg/ml, 4-20 mg/ml, 4-15 mg/ml, 5-35 mg/ml, 5-30 mg/ml, 5-25 mg/ml, 5-20 mg/ml, 5-15
mg/ml, 6-35 mg/ml, 6-30 mg/ml, 6-25 mg/ml, 6-20 mg/ml or 6-15 mg/ml. In embodiments,
gedatolisib is incorporated into the formulation at a concentration of 0.01 mg/ml, 0.1mg/mL, 0.2
20 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1
mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml,
15 mg/ml, 18.5 mg/ml, 20 mg/ml, 25 mg/ml, 30 mg/ml or 35 mg/ml.

In a non-limiting exemplary embodiment, a topical formulation comprises gedatolisib at a
concentration in a range of about 0.01 ug/ul-4 ug/ml for delivery of a dosage of, for example 1 to
25 2 drops topically to the eye. At a concentration range of 0.01 ug/ul-4 ug/ul and a drop volume of
30-50 ul, this provides a dosage of 0.3-400 ug per administration.

In embodiments, a topical dosage of gedatolisib is delivered at least one a day, at least
twice a day, at least three times a day, at least four times a day or more. In embodiments, a
topical dosage of gedatolisib is delivered every other day, every third day, once per week, once

every two weeks, once a month, once every two months or by intermittent dosing, e.g., weekly or monthly.

In embodiments, a formulation includes at least one cyclodextrin (CD). In embodiments, the CD is a betaCD (β CD) or a gamma CD (γ CD). In embodiments, the CD is a hydroxypropyl
5 (HP) cyclodextrin, such as hydroxypropyl-beta-CD (HP β CD) or hydroxypropyl-gamma-CD (HP γ CD). In embodiments, the CD is sulphobutylether- β -cyclodextrin.

In embodiments, a formulation comprises at least one pharmaceutically acceptable organic acid, or salt thereof. Non-limiting examples of pharmaceutically acceptable organic acids include lactic acid, tartaric acid, malic acid, citric acid, succinic acid, acetic acid, maleic
10 acid and orthophosphoric acid. The acid may be used in its racemic form, or as a single stereoisomeric form (or mixtures thereof), where applicable. In embodiments, the pharmaceutically acceptable organic acid, or salt thereof, is lactic acid, or salt thereof. In embodiments, lactic acid is present in the formulation at a concentration in a range of 10-200 mM, 25-200 mM, 50-200 mM, 10-100 mM, 25-100 mM or 50-100 mM, or at a concentration of
15 50 mM or 100 mM.

Injectable formulations or formulations for infusion of the pharmaceutical compositions used in the methods disclosed herein may be prepared by known methods. For example, the injectable or infusible formulation may be prepared, e.g., by dissolving, suspending or emulsifying gedatolisib or its salt in a sterile aqueous medium or an oily medium conventionally
20 used for injections. As the aqueous medium for injections or infusions, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant (e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)),
25 etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injectable or infusible formulation thus prepared is preferably filled in an appropriate injection ampoule or in a vial or bag suitable for infusion.

A pharmaceutically acceptable excipient can be a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, carrier, manufacturing aid (e.g.,
30

lubricant, talc magnesium, calcium or zinc stearate, or steric acid), solvent or encapsulating material, involved in carrying or transporting the therapeutic compound for administration to the subject, bulking agent, salt, surfactant and/or a preservative. Some examples of materials which can serve as pharmaceutically acceptable excipients include: sugars, such as lactose, glucose and sucrose and various polymers thereof; cyclodextrins, starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; gelatin; talc; waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as ethylene glycol and propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents; water; isotonic saline; pH buffered solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

A bulking agent is a compound that adds mass to a pharmaceutical formulation and contributes to the physical structure of the formulation in lyophilized form. Suitable bulking agents according to the present invention include mannitol, glycine, polyethylene glycol and sorbitol.

The use of a surfactant can reduce aggregation of a reconstituted protein and/or reduce the formation of particulates in the reconstituted formulation. The amount of surfactant added is such that it reduces aggregation of the reconstituted protein and minimizes the formation of particulates after reconstitution. Suitable surfactants according to the present invention include polysorbates (e.g. polysorbates 20 or 80); poloxamers (e.g. poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g. Pluronics, PF68, etc.).

Preservatives may be used in formulations provided herein. Suitable preservatives for use in the formulation of the invention include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyl-dimethylammonium

chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol. Other suitable excipients can be found in standard pharmaceutical texts, e.g. in

5 "Remington's Pharmaceutical Sciences", The Science and Practice of Pharmacy, 19th Ed. Mack Publishing Company, Easton, Pa., (1995).

In some embodiments, the gedatolisib used in the methods disclosed herein may be lyophilized and provided in a composition for reconstitution prior to administration.

10 **IV. Methods of Treatment**

Provided herein are methods for treating ocular diseases (*e.g.*, neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies,

15 rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa) by administering a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or

20 ester thereof, either alone or in combination with other therapies or agents to a patient in need thereof. In embodiments, gedatolisib is delivered by a route of administration selected from the group consisting of intravenous injection, intraocular injection (including to the back or posterior of the eye, suprachoroidal, periocular), intravitreal injection, intravascular injection and topical delivery. In embodiments gedatolisib is delivered to the uveal tract, or choroid layer, or

25 intravitreal space, or retinal layer, or suprachoroid space, or periocular space, or combinations of places thereof depending on the ocular disease location(s).

A "therapeutically effective amount" means an amount of gedatolisib, or other active agent, set forth herein that, when administered to a subject, is effective in producing a therapeutic effect.

As used herein, the terms “treatment,” “treating,” “treat”, or the like, mean to alleviate or reduce the severity of at least one symptom or indication, to eliminate the causation of symptoms either on a temporary or permanent basis, or to obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; 5 diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting 10 a clinically significant response without excessive levels of side effects.

As used herein, “administering” refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Administering also refers to the physical introduction of a composition comprising a therapeutic agent to a subject’s eye at the specific sublocation 15 within the eye (for nonlimiting examples, posterior, anterior, intravitreal) required to attain efficacy against the disease of interest. Preferred routes of administration for the therapeutic agents described herein include intravenous and ocular administration, including intraocular and periocular administration. As used herein, “intraocular administration” refers to administration into the eye and includes intravitreal and suprachoroidal administration, whereas “periocular 20 administration” refers to administration to the area surrounding the eyeball (periorbital). Other routes of administration include intraperitoneal, intramuscular, subcutaneous, spinal, suprachoroidal, periocular, intravitreal, topical, or other parenteral routes of administration, for example by injection or infusion. The phrase “parenteral administration” as used herein means modes of administration other than enteral administration, usually by injection, and includes, 25 without limitation, intravenous, intraperitoneal, intramuscular, intra-arterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation and preferred routes described above. Alternatively, gedatolisib or formulation thereof described herein can be 30 administered via a non-parenteral route, such as a topical, epidermal or mucosal route of

administration, for example, intranasally, orally, vaginally, rectally, sublingually, or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

The term “inhibition” or “reduction” as used herein, refers to any statistically significant decrease in biological activity, including partial and full blocking of the activity. For example, “inhibition” or “reduction” can refer to a statistically significant decrease of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% in biological activity. The terms “inhibits” or “blocks” (*e.g.*, referring to inhibition/blocking of binding or activity) are used interchangeably and encompass both partial and complete inhibition/blocking.

It will be appreciated that the exact dosage of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is chosen by the individual physician in view of the patient to be treated. In general, dosage and administration are adjusted to provide an effective amount of the gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, to the patient being treated. As used herein, the “effective amount” of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, refers to the amount necessary to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, may vary depending on such factors as the desired biological endpoint, the target tissue, the route of administration, *etc.* For example, the effective amount gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, might be the amount that results in a reduction or cessation of one more symptoms of the ocular disease (*e.g.*, diabetic macular edema, choroidal neovascularization (including myopic choroidal neovascularization (mCNP)), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa) over a desired period of time. Additional factors which may be taken into account include the severity of the disease state; age, weight and gender of the patient being treated; diet,

time and frequency of administration; drug combinations; reaction sensitivities; and tolerance/response to therapy.

In embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient systemically at a dose of 0.001-10 mg/kg, 0.01-5 mg/kg, 5 0.1-5 mg/kg, 1-10 mg/kg, 1-5 mg/kg or at 5 mg/kg. In embodiments, the patient receives at least one, two, three, four, five, six, seven, eight, nine, ten or more dosages during treatment.

In embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient eye(s) at a 5 μ L to 60 μ L dose of 0.1 μ g/mL -10 mg/mL gedatolisib concentration. In embodiments, the patient receives at least one, two, three, four, five, 10 six, dosages per day, or one or two doses per month, or one, two, three, four, five, six, seven, eight, nine, ten or more dosages during treatment depending on whether the route of administration is topical.

In embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered intraocularly to the patient, for example a dose in a volume of ~0.5 μ L to 15 50 μ L by intravitreal injection, or periocular injection, or suprachoroidal injection (10 μ L-50 μ L) into the human eye. In embodiments, the patient receives a dose that achieves at least a minimum intraocular concentration of ~10-100nM (IC50 range for gedatolisib on different cells). In embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient intraocularly at a dose of 0.001mg/mL-20 mg/mL, 1mg/mL – 20 10mg/mL, 0.001 mg/mL-1mg/mL, 0.001 mg/mL-0.1mg/mL or 0.005 mg/mL-0.1mg/mL.

In some embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient at a dose of between 100-10,000 μ g/ml gedatolisib concentration. For example, the dose may be about 0.5 μ mol to 10 μ mol per week or 0.25 mg to 6.5 mg per week, or about 20 μ g/week to 500 μ g/week, about 1100 μ g/week, about 1200 μ g/week, 25 about 1300 μ g/week, about 1400 μ g/week, about 1500 μ g/week, about 1600 μ g/week, about 1700 μ g/week, about 1800 μ g/week, about 1900 μ g/week, 2000 μ g/week, about 2100 μ g/week, about 2200 μ g/week, about 2300 μ g/week, about 2400 μ g/week, about 2500 μ g/week, about 2600 μ g/week, about 2700 μ g/week, about 2800 μ g/week, about 2900 μ g/week, 3000 μ g/week, about 3100 μ g/week, about 3200 μ g/week, about 3300 μ g/week, about 3400 μ g/week, about

3500 $\mu\text{g}/\text{week}$, about 3600 $\mu\text{g}/\text{week}$, about 3700 $\mu\text{g}/\text{week}$, about 3800 $\mu\text{g}/\text{week}$, about 3900 $\mu\text{g}/\text{week}$, or 4000 $\mu\text{g}/\text{week}$.

In other embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient at a dose of about 100-10,000 $\mu\text{g}/\text{mL}$ concentration of gedatolisib per week. For example, the dose may be about 100 $\mu\text{g}/\text{mL}$ per week, about 110 $\mu\text{g}/\text{mL}$ per week, about 120 $\mu\text{g}/\text{mL}$ per week, about 130 $\mu\text{g}/\text{mL}$ per week, about 140 $\mu\text{g}/\text{mL}$ per week, about 150 $\mu\text{g}/\text{mL}$ per week, about 160 $\mu\text{g}/\text{mL}$ per week, about 170 $\mu\text{g}/\text{mL}$ per week, about 180 $\mu\text{g}/\text{mL}$ per week, about 190 $\mu\text{g}/\text{mL}$ per week, 200 $\mu\text{g}/\text{mL}$ per week, about 210 $\mu\text{g}/\text{mL}$ per week, about 220 $\mu\text{g}/\text{mL}$ per week, about 230 $\mu\text{g}/\text{mL}$ per week, about 240 $\mu\text{g}/\text{mL}$ per week, about 250 $\mu\text{g}/\text{mL}$ per week, about 260 $\mu\text{g}/\text{mL}$ per week, about 270 $\mu\text{g}/\text{mL}$ per week, about 280 $\mu\text{g}/\text{mL}$ per week, about 290 $\mu\text{g}/\text{mL}$ per week, 300 $\mu\text{g}/\text{mL}$ per week, about 310 $\mu\text{g}/\text{mL}$ per week, about 320 $\mu\text{g}/\text{mL}$ per week, about 330 $\mu\text{g}/\text{mL}$ per week, about 340 $\mu\text{g}/\text{mL}$ per week, about 350 $\mu\text{g}/\text{mL}$ per week, about 360 $\mu\text{g}/\text{mL}$ per week, about 370 $\mu\text{g}/\text{mL}$ per week, about 380 $\mu\text{g}/\text{mL}$ per week, about 390 $\mu\text{g}/\text{mL}$ per week, or 400 $\mu\text{g}/\text{mL}$ per week, or ideally between 500 $\mu\text{g}/\text{mL}$ per week up to 10 mg/mL per week.

In other embodiments, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered to the patient at a dose sufficient to produce a concentration of about 2nM or 2nM - 100 nM (e.g., 2nM-10nM, 10nM-50nM, 50-200 nM, 75-150 nM, 80-120 nM, 90-110 nM or 100 nM) topically, intravitreally or other intraocular (e.g., suprachoroidally) or periocular site. For example, since a typical adult human eye is about 6 mL in volume, and a concentration of 100 nM corresponds to about 61 ng/mL, in a case of 100% efficiency of delivery, approximately 366 nanograms of gedatolisib can be used as the dose introduced into the eye in 30 μL . In another example with a less than 100% efficiency of delivery, 0.1 $\mu\text{g}/\mu\text{L}$ to 10 $\mu\text{g}/\mu\text{L}$ gedatolisib in about 30 μL (3 μg to 300 μg gedatolisib) is administered topically to the eye one to four times daily. Reduced efficiency of topical therapeutic delivery is known by those practiced in ocular sciences for example in the case of delivery of eye drops being only about 5% efficient in some cases. In embodiments, the dose of gedatolisib introduced into the eye can range from 1-600 nanograms, 100-600 nanograms or 200-400 nanograms or can be a dose of about 100, 200, 300, 400, 500 or 600 nanograms or a dose of 100, 200, 300, 400, 500, or 600

nanograms. In another embodiment, for a dose sufficient to produce an intraocular concentration of about 2 micromolar, a concentration range of about 3-1200 micrograms can be used.

In other embodiments, for topical administration, gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, is administered in the form of eyedrops wherein the concentration is at, for example, 0.1 µg/µL, 1-10 µg/µL, 2.5-7.5 µg/µL, 4-6 µg/µL, about 5 µg/µL or 5 µg/µL. For example, since a typical eyedrop is about 30 µL, at a concentration of 5 µg/µL gedatolisib, each eyedrop provides 150 µg/drop, which when used 1-4 times per day provides a dose of 600 µg/day or 4.2 mg/week.

In other embodiments, gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, 4 years, 5 years or longer or between 1-5 years running consecutively or intermittently on a daily or two to four times daily, weekly or monthly administration schedule. In embodiments, gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered for up to 5 years, up to 10 years, or for the remaining lifetime of the patient on an as needed basis as determined by clinical analysis by an ocular health care professional.

In one embodiment, a method of treating is neovascular age-related macular degeneration in a human subject is provided, wherein the method comprises: (A) selecting a human subject in need of treatment of neovascular age-related macular degeneration; and (B) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.

In another embodiment, a method for treating a human patient who has been determined to have neovascular age-related macular degeneration is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the neovascular age-related macular degeneration.

In one embodiment, a method of treating glaucoma in a human subject is provided, wherein the method comprises: (A) selecting a human subject in need of treatment of glaucoma;

and (B) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.

In another embodiment, a method for treating a human patient who has been determined to have glaucoma is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency
5 sufficient to treat the glaucoma.

In one embodiment, the glaucoma is open-angle glaucoma. In another embodiment, the glaucoma is angle-closure glaucoma. In another embodiment, the glaucoma is low-tension or normal-tension glaucoma. In another embodiment, the glaucoma is congenital glaucoma. In
10 another embodiment, the glaucoma is uveitic (inflammatory) glaucoma. In another embodiment, the glaucoma is neovascular glaucoma.

In one embodiment, a method of treating diabetic retinopathy in a human subject is provided, wherein the method comprises: (A) selecting a human subject in need of treatment of diabetic retinopathy; and (B) administering to the human subject a therapeutically effective
15 amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.

In another embodiment, a method for treating a human patient who has been determined to have diabetic retinopathy is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the diabetic retinopathy.

In one embodiment, a method of treating retinitis pigmentosa in a human subject is provided, wherein the method comprises: (A) selecting a human subject in need of treatment of retinitis pigmentosa; and (B) administering to the human subject a therapeutically effective
20 amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.

In another embodiment, a method for treating a human patient who has been determined to have retinitis pigmentosa is provided, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the retinitis pigmentosa.
25

In embodiments, gedatolisib treatment is administered upon detection of advancing ocular disease following a previous treatment with gedatolisib or other ocular treatment.
30 Advancing ocular disease can be as determined by an ocular health care professional, for

example employing current non-invasive clinical ocular methods such as OCT, ERG, fundus photography, cyc chart exam and the like.

While the methods of treating ocular diseases have been described herein with respect to gedatolisib (a pan-PI3K/mTOR inhibitor), the demonstration herein that a targeted therapeutic
5 that inhibits these signaling pathways can be effectively delivered to the eye, in particular by topical administration, and can effectively inhibit neovascularization suggests that other compounds that target a PI3K, AKT and/or mTOR signaling pathway similarly can be used for treatment of ocular diseases involving neovascularization and in particular could be effective through topical administration, given the gedatolisib results provided herein. Such compounds
10 include pan-PI3K inhibitors, PI3K p110 α inhibitors, PI3K p110 β inhibitors, PI3K p110 γ inhibitors, PI3K p110 δ inhibitors, AKT inhibitors, pan mTOR inhibitors, mTORC1 inhibitors and mTORC2 inhibitors.

Non-limiting examples of compounds known in the art to affect a PI3K pathway include idelalisib, copanlisib, duvelisib, alpelisib, umbralisib, leniolisib, buparlisib, dactolisib,
15 parsaclisib, paxalisib, taselisib, zandelisib, inavolisib, apitolisib, bimiralisib, eganelisib, fimepinostat, linperlisib, nemiralisib, pictilisib, pilaralisib, samotolisib, seletalisib, serabelisib, sonolisib, tenalisib, voxalisib, acalisib and omipalisib, PQR-309, GNE-317, GNE-477, GNE-493, GNE-947, TAK117, AZD8186, onatasertib, RLY-2608, WX390, STX-478, LOX-783, BBO-10203, ETX-636, OKI-219, LY4045004, LAE118, TAK288 and MEN1611. Non-limiting
20 examples of compounds known in the art to affect an AKT pathway include capivasertib, GSK690693, sapanisertib, ipatasertib, M2698, ALTA2618 and ATV-1601. Non-limiting examples of compounds known in the art to affect an mTOR pathway include sirolimus, temsirolimus, everolimus, ridaforolimus, umirolimus, zotarolimus, torin-1, torin-2, vistusertib, AZD8055, XL388, AZD2014, MLN0128, MLN2480, AP23573, CCI-779, nab-sirolimus and
25 RMC-552.

V. Outcomes

The efficacy of the treatment methods provided herein can be assessed using any suitable means. For example, the treatment can be assessed by one or more recognized clinical scales for
30 evaluating the particular ocular disease, *e.g.*, diabetic macular edema, choroidal

neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, 5 glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.

In some embodiments, the treatment results in an improvement in as assessed by one or more known visual acuity tests or assessments.

10 In some embodiments, the treatment results in an improvement in as assessed by the Early Treatment Diabetic Retinopathy Study (“ETDRS”) test, which was developed to aid in evaluating the changes in vision following treatment for patients with diabetic retinopathy. The ETDRS test incorporates specific design criteria to make it more accurate than previous tests. These include: the same number of letters per row (five letters per row), equal spacing of the 15 rows on a log scale (the rows are separated by 0.1 log unit), equal spacing of the letters on a log scale, and individual rows balanced for letter difficulty. To properly evaluate ETDRS, the test should be conducted under standardized lighting conditions.

ETDRS tests are also known as LogMAR tests. This designation derives from the fact that the spacing of the letters and of the rows are equal on a logarithmic scale (Log scale). The 20 rows are separated by 0.1 Log unit, and each letter has a value of 0.02 Log unit.

The two most common methods for scoring the ETDRS test results are the “Number of Letters” scoring method and the “Logmar” scoring method. Both of these methods provide scores that can be used for statistical analysis, unlike the scores provided by previous visual tests (*e.g.*, Snellen or Sloan Acuity tests). In the “Number of Letters” scoring method, the patient 25 starts at the top of the chart and reads down until he or she reaches a row where a minimum of three letters on a line cannot be read. The patient is scored by how many letters could be correctly identified. “Maintaining vision” is defined as losing fewer than 15 letters of visual acuity according to the ETDRS test via the “Number of Letters” scoring method. An “improvement in vision” is defined as gaining 15 or more letters of visual acuity according to the 30 ETDRS test via the “Number of Letters” scoring method.

For the “Logmar” scoring method, the patient starts on the last row where he or she can read all 5 of the letters, and then reads down until he or she reaches a row where a minimum of three letters cannot be read. For these patients, a decimal ETDRS acuity score can be determined. To calculate the decimal acuity score, determine the last row where the patient can correctly identify all 5 letters on that row and then: (1) determine the log score for that row (these scores are shown in the margin of the ETDRS test, e.g., the 20/25 line has a log score of 0.1) and (2) subtract 0.02 log units for every letter that is correctly identified beyond the last row where all of the letters are correctly identified. For example, if the patient reads all of the letters correctly on the 20/32 row and then 3 letters correctly on the 20/25 row, the Log Score would be calculated as follows: $20/32 \text{ Row} = 0.20 / 3 \text{ letters} \times 0.02 \text{ log/letter} = -0.06$ / ETDRS Acuity Log Score: $0.2 - 0.06 = 0.14$. In some embodiments, the treatment results in an improvement, as assessed by the Early Treatment Diabetic Retinopathy Study Diabetic Retinopathy Severity Scale (ETDRS-DRSS) (see, e.g., Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airle House classification. ETDRS report number 10. Ophthalmology. 1991;98:786–806; and Early Treatment Diabetic Retinopathy Study Research Group. Fundus photographic risk factors for progression of diabetic retinopathy. ETDRS report number 12. Ophthalmology. 1991;98:823–833). The ETDRS-DRSS is based on grading of fundus stereo photographs of 7 fields, and classifies DR per eye into 13 complex levels, ranging from absence of retinopathy to severe vitreous hemorrhage or retinal detachment involving the macula. The masked grading of fundus stereo photographs has proven to be a reliable and reproducible assessment.

In some embodiments, the treatment results in an improvement in one or more symptoms of compared to baseline, as assessed by a “Best-Corrected Visual Acuity” test. “Best-Corrected Visual Acuity” refers to the measurement of the best vision correction that can be achieved using glasses or contact lenses. The technique for determining this is the same as standard accuracy, but instead of using the patient’s regular eyesight, the score is determined when the patient is wearing corrective prescription lenses. For example, if the uncorrected visual acuity is measured choroidal neovascularization as being 20/200, but the patient can see 20/20 with glasses, the best-corrected visual acuity (or BCVA) is determined as 20/20. This means that using glasses, the patient’s vision is considered normal. In some embodiments, the treatment results in an

improvement in one or more symptoms of compared to baseline, according to the “Best-Corrected Visual Acuity” test, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method).

In some embodiments, the treatment results in a “doubling of the visual angle” in best corrected distance visual acuity at 9 months or later after the start of treatment. The phrase “doubling of the visual angle” is equivalent to 15 letters or more decrease on an ETDRS visual acuity chart measured at a distance of 4 meters or longer. Best corrected distance visual acuity can be measured at 3 meters instead of 4 meters if measured using an automated threshold testing system.

In some embodiments, the treatment results in a “halving of the visual angle” in best corrected distance visual acuity at 9 months or later after the start of treatment. The phrase “halving of the visual angle” is equivalent to 15 letters or more improvement on an ETDRS visual acuity chart measured at a distance of 4 meters or longer. Best corrected distance visual acuity can be measured at 3 meters instead of 4 meters if measured using an automated threshold testing system.

In some embodiments, the treatment results in an improvement, as assessed by an Amsler grid. The Amsler grid is a tool that can be use at home to test a person’s eyes for vision problems. The basic Amsler grid is a 10-centimeter by 10-centimeter square filled with evenly spaced straight lines in a grid pattern. The lines form very small squares that measure 5 millimeters on each side. There’s a dot to mark the center. The basic grid is typically black lines on a white background, but variations exist. When vision is normal, the person should be able to see all areas on the grid, and all lines should appear straight. If the person has, for example, AMD, the person could notice defects on the grid, including blank spots, warping lines that converge at a spot, wavy lines, and blurriness in parts of the grid.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of neovascular age-related macular degeneration compared to baseline. Exemplary symptoms of neovascular age-related macular degeneration include, but are not limited to visual distortions, reduced central vision in one or both eyes, the need for brighter light when reading or doing close-up work, difficulty adjusting to low light levels,

increased blurriness of printed words, difficulty recognizing faces, and a well-defined blurry spot or blind spot in the field of vision, compared to baseline.

In some embodiments, the patient maintains vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the patient maintains vision, as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “maintaining vision” is defined as losing 15 letters of visual acuity (*e.g.*, at 12 months compared to baseline).

In some embodiments, the treatment results in an improvement in vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS or (ETDRS-DRSS) test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more letters (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 6, 9, or 12 months compared to baseline). In some embodiments, the treatment results in an improvement, as assessed by a “Best-Corrected Visual Acuity” test. In some embodiments, the treatment results in an improvement, according to the “Best-Corrected Visual Acuity” test, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more letters (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 6, 9, or 12 months compared to baseline).

In some embodiments, the treatment results in a “doubling of the visual angle” in best corrected distance visual acuity at, for example, 6, 9, or 12 months after the start of treatment.

In some embodiments, the treatment results in a “halving of the visual angle” in best corrected distance visual acuity at, for example, 6, 9, or 12 months after the start of treatment.

In some embodiments, the treatment results in an improvement in neovascular age-related macular degeneration as assessed by visual examination of the back of the eye. In embodiments, an advanced optical system that allows fundus photography is used (e.g., OCT, ERG), which does not require eye dilation, is used. Alternatively, an examination that requires eye dilation can be used. During such an examination, the eyes are dilated with drops. Then a special instrument is used to examine the back of the eye, *e.g.*, for fluid or blood or a mottled appearance that is caused by yellow deposits that form under the retina, called drusen. People with macular degeneration often have many drusen. In some embodiments, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improved compared to baseline, as assessed by eye dilation and visual examination. In some embodiments, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% reduction in fluid, blood, and/or drusen compared to baseline.

In some embodiments, the treatment results in an improvement in neovascular age-related macular degeneration as assessed by an Amsler grid. An Amsler grid is a simple square containing a grid pattern and a dot in the middle. When used correctly (*e.g.*, once a day, every day), the Amsler grid can show problem spots in the field of vision. For example, in instances of macular degeneration, some of the straight lines in the grid may look faded, broken or distorted. In one embodiment, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement compared to baseline, as assessed by an Amsler grid.

In some embodiments, the treatment results in an improvement in neovascular age-related macular degeneration as assessed by fluorescein angiography. During this test, a dye is injected into a vein in the arm. The dye travels to and highlights the blood vessels in the eye. A special camera takes pictures as the dye travels through the blood vessels. The images show if there are leaking blood vessels or retinal changes. In some embodiments, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement compared to baseline, as assessed by fluorescein angiography.

In some embodiments, the treatment results in an improvement in neovascular age-related macular degeneration as assessed by indocyanine green angiography. Like fluorescein angiography, this test uses an injected dye. It can be used to confirm the findings of a fluorescein angiography or to identify problem blood vessels deeper in the retina. In some
5 embodiments, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement compared to baseline, as assessed by fluorescein angiography.

In some embodiments, the treatment results in an improvement in neovascular age-related macular degeneration as assessed by optical coherence tomography. This noninvasive imaging
10 test displays detailed cross sections of the retina. It identifies areas of thinning, thickening or swelling. This test also is used to help monitor how the retina responds to macular degeneration treatments. In some embodiments, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement compared to baseline, as assessed by optical
15 coherence tomography.

In some embodiments, the treatment results in an improvement in neovascular age-related macular degeneration as assessed by optical coherence tomography (OCT) angiography. This is a noninvasive test. In certain cases, OCT allows one to see unwanted blood vessels in the macula. Though still used primarily as a research tool, its use is increasing in clinics. In some
20 embodiments, an improvement in neovascular age-related macular degeneration results in at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement compared to baseline, as assessed by optical OCT angiography.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of glaucoma. Exemplary symptoms of glaucoma, include
25 but are not limited to, eye pain, eye pressure, headaches, rainbow-colored halos around lights, low vision, blurred vision, narrowed vision (tunnel vision), blind spots, nausea, vomiting, and/or red eyes. In one embodiment, the treatment results in an improvement in one or more symptoms of glaucoma compared to baseline, as assessed by a visual acuity test, eye dilation and visual examination, a visual field test to measure peripheral vision, tonometry, optic nerve imaging,
30 gonioscopy and/or pachymetry. In one embodiment, the glaucoma is open-angle glaucoma. In

another embodiment, the glaucoma is angle-closure glaucoma. In another embodiment, the glaucoma is low-tension or normal-tension glaucoma. In another embodiment, the glaucoma is congenital glaucoma. In another embodiment, the glaucoma is uveitic (inflammatory) glaucoma. In another embodiment, the glaucoma is neovascular glaucoma.

5 In some embodiments, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement in one or more symptoms of glaucoma compared to baseline, *e.g.*, as assessed by a visual acuity test, eye dilation and visual examination, a visual field test to measure peripheral vision, tonometry, optic nerve imaging, gonioscopy and/or pachymetry.

10 In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of diabetic retinopathy. Exemplary symptoms of diabetic retinopathy include, but are not limited to, floaters, blurry vision, vision that changes sometimes from blurry to clear, seeing blank or dark areas in the field of vision, poor night vision, distortion of color vision, and/or loss of vision. In some embodiments, the diabetic retinopathy is non-
15 proliferative diabetic retinopathy (NPDR). In some embodiments, the diabetic retinopathy is proliferative diabetic retinopathy (PDR).

 In some embodiments, the treatment results in an improvement in DR by at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, wherein the improvement is a ≥ 2 step improvement according to an
20 ETDRS-DRSS test. In some embodiments, the treatment results in an improvement in DR by 12 months compared to baseline, wherein the improvement is a ≥ 2 step improvement according to an ETDRS-DRSS test. In some embodiments, the treatment results in an improvement in DR by at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, wherein the improvement is a ≥ 3 step improvement
25 according to an ETDRS-DRSS test. In some embodiments, the treatment results in an improvement in DR by 12 months compared to baseline, wherein the improvement is a ≥ 3 step improvement according to an ETDRS-DRSS test.

 In some embodiments, the treatment results in an improvement in one or more symptoms of diabetic retinopathy compared to baseline, as assessed by eye dilation and visual examination,
30 optical coherence tomography (OCT), and/or fluorescein angiography. In some embodiments,

the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of diabetic retinopathy compared to baseline, as assessed by eye dilation and visual examination, optical coherence tomography (OCT), and/or fluorescein angiography.

5 In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of diabetic macular edema. Exemplary symptoms of diabetic macular edema include, but are not limited to, decreased visual acuity (VA), blurred vision, metamorphopsia, changes in color perception, and/or difficulty reading.

10 In some embodiments, the treatment results in an improvement in one or more symptoms of diabetic macular edema compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of diabetic macular edema compared to baseline, *e.g.*, as assessed by as assessed by eye dilation and visual examination, OCT, and/or
15 fluorescein angiography.

 In some embodiments, the patient having diabetic macular edema maintains vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the patient
20 maintains vision, as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “maintaining vision” is defined as losing 15 letters of visual acuity (*e.g.*, at 12 months compared to baseline).

 In some embodiments, the treatment results in an improvement in vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or
25 more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method) in a patient having diabetic macular edema. In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more letters

(*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 12 months compared to baseline).

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of retinitis pigmentosa. Exemplary symptoms of retinitis pigmentosa include, but are not limited to, loss of night vision, loss of peripheral (side) vision, and/or difficulty seeing different colors.

In some embodiments, the treatment results in an improvement in one or more symptoms of retinitis pigmentosa compared to baseline, as assessed by electroretinography, visual field teasing, and/or optical coherence tomography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of retinitis pigmentosa compared to baseline, as assessed by electroretinography, visual field teasing, and/or optical coherence tomography.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of choroidal neovascularization (including myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma). Exemplary symptoms of choroidal neovascularization include, but are not limited to, an acute decrease in visual acuity, relative scotoma, and/or metamorphopsia.

In some embodiments, the treatment results in an improvement in one or more symptoms of choroidal neovascularization compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of choroidal neovascularization compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In some embodiments, the patient having choroidal neovascularization maintains vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method). In one embodiment, the

patient maintains vision, as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “maintaining vision” is defined as losing 15 letters of visual acuity (*e.g.*, at 12 months compared to baseline).

In some embodiments, the treatment results in an improvement in vision for at least 3 months (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more) compared to baseline, as assessed by an ETDRS test (*e.g.*, via the “Number of Letters” scoring method and/or the “Logmar” scoring method) in a patient having choroidal neovascularization. In one embodiment, the treatment results in an improvement in vision (*e.g.*, a clinically significant improvement in vision), as assessed by an ETDRS test via the “Number of Letters” scoring method, wherein “an improvement in vision” is defined as gaining 15 or more letters (*e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more letters) of visual acuity (*e.g.*, at 12 months compared to baseline).

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of central retinal vein occlusion. Exemplary symptoms of central retinal vein occlusion include, but are not limited to blurry or distorted vision, transient visual obscurations, pain, redness, irritation and/or other problems.

In some embodiments, the treatment results in an improvement in one or more symptoms of central retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of central retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of macular edema following retinal vein occlusion. Exemplary symptoms of macular edema following retinal vein occlusion include, but are not limited to, the sudden appearance of floaters or severe loss of vision.

In some embodiments, the treatment results in an improvement in one or more symptoms of macular edema following retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the

treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of macular edema following retinal vein occlusion compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

5 In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of cystoid macular edema include. Exemplary symptoms of cystoid macular edema include, but are not limited to, a decrease in visual acuity that is associated with retinal edema, loss of contrast sensitivity and color vision, metamorphopsia (*e.g.*, that can be demonstrated on Amsler grid), micropsia, and central scotoma.

10 In some embodiments, the treatment results in an improvement in one or more symptoms of cystoid macular edema compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement of one or more symptoms of cystoid macular edema compared to
15 baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of polypoidal choroidal vasculopathy. Exemplary symptoms of polypoidal choroidal vasculopathy include, but are not limited to, blurred vision or a blind spot in (or near) the center of vision in one or both eyes. These symptoms may appear
20 suddenly and tend not to vary throughout the day.

 In some embodiments, the treatment results in an improvement in one or more symptoms of polypoidal choroidal vasculopathy compared to baseline, *e.g.*, as assessed by indocyanine green angiography (ICGA). In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%
25 improvement of one or more symptoms of polypoidal choroidal vasculopathy compared to baseline, *e.g.*, ICGA.

 In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of retinopathy of prematurity. Exemplary symptoms of retinopathy of prematurity include, but are not limited, to abnormal blood vessels grow in the
30 retina in babies who are premature or who weigh less than 3 pounds at birth.

In some embodiments, the treatment results in an improvement in one or more symptoms of retinopathy of prematurity compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography. In some embodiment, the treatment results in an at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
5 95%, or 100% improvement of one or more symptoms of retinopathy of prematurity compared to baseline, *e.g.*, as assessed by eye dilation and visual examination, OCT, and/or fluorescein angiography.

In some embodiments, the treatment results in an improvement (*e.g.*, reduction or cessation) of one or more symptoms of Ocular von Hippel Lindau Disease.
10

VI. Combination Therapies

Gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, can be
15 administered (1) alone or (2) in combination with one or more therapies or therapeutic agents, according to the methods described herein. For example, gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, can be administered prior to, after, or concurrently with one or more therapies or therapeutic agents.

Any other suitable therapy or therapeutic agent appropriate for the ocular disease or
20 disorder to be treated can be administered, such as an additional therapy or therapeutic agent that reduces ocular neovascularization.

In embodiments, gedatolisib treatment is combined with photodynamic therapy (*e.g.*, with verteporfin (PDT-V), laser ablation therapy and/or cauterization.

In embodiments, gedatolisib treatment is combined with one or more VEGF inhibitors.
25 Non-limiting examples of VEGF inhibitors known in the art include ranibizumab (Lucentis™), aflibercept (Eylea™), bevacizumab (Avastin™), pegaptanib (Macugen™), brolucizumab (Beovu™), faricimab-svoa (Vabysmo™), conbercept (FP3/KH902), KSI-301 and ziv-aflibercept, and receptor kinase inhibitors axitinib.

In embodiments, gedatolisib treatment is combined with one or more additional agents of use in the treatment of ocular diseases or disorders, such as complement inhibitors, MMP inhibitors (e.g., doxycycline), CW-703, rAPN, RC-28-E, DAVP2 and DAVP3.

5 **VII. Kits and Unit Dosage Forms**

Also provided herein are kits that include a pharmaceutical composition containing gedatolisib or a pharmaceutically acceptable salt, solvate, or ester thereof, in a therapeutically effective amount adapted for use in the methods described herein *e.g.*, for effective treatment of an ocular disease, such as diabetic macular edema, choroidal neovascularization (including
10 myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (including choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma), macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel
15 Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa. The kits optionally also can include instructions, *e.g.*, comprising administration schedules, to allow a practitioner (*e.g.*, a physician, nurse or patient) to administer the composition contained therein to a patient having an ocular disease. The kit also can include a syringe.

Optionally, the kits include multiple packages of the single-dose pharmaceutical
20 compositions each containing an effective amount of the gedatolisib for a single administration (*e.g.*, 180 mg) in accordance with the methods provided above. Instruments or devices necessary for administering the pharmaceutical composition(s) also may be included in the kits. For instance, a kit may provide one or more pre-filled syringes containing an amount of the liquid necessary for reconstitution of the gedatolisib.

25 In one embodiment, the kit comprises: (a) a dose of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, and (b) instructions for using gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in the methods described herein.

The following examples are merely illustrative and should not be construed as limiting
30 the scope of this disclosure in any way as many variations and equivalents will become apparent

to those skilled in the art upon reading the present disclosure. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

5

EXAMPLES

EXAMPLE 1: ASSESSMENT OF EFFICACY OF GEDATOLISIB IN TREATING NEOVASCULAR AGE-RELATED MACULAR DEGENERATION (“WET” AMD)

10

The first aim of the study was to determine an 8-day concentration of gedatolisib in the eye (intravenous (i.v.) administered API-gedatolisib), at three different single dose concentrations. A second aim was to determine if prophylactically i.v. administered API-gedatolisib can delay the onset of neo-vascularization in the rat L-CNV model as well as or better than historical data for bevacizumab administered by direct injection into the vitreous of the rat eye. A further aim was to measure changes in VEGF and Ang2, angiogenic proteins’ concentration in drug treated eyes compared to untreated controls.

15

A. Study Overview

6-week-old male Brown Norway rats received tail vein injections of either vehicle or Gedatolisib at one of three doses outlined in Table 2.

20

Table 2. Study Design

Arm	Treatment	Planned ANG-2 ELISA (n = samples)	Actual VEGF ELISA (n = samples)	Planned VEGF ELISA (n = samples)	Actual VEGF ELISA (n = samples)	Planned CNV (n = rats)	Actual CNV (n = rats)
1	Vehicle control	5	–	5	3	5	6
2	Gedatolisib 1.5mg/kg	5	–	5	4	5	6
3	Gedatolisib 5mg/kg	5	–	5	5	5	6
4	Gedatolisib 15mg/kg	5	–	5	4	5	6

Four hours after gedatolisib was injected, a laser procedure to induce the rupture of Bruch's membrane was performed to generate choroidal neovascularization (CNV) in all animals. Rats were anesthetized with an intraperitoneal injection of ketamine/xylazine, and a 1% tropicamide solution was used to dilate the eyes. Using a hand-held cover slip as a contact lens and GenTeal lubricating eye gel as a medium contacting the cover slip to the surface of the cornea, a Nidek GYC-500 green laser photocoagulator coupled to a Nidek SL-1800 slit-lamp was used to create four lesions equidistant from the optic nerve head in the retinal mid-periphery. Lesions were created with laser parameters that include: 532 nm wavelength, 100 μ m spot size ($\pm$ 7 μ m), 0.1 sec duration, and 120 mW. Rats in the Vehicle control, 1.5mg/kg, and 5mg/kg Gedatolisib treatment groups received a second dose 24 hours post-laser. Rats in the 15mg/kg Gedatolisib treatment group received a second dose of the asset four days post-laser.

Eight days after the laser procedure, rats were sacrificed, and the eyes were enucleated and dissected. The extent of CNV at the Bruch's membrane rupture sites were measured using computer-assisted image analysis of choroidal flat-mounts stained using FITC-conjugated isolectin B4 (IB4). Gedatolisib was administered systemically, therefore, the average area for all lesions in both eyes is reported as a single data point. Area measurements were analyzed by ANOVA and an appropriate post hoc test. As a secondary terminal readout, eye tissue was collected for analysis by ELISA for angiopoietin-2 and VEGF.

Several optimization steps were attempted to maximize the likelihood that both ANG-2 and VEGF would be detected in tissue samples from lasered and non-lasered eyes. First, dissection of choroidal tissue was optimized by selecting and dissecting only the portion of tissue to which the laser had been applied. This was done to minimize interference by high abundance protein in non-lasered areas of the same choroid. Additional optimization was performed by microdissection and removal of scleral tissue from the sample. This also allowed for elimination of protein sources of high abundance but little interest.

Dissected choroidal tissue from two eyes was pooled into each sample for protein extraction and measurement by conventional ELISA. This experimental parameter also was optimized in order to meet criteria suitable for detection.

After these and multiple other troubleshooting and optimization steps, across all treatment groups choroidal angiopoietin-2 fell below the lower limit of detection (39.1pg/mL) in the commercially-available ELISA manufactured by R&D Systems (Cat. No. MANG20).

Previous measurement of choroidal VEGF by ELISA in unlasered and untreated eyes show levels consistent with the concentration of VEGF in the gedatolisib-treated groups in this study.

B. Analytical Method

a. Chemicals and Solvents

All chemicals used in this study were analytical grade or HPLC grade. All organic solvents for sample preparation reagents such as methanol (MeOH), acetonitrile (ACN), dimethyl sulfoxide (DMSO), were supplied from Fisher Scientific (Hampton, NH, USA).

b. Standards and Sample Preparation

Gedatolisib was prepared as an individual stock solution at 0.5 mg/mL in dimethylsulfoxide (DMSO) by sonication for approximately 10 minutes (until solution became clear. The stock solution was then further diluted in DMSO to create a 2 µg/mL diluted stock solution. Working solutions for use in calibration and quality control samples were prepared in DMSO:Acetonitrile (1:1, vol:vol).

Calibrators, quality control samples (QCs), and blanks were made by dilution of working solutions using Methanol:Water, (1:1, vol:vol). The calibration range for Gedatolisib used in this method was 5 pg/mL to 2500 pg/mL. Stable isotopic labelled internal standard was added to calibration standards, quality control, and matrix samples.

Tissue samples were processed by disruption with Qiagen Tissuelyser. Internal standard solution was added to each eye in a 2 mL round bottom tube and incubated for 10 minutes at room temperature. Addition of a 5mm stainless steel bead and 95%

Methanol:5% DMSO was followed by processing at 30/sec for 1 minute and a 10-minute cycle at 20/sec. Supernatant from centrifugation of homogenates were transferred for LC-MS/MS analysis.

c. Chromatographic and Mass Spectrometer Conditions

5 Chromatographic separations were performed using a 1290 series high pressure liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) with a 3 X 50 mm reversed phase column at 40°C.

Mass spectrometric analysis was performed using an Agilent 6495 Series Triple
Quadrupole tandem mass spectrometer equipped with an Agilent Jet Stream
10 Electrospray source operated in the positive ion mode and using nitrogen as the carrier gas set at the flow rate of 12 L/min. The gas temperature was set at 200°C, nebulizer at 45 psi, and capillary voltage at 3500 V. At these settings, the analyte was detected in its protonated form ($[M + H]^+$). Sample analysis was carried out in the Dynamic MRM mode. The transitions monitored for Gedatolisib were 616.3→488.3 (Quantifier) and
15 616.3→367.2 (Qualifier); similarly for [2H9] Gedatolisib, the Quantifier transition was 625.0→492.2 and the Qualifier was 625.0→124.2.

d. Data Acquisition and Processing

HPLC/MS-MS data were acquired using the proprietary software application MassHunter Workstation Data Acquisition for Triple Quad B.07.01/Build 7.1.7112.0,
20 Agilent Technologies, Inc.). Data were processed (integrated) using the software application MassHunter Quantitative Analysis for QQQ (version B.07.01/Build 7.1.524.0. Agilent Technologies, Inc.). Calibration plots of area ratio versus analyte concentrations were constructed and the analyte-specific model was applied to the data using MassHunter Quantitative Analysis for QQQ. The regression model used was:
25 power, weighted $1/x^2$.

Samples were tested against calibration curve requested for original development efforts (5 - 2500 pg/mL) and were all found to be acceptable quality level (AQL). Interpolated results (extrapolated) indicated results 10-40x the upper limit of quantification (with only replicate one of the calibration curve analyzed). Additional
30 Calibration Standards were prepared to extend the range for analysis to 125,000 pg/mL.

Replicate injections of the additional standards were included at the end of the run. Extension of the calibration range generated calibration curve with good fit. Sample results were not impacted significantly by carryover, and the extended calibration curve was not impacted.

5 *e. Laser Choroid Neovascularization (LCVN) Image Intensity Analysis*

The analysis sought to determine whether treated CNV images exhibit different FITC-conjugated isolectin B4 intensity from vehicle CNV images.

Images were processed as follows. Image labeling was cut. Color was split and green image retained as 8-bit grey scale. Average full image intensity was recorded.

10 Auto-threshold was determined using the 'Triangle' algorithm. This algorithm did the best job of identifying CNV areas in both vehicle and treated. Particle/blob analysis was performed with size threshold 500 to infinity pixels and 0 to 1 circularity. Measurements were redirected to the grey scale image. The largest blob was selected, after verifying it occurred in the CNV area, and the size and intensity was recorded.

15 The table below reports these results showing that the intensities do not differ significantly between the two analyses.

Table 3: Analysis Intensity and Area for Sixteen Sample Images

			Full Image	Threshold Area	Threshold Area
Treatment		Image	Avg Intensity	# 20x Pixels	Avg Intensity
Vehicle	Rat 1	1	26	60,951	53
		2	29	9,099	70
		3	39	148,520	82
		4	34	46,588	68
	Rat 4	1	29	202,432	57
		2	47	44,668	92
		3	38	146,216	91
		4	33	74,456	75
5 mg/kg	Rat 3	1	34	11,141	81
		2	36	11,419	87
		3	39	3,512	75
		4	41	10,151	85
	Rat 5	1	54	5,997	120
		2	35	2,929	76
		3	24	17,487	64

		4	27	15,591	55
Vehicle		Average	34	91,616	74
		Stdev	7	66,305	14
5mg/kg		Average	36	9,778	80
		Stdev	9	5,323	19

FIGS. 1A-1J depict the analysis process with two images. Specifically, **FIG. 1A** is the original vehicle-treated image. **FIG. 1B** is the original 5 mg/kg gedatolisib-treated image. **FIG. 1C** is the green channel (8 bit 0 to 256) threshold analysis for the vehicle-treated image. **FIG. 1D** is the green channel (8 bit 0 to 256) threshold analysis for the 5 mg/kg gedatolisib-treated image. **FIG. 1E** is the green channel (8 bit 0 to 125) visual inspection only for the vehicle-treated image. **FIG. 1F** is the green channel (8 bit 0 to 125) visual inspection only for the 5 mg/kg gedatolisib-treated image. **FIG. 1G** is the triangle thresholded image for the vehicle-treated image. **FIG. 1H** is the triangle thresholded image for the 5 mg/kg gedatolisib-treated image. **FIG. 1I** is the particle analysis (500 infinity pixels – intensity taken from largest blob) for the vehicle-treated image. **FIG. 1J** is the particle analysis (500 infinity pixels – intensity taken from largest blob) for the 5 mg/kg gedatolisib-treated image.

The graph set forth in **FIG. 2** plots CNV area data for each laser hole image to visualize the distribution of CNV areas. The graph in **FIG. 4** reports the average CNV area at each laser site by eye prior to applying statistical evaluation. The graph in **FIG. 2** reflects variability within all CNV areas. The rats treated with 5 mg/kg doses had significantly smaller CNV area than rats treated with Vehicle. The rats treated with 1.5 mg/kg doses also had significantly smaller CNV area than vehicle.

20

C. Rat Gedatolisib Pharmacokinetics (PK) Study

A pharmacokinetic (PK) study was conducted in Charles River Brown Norway Rats (male, 8-12 weeks, 150-300g). Gedatolisib was administered intravenously at a dose of 1.5mg/kg, 5mg/kg or 15mg/kg via lateral tail vein (100-200 ul volume). Necropsies were performed at 2 hours, 4 days and 8 days post-dose.

25

For 1.5 and 5 mg/kg doses, rats were dosed on day -1 (loading dose) and day 0. First necropsy was 2 hours after the second dose on day 0. Second and third necropsies were 4 days and 8 day post-second dose on day 0.

For the 15 mg/kg dose, the first dose was administered on day 0, and the second
5 dose on day 4. First necropsy 2 hours post-dose day 0. Second necropsy 2 hours post dose day 4. Third necropsy 8 days from day 0.

Three rats were used for each time point and each dose (27 rats), plus 3 naïve rats (for T0 drug levels in the plasma, eyes and other tissues), for a total of 30 rats. Blood samples were collected prior to euthanasia for plasma drug levels (EDTA plasma).
10 Euthanasia was performed via CO2 asphyxiation. Eyes were collected and flash frozen. Liver, pancreas, kidney, spleen, lung, heart, thyroid, brain were weighed, collected, and flash frozen for potential future analysis of drug levels.

For IV administration, lateral tail veins were dilated prior to IV injection by warming the tail under an infra-red lamp or by immersing the tail into a warm (not hot)
15 water bath. The rats were constantly attended during warming to dilate veins so that no rats experienced burns or heat stress. One person was assigned to only this task while another person performed injections.

In a separate analysis, 5mg/kg gedatolisib was administered intramuscularly (IM) in gluteal muscle (100-200 ul volume). Time Points analyzed were 2 hours, 4 days and
20 8 days post dose. One rat was used for each time point and dose, for a total of 3 rats. Blood samples were collected prior to euthanasia for plasma drug levels (EDTA plasma). Euthanasia was performed via CO2 asphyxiation. Eyes were collected and flash frozen. Liver, pancreas, kidney, spleen, lung, heart, thyroid, brain were weighed, collected, and flash frozen for potential future analysis of drug levels.

25 Gedatolisib was administered to rats as a pharmaceutical grade drug and excipients. The drug was supplied in pre-formulated vials containing dry chemical that is water soluble so that concentration could be adjusted with USP water for injection to requirements for dose volume in each route of administration.

Animals were given a single dose of gedatolisib at the dose level and by the route in the group to which the rat is assigned. Rats had a single terminal blood collection followed by euthanasia and tissue harvest at the time point assigned to the group.

5 D. Results

a. Results for Rat L-CNV Model of Wet AMD

FIGS. 3A-3D depict representative single L-CNV lesion images from rats administered intravenous dosages of vehicle (**FIG. 3A**), gedatolisib at a 1.5 mg/kg dose (**FIG. 3B**), gedatolisib at a 5 mg/kg dose (**FIG. 3C**) or gedatolisib at a 15 mg/kg dose (**FIG. 3D**). At 1.5mg/kg and
10 5mg/kg doses, clear evidence of inhibition of neovascularization is demonstrated by reduction of intensity and size of the area stained with endothelial marking Isolectin B-4 dye (lighter color).

IB4 stain causes damaged endothelial cells to have a brighter intensity green color. The tissue mounts shown **FIGS. 3A-3D** are looking straight back through a dissected choroid mount (retinal layers have NOT been dissected away). Layers of the imaged slides are from the top
15 RPE, Bruchs Membrane (Basement Membrane [mostly collagen] and choriocapillaris), choroid layer is at bottom of image (“viewed through the laser hole). Immediately after the lasing, there is edema and inflammation that tends to fill in the hole quickly with liquid proteinaceous materials. The heat of lasing is focused on the RPE and Bruchs layers, and heat is dissipated quickly at the choroid layer where there is considerable blood volume (higher heat capacity).

20 Gedatolisib tested at the 2x 5 mg/kg dose exhibited significant efficacy against choroidal neovascularization. Administration of two 5 mg/kg doses separated by 24 hours caused at least a 76.3% reduction in CNV lesion size ($p = 0.0067$) at 8-days post-laser when compared to the vehicle control (IV administered, NOT intravitreal), as shown in **FIG. 4**. Significant efficacy as determined by lesion reduction vs control was not observed at the other two doses.

25 A separate analysis was performed to determine if significant differences in IB4 intensity existed between treatment groups. A sampling analysis of the intensity for incorporation into the area information analysis using 4 rats’ worth of images, i.e., 16 total images, 8 from Vehicle and 8 from 5 mg/kg. Rats: 1 and 4 (Vehicle), 3 and 5 (5mg/kg) employing the triangle threshold method and ‘blob’ analysis, area and intensity data was collated.

There was no evidence of consistent intensity difference either across the entire image or in the threshold area when comparing the 5mg/Kg and control treatment groups. The thresholding method tends to overestimate Vehicle and underestimate 5 mg/kg giving a more significant difference in intensity and area between the two treatment groups. Appendix D contains the numerical results and analytical details of this analysis.

VEGF concentration was measured in the choroid layer for each treatment condition. VEGF was detected in choroidal tissue by ELISA in all treatment arms. VO-CRO, a contract research organization specializing in drug studies for eye therapeutics, report that historically, the normal level of VEGF in choroid tissue is 299 pg/mg for no lesions, no treatments, sclera scraped, in normal adult rat eyes. Thus, this data suggests that there is about ~28% increase in VEGF concentration (mean 382.4+ 62.5 pg/mg) in the choroid of the Vehicle treated animals in this study and between 16%-19% reduction of VEGF concentration in the gedatolisib treated animal eyes compared to the vehicle control condition. This trend is seen with two normalization methods (mL lysate and mg protein) although it's significant only when normalizing to mL lysate.

The concentration of VEGF in the choroids from eyes that received the gedatolisib 15 mg/kg dose was significantly lower than VEGF in the choroids from the vehicle group ($p = 0.0251$), as shown in **FIGS. 5-6**. Gedatolisib at the 15mg/kg dose caused a 28.0% reduction in the concentration of VEGF in choroidal tissue.

b. Measurement of Gedatolisib Concentration in Rat Eyes

Adult male Brown Norway rats (BNR) were treated with 3 different dose levels of gedatolisib. Briefly, 2 doses of each dose level were administered ~24 hours apart by tail IV injection to three different groups of animals. At 2 hours, Day 4 and Day 8, following the second injections, the eyes were harvested from animals. Therefore, the data from this part of the study represent the drug concentrations and two doses administered to BNR in the L-CNV portion of this study.

Gedatolisib was measured in whole rat eyes. CRO results are reported for two IV injections/animal of 1.5 mg/kg, 5 mg/kg, or 15 mg/kg (administered on day 0 and day 4) on day 0, harvest days 0 (2 hours), 4, and 8 in adult male Brown Norway rats.

Table 4. Analytical Standards Testing for Gedatolisib by Mass Spectroscopy

LLOQ*	5.00 pg/mL
ULOQ	125,000.00 pg/mL

Summary data for quantification of gedatolisib concentration found in total Brown Norway rat whole eye following two doses injected at the indicated concentrations (mg/Kg), at each point (N=6 eyes each point for IV administration route, N=2 eyes for IM administration route) measured by mass spectroscopy is set forth in **FIG. 7**.

A conversion of pg/g to pg/ml for the entire eye works out to ~100nM gedatolisib concentration from the Day 8 data (~60,000 pg/g) for the 5mg/Kg dose and 30nM for the 1.5mg/Kg treatment group. Gedatolisib has an IC₅₀ for various signaling, viability readouts of about 10nM in cells.

Concentration loss in tissue over time: (A) Day 4/Day 0 ratios are 0.31 (69% reduction) and 0.42 (58% reduction) for 1.5mg/Kg and 5mg/Kg treatment groups, respectively, and (B) Day 8/Day 4 ratios are 0.84 (16% reduction) and 0.77 (23% reduction) for 1.5mg/Kg and 5mg/Kg treatment groups, respectively. Thus, during the first 4 days, there is a 2-3x larger loss in concentration in the whole eye than in the next 4 days. This is consistent with previous studies.

FIGS. 8A-8B depict the linearity of dosing for three concentrations measured on Days 4 and 8 post injection of two doses in BNR. The doses stack in a very linear manner from 1.5mg/Kg to 15mg/Kg for both Day 4 and Day 8 timepoints. Comparison of the slopes for Day 4 vs Day 8 shows an apparent 57% decline in the dose vs quantification in tissue graph slope over the 4 days and is consistent with the decline in the rate of loss of gedatolisib from the tissue.

FIG. 9 shows a comparison of Day 4 versus Day 8 dose versus measured concentration graphs for two dose administration of gedatolisib. The dose linearity and stability at Day 8 in these rats is consistent with information from other animals using radioactive carbon-labeled gedatolisib, demonstrating retention of gedatolisib at efficacious levels in the eye.

FIGS. 10A-10B show the distribution to ocular tissue data with ¹⁴C-labeled gedatolisib following a single intravenous administration of 5mg/Kg dose in Long Evans rats. This data demonstrates about 10x higher concentration of gedatolisib in the uveal tract vs. the whole eye. The largest drop in concentration in the eye happens within the first 8-24 hours. After 168 hrs (7

days), the drug concentration levels off in the eye and uveal tract and remains relatively unchanged out to the last time point measured at day 28 post injection, demonstrating retention of gedatolisib at efficacious levels in the eye.

c. In vitro impedance assessment of endothelial cell function

5 During the course of the present study, *in vitro* tools were used to study endothelial cell function. Impedance has become a standard for *in vitro* study of vascular barrier function (e.g. blood brain and retinal), as well as critical barriers formed by other cell types such as Retinal Pigment Epithelial (RPE) cells.

10 **FIG. 11** shows the impedance response of a Human Vascular Endothelial Cell (HUVEC) confluent cell layer formed from addition of 25,000 cells to a 96-well ACEA E-plate. This condition should mimic normal endothelial cells with tight gap junctions formed between cells. Individual colored traces demonstrate impedance cell-based change compared to vehicle media upon addition of four inflammatory cytokines, known to cause reversible permeabilization of vascular intercellular tight gap junctions and thus reduction of impedance. The expression of
15 these cytokines are also known to be linked to vascular leakage (weakened tight gap junctions) leading to various forms of ocular dysfunction such as found in wet AMD. This figure demonstrates that HUVEC barrier function *in vitro* impedance measurements can model vascular endothelial cell response to factors that affect endothelial cell function such as inflammatory diseases.

20 **FIG. 12** shows the results of Primary Human Retinal Microvascular Endothelial Cells (ACBRI 181) from Cell Systems. The Human Retinal Pigmented Micro Vascular Endothelial cells seeded at 25k per well into x-CELLigence E-plate wells form a confluent barrier. The data shows the impedance response of the cell barrier to VEGF added at hour 44 and with some wells additionally receiving gedatolisib at hour 25. The data for four wells each condition, normalized
25 just prior to the VEGF timepoint, show weakening of the barrier (reduction of the impedance) by VEGFA-165 50 ng/mL (~2.5 nM) and inhibition of the VEGFA-165 response by 100 nM Gedatolisib. The entire experiment took place in R&D Systems endothelial growth medium with supplement minus VEGF.

d. Discussion

Gedatolisib was shown to reduce neovascularization in the L-CNV model (~76%) and significantly impede choroidal neovascularization, especially compared to historical data for administration of intravitreal anti-VEGF therapy (~40% inhibition in the L-CNV model). The 76% reduction of neovascularization represents a conservative limit on the success of this study owing to the present inability to confirm that the IB4 staining in the 5mg/kg treatment group had no choroidal neovascularization. The current choroid flat mount staining data does not discriminate between normal vascularization of the choroid layer (on the other side of Bruch's membrane) from neovascularization of the retinal layer (above Bruch's membrane).

In FIG. 1, there are clear differences between the different treatment groups in terms of size of the IB4 stain area and intensity. Differences in intensity can be due to vascular leakage or more likely endothelial cell expression of the IB4 lectin-binding to N-acetylglucosamine/ α -D-galactose residues in glycoconjugates on the cell surface and Golgi apparatus of damaged endothelial cells. The carbohydrate composition on cell membranes can change in response to an injury, infection, or pathological state, and lectin-binding patterns may change accordingly.

There was some difference in the concentration found in the Long Evans rat eyes on Day 7 (0.3uM, single 5mg/Kg dose, N= 1 animal, method: ^{14}C -labeled gedatolisib in scintillation counter) versus the Brown Norway rat eyes ($0.1\text{uM} \pm 0.016$, two 5mg/Kg doses, N= 3 animals, method: LC-MS direct) on Day 8 in the present study. The theoretical differences represent a six-fold discrepancy. Although the age of the rats was similar in the 2 studies (7-8 weeks), differences in the tissue concentrations may be attributed to differences in rat species (*e.g.*, different degrees of pigmented ocular tissue), differences in sample preparation, or radioactive metabolite. Due to the shallowness in the time versus concentration plots for both the ^{14}C data and the LC-MS data, significant differences are not likely due to one measurement being made on Day 7 and another on Day 8. Identification is possible of whether the discrepancy is eye-specific or is a general phenomenon as tissue samples were archived from the rats as well as eyes and could be analyzed for gedatolisib.

The present study and previous ^{14}C -labeled gedatolisib study confirm the presence of the drug in the timeframe of the study and confirm the concentration of the drug is at or above a level expected to be efficacious.

e. Conclusion:

Gedatolisib was detected in the eyes of rats following intravenous administration. A linear relationship was demonstrated for gedatolisib concentrations detected in whole rat eyes at 4 and 8 days post intravenous injection following a two-dose i.v. administration of gedatolisib. Gedatolisib administered by intramuscular injection also reached the same concentration in the eye as intravenous administration at the 5mg/kg dose.

Gedatolisib significantly affected neovascularization when administered by intravenous injection prophylactically in the L-CNV model. Gedatolisib provided superior benefit inhibiting neovascularization in the L-CNV rat model compared to historical data using standard of care anti-VEGF antibody.

Finally, gedatolisib significantly reduced choroidal VEGF in the L-CNV model. At 15mg/Kg, gedatolisib reduced choroidal VEGF concentration. One caveat is that the level of VEGF in the choroid of the untreated eye was found to be only slightly above normal. Apparently, an intravitreal (i.vit) injection of vehicle can raise VEGF levels in the choroid/retina as is commonly found with using i.vit administration route.

EXAMPLE 2: GEDATOLISIB TREATMENT PREVENTS NEOVASCULARIZATION IN A WET AMD MODEL BY DRUG THAT IS RETAINED IN EYE TISSUE

The aim of the study described in this example was to determine whether circulating gedatolisib resulted in inhibition of neovascularization in the L-CNV model described in Example 1 and/or whether gedatolisib that had become resident in eye tissue resulted in inhibition of neovascularization in the L-CNV model.

As described herein, the data demonstrated that gedatolisib that was administered by iv injection likely becomes resident in the eye three days before laser treatment and significantly reduced the amount of L-CNV lesion area (26%, $p=0.012$) measured at 8 days after laser treatment compared to vehicle injected rats.

In this study, the size of the laser hole that was made was intentionally increased compared to an initial feasibility study in an attempt to increase the dynamic range of the test. From the studies it was concluded that there is a non-linear relationship between the hole diameter and the lesion size at Day 8 after laser treatment (20% larger diameter hole or 44% area increase leads to a 2.19x larger CNV lesion at D8 after laser treatment).

In a first study, gedatolisib treatment reduced lesion area by (0.0108mm² or 10,800µm²) and in a second study reduced lesion area by (0.00969 mm² or 9,690µm²). The effects were statistically equivalent on a lesion area “protected” from endothelial expansion basis. Details of the studies are described below.

5

Background and Purpose

Previous data indicated that the male rat uveal tract maintained significant levels of gedatolisib above the IC₅₀ (in tissue ~30nM-50nM, in cell free assays 0.4nM-6nM) to 672hr (28 days) when administered one 5mg/kg dose of gedatolisib by iv. It was also established that gedatolisib T_{1/2} in rat blood was 12.8 hours. Somewhere between 24hr (140nM) and 48hr (BLQ) post administration, the concentration dropped below the limit of quantification (BLQ) for the test (~90nM). It was estimated that at the AUC_{0-∞} values 9858 ng eq-hour/g that 72hrs is 5.6 half-lives and this time length should clear gedatolisib from circulation below the IC₅₀ concentration. The estimations provided some margin of error and did not take into account all of the various organs that can be shedding gedatolisib over time into circulation.

In a first study described by EXAMPLE 1 immediately above, administration of two 5mg/Kg doses of gedatolisib, one dose at 4hrs before laser treatment (H-4) and the second dose at 20hrs (H+20) after laser treatment of rat eyes, was found to significantly inhibit (76%) formation of leaky neovascularization quantified at 8 days (D8) post laser treatment. This protocol showed that gedatolisib could inhibit the L-CNV, although it did not isolate the effect with respect to the timing of the laser treatment or whether the effect was from circulating drug or tissue resident drug.

The present study used the dose which previously demonstrated effective inhibition of CNV (2x5mg/kg, for a total of 10 mg/kg) administered on contiguous days 3 days prior to laser treatment to establish whether the eye can serve as drug depot to prevent NV for up to 3 days and during the post-laser 8 days.

Experimental Procedures

Eye Depot Determination Methods

Laser treatment- 6-week-old male Brown Norway rats received standardized Laser-induced choroidal neovascularization (LCNV) treatment on experiment Day 0 (D0). During treatment, animals were anesthetized with an intraperitoneal (IP) injection of ketamine/xylazine and topical application of 1% tropicamide was used to dilate the pupils. Using a hand-held cover slip as a contact lens and GenTeal lubricating eye gel as a medium contacting the cover slip to the surface of the cornea, a Nidek GYC- 500 green laser photocoagulator coupled to a Nidek SL-1800 slit-lamp was used to create four lesions equidistant from the optic nerve head in the retinal midperiphery, avoiding laser ablation of major retinal vasculature. Laser parameters included 532 nm wavelength, 120 µm spot size, 0.1 sec duration, and 120 mW power.

Dosing- Gedatolisib and vehicle treatments were administered via i.v. injection, with dosing as outlined below in Table 5.

Table 5: Experimental Design Arm	Treatment	Treatment Timing	CNV Area (n = eyes)
1	Vehicle	Day -4, Day -3	6
2	Gedatolisib (5mg/kg)	-4hr, +20hr	6
3	Gedatolisib (5mg/kg)	Day -4, Day -3	6

Randomization of animals and housing cages occurred prior to drug treatments.

CNV area- To calculate the CNV lesion area, animals were euthanized on day 8 and their eyes enucleated. The extent of CNV at the Bruch’s membrane rupture sites was measured by masked observers using computer-assisted image analysis of FITC-conjugated isolectin B4-stained choroidal flat-mounts. The areas for all lesions in a single eye were averaged and reported as a single data point. One-way ANOVA analysis was used to report p-value significances between treatment groups.

Acceptance Criteria

For each metric, statistically significant differences between treated and untreated groups had to demonstrate $p \leq 0.05$.

Results and Discussion

Results for the lesion area for treatment according to design arm 1, 2 or 3 are shown in **FIG. 14**. One way ANOVA analysis demonstrated significant differences between vehicle treatment (arm 1) and the two treatment arms (arms 2 and 3), as shown below in Table 6.

5 **Table 6: Statistical comparison of differences between treatment groups in this study**

Group 1	Group 2	Absolute Difference	p-value
Vehicle	5mg/Kg D-3,D-4	0.0072	0.0120
Vehicle	5mg/Kg H-4, H+20	0.0108	0.0003

The “depot” group (receiving gedatolisib treatments on Day -3 and Day -4, before laser treatment) had 26% inhibition and the “standard” treatment group had 39% inhibition (difference 13%), suggesting that transient levels of circulating gedatolisib may inhibit CNV development.

10 However, there is no statistical difference ($p \sim 0.21$) between the two groups of rats that received gedatolisib on these two different administration schedules.

In summary, the results demonstrated that not only did gedatolisib treatment 4 hours prior and 20 hours after laser treatment lead to inhibition of NV, but treatment with gedatolisib 3 days prior to laser treatment also resulted in inhibition of NV, thereby demonstrating that the eye can
15 serve as a depot for gedatolisib to inhibit L-CNV development.

EXAMPLE 3: GEDATOLISIB TREATMENT REGRESSES ESTABLISHED
NEOVASCULARIZATION IN A WET AMD MODEL

Laser induced lesions in the rat eye, under different conditions such as ivt injections of
20 controls or drugs, spontaneously heal in a 4-8 week timeframe. The aim of the study described in this example was to demonstrate that gedatolisib, applied to the rat L-CNV model (described in Example 1) at days 6 and 7 after laser treatment (L6 and L7, typically when CNV is at a peak lesion area) significantly regresses leaky neovascularization (NV) in the period after laser-induced NV peaks and before natural NV regression occurs to any significant extent. Lesion data
25 was collected using two metrics. The first metric was using IB4 staining of choroid flat mounts of the eyecups harvested at the end of the study (Day 15 or D15). The second metric was to use quantitative fluorescein angiograms (qFA) to measure vascular leakiness on live animals during the course of the study.

As described in detail below, the data demonstrates that gedatolisib (at a dose of 5mg/kg x2) can regress established NV lesions as compared to vehicle control as measured using either qFA or IB4 flat mount metrics.

5 **Methods**

Model Generation and Treatment

Laser-induced choroidal neovascularization (LCNV) was generated in 6-week-old male Brown Norway rats on experiment Day 0 (D0). Animals were anesthetized with an intraperitoneal (IP) injection of ketamine/xylazine, and 1% tropicamide was applied topically to dilate the pupils. Using a hand-held cover slip as a contact lens and GenTeal lubricating eye gel as a medium contacting the cover slip to the surface of the cornea, a Nidek GYC- 500 green laser photocoagulator coupled to a Nidek SL-1800 slit-lamp was used to create four lesions equidistant from the optic nerve head in the retinal midperiphery. Laser parameters included 532 nm wavelength, 120 µm spot size, 0.1 sec duration, and 120 mW power. Treatments were administered via tail vein injection, with dose timing and doses outlined in Table 7:

Table 7: Experimental Design

Arm	Treatment	Treatment Timing	FA* at 4 times (n = eyes)	HistologyIB4 (n = eyes)	Histology RPE-65/ α -SMA/DAPI (n = eyes)
1	Vehicle Control	D6, D7	6	6	6
2	Gedatolisib (2mg/kg)	D6, D7	6	6	6
3	Gedatolisib (5mg/kg)	D6, D7	6	6	6

Treatments were randomized between animals and housing cages.

20 *Fluorescein Angiography*

On D6, D9, D12, and D15, fluorescein leakage area were assessed on live animals via fluorescein angiography (FA) using the Phoenix Micron IV. Animals were anesthetized with an IP injection of ketamine/xylazine and 10% sodium fluorescein was administered via IP injection at 1µL/g BW. Fluorescent fundus images were captured with a Micron IV imaging system for the right eye of each animal at three minutes and five minutes post-fluorescein injection. ImageJ

software was used by a masked observer to quantify the area of fluorescein leakage for each lesion. As FA is a survival procedure, the same animals were also used for later histology.

Histology

On D15, animals were euthanized, their eyes were enucleated, and choroidal tissue was dissected. For one eye per rat, the choroid was stained with anti-RPE-65 antibody, anti- α -SMA antibody, and DAPI and the non- RPE portion of the lesion were quantified. The contralateral choroid was stained with IB4 for computer- assisted quantification of the lesion area.

Acceptance Criteria

For each metric, statistically significant differences between treated and untreated groups had to be demonstrated $p \leq 0.05$.

Results and Discussion

The results for lesion size as measured by qFA over the 15 day experimental study are shown in **FIG. 15A**. The largest change in difference between vehicle and gedatolisib treated lesions occurred between D6 (first treatment day) and D9 for the 5mg/Kg treated lesions. The rate of mean area change for the vehicle lesions was $8,572 \mu\text{m}^2$ per day during this time period whereas the drug treatment demonstrated a rate of mean area change for the 5mg/Kg gedatolisib treated lesions of $14,651 \mu\text{m}^2$ per day, reflecting a 71% increase in lesion regression rate over this time period.

The percent regression in the gedatolisib treated lesions, as compared to vehicle, measured by qFA analysis is summarized below in Table 8:

Table 8: % inhibition by qFA analysis of NV in rat regression model

Day post laser	% regression compared to vehicle	
	Gedatolisib 2X2mg/kg	Gedatolisib 2X5mg/kg
Day 6#	0	0
Day 9	0.95	46.22*
Day 12	8.24	39.61
Day 15	0.96	49.93*
* $p < 0.05$		

#Day 6 measurements taken prior to dosing with gedatolisib
--

The results for lesion size as measured by IB4 staining of flatmounts on day 15 are shown in **FIG. 15B**. Statistically significant regression was demonstrated for the 2X5mg/kg dose

Thus, both the qFA measurements as well as the IB4 staining demonstrated significant
5 inhibition of NV during the 15 day study. Although some normal (vehicle-treated) decreased NV was evident at all the timepoints in the qFA analysis, statistical significance was only shown on D15 between the 5mg/kg arm and the vehicle and between the 2mg/kg arm on D9. Analysis of the slopes of the vehicle and 5mg/ml groups in the qFA data indicates that the differences in slope are not statistically significant due to variances for both treatment groups. IB4 staining at
10 D15 showed 24% regression ($p < 0.05$).

In summary, the data demonstrates that gedatolisib treatment (at 5 mg/kg dosage) was able to regress established NV in the L-CNV model, particularly at the early stage of regression, as compared to the vehicle control.

15

EXAMPLE 4: GEDATOLISIB EXHIBITS EFFICACY DOSE RESPONSE RELATIONSHIP IN DEPOT RAT LCNV MODEL

The present study evaluated several criteria for the treatment effect of gedatolisib on CNV area using the depot dosing described in Example 2 above:

- 20
1. Determined the IC₅₀ of ~8mg/Kg total exposure (basis of 2x 4mg/Kg) when gedatolisib is resident as depot in the eye and there is no gedatolisib in the circulatory system using an intravenous (i.v.) route of administration.
 2. Determined the amount and locations of gedatolisib within the eye when administered i.v. at 12mg/kg.
 - 25 3. Evaluated the influence of how creating a laser hole affects the distribution of gedatolisib within the eye when administered by iv 3 days before laser treatment.
 4. Evaluated intramuscular route of administration of gedatolisib.

The present study used the “depot” dosing described in Example 2 above to generate a gedatolisib dose response curve vs efficacy (reduction of L-CNV compared to vehicle control), to confirm the efficacy of the intramuscular route of gedatolisib administration compared to the i.v. administration route, and to determine the locations and concentrations (pre-retinal or post-Bruchs, i.e. either side of the blood brain barrier) of the depot for gedatolisib within different sections of eye tissue.

Experimental Design

The experimental design was as shown below in Table 9:

Table 9 -Description of Study Arms

Experimental Design Arm	Laser Application, each rat	RoA and Treatment	Treatment Timing	# Rats	CNV Area (n = eyes)	Tissue Collection* (n = eyes)
1	Both eyes	IV Vehicle	D-4, D-3	3	6	0
2	Both eyes	IV Gedatolisib (1 mg/kg)	D-4, D-3	3	6	0
3	Both eyes	IV Gedatolisib (3 mg/kg)	D-4, D-3	3	6	0
4	Both eyes	IV Gedatolisib (6 mg/kg)	D-4, D-3	3	6	0
5	Both eyes	IV Gedatolisib (9 mg/kg)	D-4, D-3	3	6	0
6	Both eyes	IM Gedatolisib (6 mg/kg)	D-4, D-3	3	6	0

*Retina, choroid, and remnant eye tissue will be dissected and collected for each eye.

Methods –

Using the methods described in Example 2 above, a dose response versus efficacy study was performed. Drug was administered 3 days before laser treatment.

Results & Discussion

Gedatolisib was administered in increasing mg/Kg and mean LCNV lesion area measured by IB4 flatmount as described previously. As shown in Table 10 below, the mean lesion area decreased at doses of 12 mg/kg or 18 mg/kg.

Table 10: Mean lesion area is reported versus the amount of drug administered.

Dose mg/Kg	Mean Lesion Area	stdev
0	0.025	0.005
2	0.026	0.009
6	0.023	0.008
12	0.015	0.007
18	0.016	0.004

As shown in **FIG. 16A**, at 2mg/Kg and 12mg/Kg a statistically significant reduction in lesion area is demonstrated.

- 5 As shown in **FIG. 16B**, a typical sigmoid dose response was demonstrated for intravenous administration of gedatolisib reduction in the lesion area. An IC₅₀ of 8mg/Kg gedatolisib and IC₈₀ of 10mg/Kg was estimated from the sigmoid dose response data.

As shown in **FIG. 17**, gedatolisib administered at 12mg/Kg by intramuscular route employing a depot method demonstrated reduction of LCNV area.

- 10 Mass spectroscopy data for laser treated and laser untreated sections of the eye are shown below in Table 11:

Table 11: Mass spectroscopy data for laser treated and laser untreated sections of eye

Sample ID	Gedatolisib Treatment	Lasered/ Unlasered	Time Point	Concentration (pg/mL)	Tissue Mass (mg)	Tissue Concentration (pg/g)	nM	Ave (nM)	SD
1L retina	12 mg/kg IV	Unlasered	--	5873.88	22.53	64612.66	107.90	130.62	16.77
1R retina	12 mg/kg IV	Lasered	--	5863.62	17.95	64499.83	107.71	131.01	18.79
2L retina	12 mg/kg IV	Unlasered	--	8042.21	17.22	88464.31	147.74		
2R retina	12 mg/kg IV	Lasered	--	6892.18	20.50	75813.94	126.61		
3L retina	12 mg/kg IV	Unlasered	--	7432.41	16.55	81756.46	136.53		
3R retina	12 mg/kg IV	Lasered	--	8296.12	16.37	91257.35	152.40		

4L retina	12 mg/kg IV	Unlasered	--	7094.54	17.77	78039.95	130.33		
4R retina	12 mg/kg IV	Lasered	--	7474.28	17.40	82217.07	137.30		
1L choroid	12 mg/kg IV	Unlasered	--	37733.16	4.35	1169727.84	1953.45	2295.32	469.8
1R choroid	12 mg/kg IV	Lasered	--	29343.84	6.03	909659.03	1519.13	2129.74	453.2
2L choroid	12 mg/kg IV	Unlasered	--	35329.50	5.60	1095214.38	1829.01		
2R choroid	12 mg/kg IV	Lasered	--	45562.60	3.95	1412440.74	2358.78		
3L choroid	12 mg/kg IV	Unlasered	--	51674.88	4.25	1601921.16	2675.21		
3R choroid	12 mg/kg IV	Lasered	--	49517.29	5.00	1535036.08	2563.51		
4L choroid	12 mg/kg IV	Unlasered	--	52609.70	3.66	1630900.64	2723.60		
4R choroid	12 mg/kg IV	Lasered	--	40129.89	5.12	1244026.48	2077.52		
1L OT	12 mg/kg IV	Unlasered	--	21812.03	99.30	87248.12	145.70	160.64	10.93
1R OT	12 mg/kg IV	Lasered	--	24236.28	99.70	96945.12	161.90	162.03	7.21
2L OT	12 mg/kg IV	Unlasered	--	23982.11	106.29	95928.46	160.20		
2R OT	12 mg/kg IV	Lasered	--	25728.00	110.36	102912.01	171.86		
3L OT	12 mg/kg IV	Unlasered	--	24768.74	105.90	99074.95	165.46		
3R OT	12 mg/kg IV	Lasered	--	23904.13	108.66	95616.53	159.68		
4L OT	12 mg/kg IV	Unlasered	--	25631.58	111.37	102526.33	171.22		
4R OT	12 mg/kg IV	Lasered	--	23156.85	119.70	92627.39	154.69		

- There is no significant difference between the amounts of drug found in the different sections of the eye between the two different laser treatment groups, treated vs untreated. The conclusion is that the study method of laser treatment does not affect the amount of drug that reaches the
- 5 different tissues of the eye when gedatolisib is administered by intravenous route three days before laser treatments.

Conclusions

- Gedatolisib exhibited a dose response relationship with efficacious effect in the rat
- 10 LCNV model.

Gedatolisib administered by intravenous or intramuscular routes reached significant levels in different tissues of the eye.

The laser method employed in the study did not interfere with gedatolisib accessing different tissue layers of the eye.

**EXAMPLE 5: GEDATOLISIB EXHIBITS EFFICACY IN TOPICAL
ADMINISTRATION RAT LCNV MODEL**

Topical administration of ocular therapies has several advantages, as described previously. This study demonstrates that a formulation of gedatolisib can be administered topically and has efficacy in a LCNV model of wet AMD.

Experimental Design

The experimental design for this study is shown below in Table 12:

Table 12: Description of Study Arms

Experimental Design Arm	Laser Application, each rat	RoA and Treatment	Treatment Timing	# Rats	CNV Area (n = eyes)	Tissue Collection* (n = eyes)
1	Both eyes	Topical drops with clinical formulation 5mg/ml BID	D-1, D0, D1, D2, D3	4	4	4

*Retina, choroid, and remnant eye tissue will be dissected and collected for each eye.

Methods

Example 2 above details the methods employed in this study. Gedatolisib was administered to rats 5mg/mL 1- 5uL drop BID for 5 days total beginning one day before laser treatment and continuing four additional days. Mass spectroscopy methodology as described in previous Examples was used to determine the amounts of gedatolisib in different sections of the eye by the topical administration method.

Results and Discussion

The data demonstrate that topically administered gedatolisib leads to a reduction in LCNV lesions by attaining nanomolar concentrations in the eye.

Mass spectroscopy data for different eye sections following topical administration of gedatolisib are shown below in Table 13:

Table 13: Mass spectroscopy data for different eye sections following topical administration of gedatolisib.

Sample ID	Gedatolisib Treatment	Lasered/ Unlasered	Time Point	Concentration (pg/mL)	Tissue Mass (mg)	Tissue Concentration (pg/g)	nM	Ave (nM)	SD
19A Plasma	5 mg/mL Topical	--	1 hr	130.63	--	--	0.22	0.22	
19B Plasma	5 mg/mL Topical	--	1 hr	124.42	--	--	0.21	0.22	
25B Plasma	5 mg/mL Topical	--	1 hr	134.20	--	--	0.22	0.18	
25A Plasma	5 mg/mL Topical	--	2 hr	133.38	--	--	0.22	0.19	
26A Plasma	5 mg/mL Topical	--	4 hr	105.62	--	--	0.18	0.23	
27A Plasma	5 mg/mL Topical	--	8 hr	113.52	--	--	0.19	0.17	
26B Plasma	5 mg/mL Topical	--	12 hr	140.19	--	--	0.23		
27B Plasma	5 mg/mL Topical	--	24 hr	100.30	--	--	0.17		
25R retina	5 mg/mL Topical	Lasered	--	71.39	16.46	785.30	1.31	0.72	0.51
26R retina	5 mg/mL Topical	Lasered	--	27.95	16.15	307.45	0.51		
27R retina	5 mg/mL Topical	Lasered	--	19.00	15.55	209.02	0.35		
25R choroid	5 mg/mL Topical	Lasered	--	49.21	4.08	1525.52	2.55	2.03	0.46
26R choroid	5 mg/mL Topical	Lasered	--	32.32	4.55	1001.81	1.67		
27R choroid	5 mg/mL Topical	Lasered	--	36.09	4.56	1118.65	1.87		
25R OT	5 mg/mL Topical	Lasered	--	4068.66	113.55	16274.65	27.18	12.97	12.34
26R OT	5 mg/mL Topical	Lasered	--	1028.21	109.60	4112.83	6.87		
27R OT	5 mg/mL Topical	Lasered	--	729.36	113.12	2917.46	4.87		

The data showed that gedatolisib reached significant nanomolar levels in different sections of the eye and did not exceed ~200pM in the plasma.

FIG. 18 shows the analysis of LCNV area of individual raw data points (laser holes) from rat eyes treated with topically administered gedatolisib. The results shows that the gedatolisib treated eyes had a ~26% reduction in mean LCNV area (vehicle arm = 0.023 mm²; gedatolisib arm = 0.017 mm²; *p*-value = 0.012), when outlier data points were excluded.

Conclusions

Topical administration of gedatolisib has efficacy in the LCNV model of AMD.

**EXAMPLE 6: ASSESSMENT OF TOPICALLY ADMINISTRATED GEDATOLISIB
‘EYE DROPS’ REACHING POSTERIOR OF EYE**

5 The aim of the study in this Example was to determine if topically administered gedatolisib could distribute to an efficacious concentration in the posterior of the eye following application of a single drop into a rabbit eye. A second aim was to determine what types of formulation excipients could facilitate the distribution of gedatolisib to the posterior tissue of the eye.

10 Rabbits have relatively large eyes, sharing many anatomical features with humans, including eyeball size, its internal structure and optical system, biomechanical and biochemical features, as well as conjunctiva cavity volume (see e.g., Peiffer et al. (2013) *Models in Ophthalmology and Vision Research in The Biology of the Laboratory Rabbit* Oct 21:409–433). Rabbits share many common traits with humans, including similar physiology and heterogeneous
15 genetic background. Indeed, phylogenetically rabbits are closer to primates than to rodents (Graur et al. (1996) *Nature* 379: 333-335; Gelatt KN. *Essentials of veterinary ophthalmology*. Third edn: 2012).

B. Study Overview

20 4-6 month-old male Dutch Belted Rabbits (pigmented eye) received topical administration of either vehicle or Gedatolisib in one of the concentrations or formulations doses outlined below. Rabbits (4 eyes total) received 1 drop (35ul containing 350ug) of gedatolisib.

 A panel of formulations were tested with varying gedatolisib concentrations,
25 ranging from 4 mg/ml to 20.5 mg/ml and with different pharmaceutically acceptable excipients. Following treatment, tissue was collected from conjunctiva, retina, retinal pigment epithelium (RPE; pooled), iris/ciliary body, choroid, vitreous humor, aqueous humor, plasma and red blood cells. Tissue and blood samples were collected at the indicated number of minutes post drop administration directly onto the rabbit eye, as
30 shown in the results below.

Blood draws and tissue dissection for distribution analysis

5 Blood was collected on day 1 at the indicated minutes post-treatment into EDTA-coated tubes to prevent coagulation. Plasma was separated via centrifugation and flash-frozen, Animals were euthanized and their eyes enucleated. Tissue samples were isolated as described in the Specific Aims. All ocular samples from each eye were flash-frozen. All blood and ocular tissue samples was stored at -80°C until shipment on dry ice for further analysis.

Quantification of Drug Concentration in Eye Sections

10 Tissue and eye fluid harvest was done at the time points as indicated in **Table 15** below following a single dose of topical gedatolisib formulation. The ocular tissue was dissected and flash-frozen. Tissue was stored at -80°C until quantitative analysis of gedatolisib.

Acceptance Criteria

15 For each metric, statistically significant differences between treated and untreated groups must demonstrate $p \leq 0.05$.

For quantitative mass spectrometry, two standard deviations above the mean LOQ must be detected for indication of the presence of gedatolisib. **Table 14** shows the OpAns analytical standards testing for gedatolisib by mass spectroscopy.

20

Table 14: OpAns Analytical Standards Testing for Gedatolisib by Mass Spectroscopy

LLOQ*	5.00 pg/mL	8pM Gedatolisib
ULOQ	125,000.00 pg/mL	200nM Gedatolisib

E. Analytical Method for Quantification of Gedatolisib

25

a. Standards and Sample Preparation

Gedatolisib was prepared as an individual stock solution at 0.5 mg/mL in dimethylsulfoxide (DMSO) by sonication for approximately 10 minutes (until solution became clear. The stock solution was then further diluted in DMSO to create a 2 µg/mL

diluted stock solution. Working solutions for use in calibration and quality control samples were prepared in DMSO:Acetonitrile (1:1, vol:vol).

Calibrators, quality control samples (QCs), and blanks were made by dilution of working solutions using Methanol:Water, (1:1, vol:vol). The calibration range for

- 5 Gedatolisib used in this method was 5 pg/mL to 2500 pg/mL. Stable isotopic labelled internal standard was added to calibration standards, quality control, and matrix samples.

Tissue samples were processed by disruption with Qiagen TissueLyser. Internal standard solution was added to each eye in a 2 mL round bottom tube and incubated for 10 minutes at room temperature. Addition of a 5mm stainless steel bead and 95%

- 10 Methanol:5% DMSO was followed by processing at 30/sec for 1 minute and a 10-minute cycle at 20/sec. Supernatant from centrifugation of homogenates were transferred for LC-MS/MS analysis.

b. Chromatographic and Mass Spectrometer Conditions

- Chromatographic separations were performed using a 1290 series high pressure
15 liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) with a 3 X 50 mm reversed phase column at 40°C.

- Mass spectrometric analysis was performed using an Agilent 6495 Series Triple
Quadrupole tandem mass spectrometer equipped with an Agilent Jet Stream
Electrospray source operated in the positive ion mode and using nitrogen as the carrier
20 gas set at the flow rate of 12 L/min. The gas temperature was set at 200°C, nebulizer at 45 psi, and capillary voltage at 3500 V. At these settings, the analyte was detected in its protonated form ($[M + H]^+$). Sample analysis was carried out in the Dynamic MRM mode. The transitions monitored for Gedatolisib were 616.3→488.3 (Quantifier) and 616.3→367.2 (Qualifier); similarly for [2H9] Gedatolisib, the Quantifier transition was
25 625.0→492.2 and the Qualifier was 625.0→124.2.

c. Data Acquisition and Processing

- HPLC/MS-MS data were acquired using the proprietary software application
MassHunter Workstation Data Acquisition for Triple Quad B.07.01/Build 7.1.7112.0,
Agilent Technologies, Inc.). Data were processed (integrated) using the software
30 application MassHunter Quantitative Analysis for QQQ (version B.07.01/Build

7.1.524.0. Agilent Technologies, Inc.). Calibration plots of area ratio versus analyte concentrations were constructed and the analyte-specific model was applied to the data using MassHunter Quantitative Analysis for QQQ. The regression model used was: power, weighted $1/x^2$.

- 5 Samples were tested against calibration curve requested for original development efforts (5 - 2500 pg/mL) and were all found to be acceptable quality level (AQL). Interpolated results (extrapolated) indicated results 10-40x the upper limit of quantification (with only replicate one of the calibration curve analyzed). Additional Calibration Standards were prepared to extend the range for analysis to 125,000 pg/mL.
- 10 Replicate injections of the additional standards were included at the end of the run. Extension of the calibration range generated calibration curve with good fit. Sample results were not impacted significantly by carryover, and the extended calibration curve was not impacted.

15 F. Results

a. *Results for Distribution*

- Table 15** below details the concentration of gedatolisib measured in eye tissue and plasma following administration of a single topical drop (35uL/eye) of Formulation #1-#5 or
- 20 Formulation #6-#10 measured at the indicated time after treatment.

Table 15: Gedatolisib Concentration in Indicated Tissues after Topical Treatment with Formulations

Study Arm	RPE (nM)		Choroid (nM)		Plasma (nM)	
	20 min	100 min	20 min	100 min	20 min	100 min
#1 GEDA at 10 mg/ml	7.25	4.66	16.62	6.00	0.95	0.56
#2 GEDA at 10 mg/ml	15.10	7.17	11.84	47.73	0.80	1.40
#3 GEDA at 10 mg/ml	2.74	1.58	5.61	6.79	1.30	0.62
#4 GEDA at 10 mg/ml	7.03	2.32	7.19	8.25	1.29	1.42
#5 GEDA at 10 mg/ml	4.75	4.35	3.01	7.97	0.92	0.39

Study Arm	RPE (nM)	Choroid (nM)	Plasma (nM)
-----------	----------	--------------	-------------

	30 min	30 min	30 min
#6 GEDA at 18.5mg/mL	385.34	143.74	0.81
#7 GEDA at 20.5 mg/mL	12.30	28.44	0.26
#8 GEDA at 4 mg/mL	8.14	18.82	1.25
#9 GEDA at 10 mg/mL	144.32	40.37	0.80
#10 GEDA at 20.5 mg/mL	3.14	14.18	5.60

f. Discussion

Gedatolisib was demonstrated to achieve efficacious concentrations in the posterior rabbit eye in tissues associated with wet AMD, including the retinal pigment epithelium (RPE) and the choroid from which the neo-vascular endothelial cells disseminate through Bruch’s membrane and then through the RPE. The result was achieved with all different formulations tested.

g. Conclusion:

The study demonstrated that gedatolisib was detectable in the posterior eye tissue of rabbits following a single drop topical administration using a variety of different formulations with gedatolisib concentrations ranging from 4 mg/ml to 20.5 mg/ml. A relationship was demonstrated for gedatolisib concentrations detected in tissues from rabbit eyes at less than 2 hrs post topical administration. Thus, the study demonstrated that gedatolisib administered by topical application achieved nanomolar levels >IC50 in RPE and choroid tissues at the posterior of the eye where dysfunction in many eye diseases is found.

EXAMPLE 7: ASSESSMENT OF TOPICALLY ADMINISTARTED GEDATOLISIB ‘EYE DROPS’ EFFICACY IN RABBIT AMINO-ADIPIC ACID TREATED RETINAL NEOVASCULARIZATION MODEL

The objective of this study is to evaluate the efficacy of gedatolisib in a AminoAdipicAcid-induced retinal leakage model in Dutch Belted rabbits. The number of

animals, data collection time points, and parameters for measurement were chosen based on the minimum required to meet the objectives of this study.

A. Study Overview

4-6 month-old female Dutch Belted Rabbits (pigmented eye) received topical
5 administration of either vehicle or Gedatolisib in a formulation at 4mg/mL
concentration. Rabbits (16 eyes total, 8 eyes each arm) received either vehicle or 2 drops
QD (2x 35ul containing 280ug) of gedatolisib for seven days.

Formulations

10 Arm #1. Vehicle
Arm #2. 4 mg/mL Gedatolisib Formulation

Abbreviations- QD- Once Daily

15 B. Methods

Model Induction Procedures: Intravitreal Dosing of DL-AAA:

Procedure: Following anesthetic induction, eyes were aseptically prepared by application
of topical 5% betadine solution, rinsing with sterile eye wash, and administration of one drop of
20 topical 0.5% proparacaine HCl and 2.5% or 10% phenylephrine HCl. The procedure will be
aseptically performed and the injection of DL-AAA (aminoadipic acid) will be made using a 30-
31 G needle, 2-3 mm posterior to the superior limbus (through the pars plana), with the needle
pointing slightly posteriorly to avoid contact with the lens. Following the injection procedure, 1
drop of Neomycin Polymyxin B Sulfates Gramicidin ophthalmic solution or Ofloxacin will be
25 applied topically to the ocular surface. Animals may be given atipamezole (1-2 mg/kg, 0.2-1.0
mL, IM) if needed to reverse the anesthetic effects. Animals will be returned to their cages and
monitored every 15 minutes until recovered.

Fluorescein Angiography (FA), Spectralis (OS-induced eye only) for Groups 1-2:

30 *Procedure:* FA was done at the timepoints indicated utilizing the Spectralis HRA OCT
(Heidelberg). An IV butterfly catheter may be placed in the ear for infusion of sodium

fluorescein (~12 mg/kg). The induced eye (OS) will be oriented so that the optic nerve head is at the 3 or 9 o'clock position to observe the retinal leakage extending from one of the medullary arrays. . Intensity settings should be kept constant for all animals at all timepoints. Initial model induction will be evaluated, and animals will be enrolled based on induction of retinal leakage.

- 5 Animals were also stratified into groups to ensure an equal balance of induction phenotypes within each group. Following completion of FA procedures, animals were given atipamezole (1-2 mg/kg, 0.2-1.0 mL, IM) if needed to reverse the anesthetic effects.

Quantitative Retinal Leakage (Corrected Total Regional Fluorescence

10 Measurements) for Groups 1-2:

- A single frame from the induced eyes from each timepoint of the fluorescein angiography image set was selected based on the retinal vasculature flanking the ONH to the left and right. Care will be taken to use the same time elapsed after fluorescein injection (30s) in each animal. Using ImageJ software, a rectangular Region of Interest (ROI) of standardized dimensions will
- 15 be placed over the secondary and tertiary vascular branches, with the long axis of the ROI set perpendicular to the plane bifurcating left and right vasculature (Measurement ROI) A second ROI of standard dimensions will be positioned alongside the Measurement ROI to capture local background signal (Background ROI). Signal thresholding functions in ImageJ will be used to bound the hyperfluorescent vasculature and leakage signal within the Measurement ROI. Metrics
- 20 quantifying fluorescein signal (including, but not limited to, mean intensity, integrated density, and area) will then be extracted. The mean fluorescence intensity from the Background ROI will also be taken. This process will be repeated for the vascular density opposite the ONH. After all measurements were collected, Corrected Total Region Fluorescence (CTRF) was computed. For each measurement (two per eye), $CTRF = (IntegratedDensityTHRESHOLD) -$
- 25 $(BackgroundMEAN \times AreaTHRESHOLD)$, to quantitate the background-subtracted sum of all hyperfluorescent pixel intensities. Secondary metrics such as Total Leakage Area (% of Thresholded Pixels within the standard Measurement ROI boundary) were computed if deemed potentially informative. Data was represented as Mean +/- Standard Deviation with data aggregated by animal.

Fluorescein Angiographic Images: Images were assessed (masked) and scored for area of maximal fluorescein leakage for each lesion. Averages per group were graphed as score vs. timepoint.

5 C. Results for Efficacy

Table 16: below details the efficacy of Gedatolisib (Group 2) compared to vehicle control (Group 1) at 68.3% reduction in FA leakage after 7 days of treatment.

Group	Condition	Day (-)3		Day 8		Day-3 to Day 8 Difference %
		FA Leak (AU)	Std Dev	FA Leak (AU)	Std Dev	
#1	Vehicle	148.9	20	143	12	N.C.
#2	4mg/mL gedatolisib	148	28.7	46.9	23.2	- 68.3

10 D. Discussion

Topically administered Gedatolisib was previously demonstrated to achieve efficacious concentrations in the posterior rabbit eye in tissues associated with wet AMD, including the retinal pigment epithelium (RPE) and the choroid from which the neo-vascular endothelial cells disseminate through Bruch’s membrane and then through the RPE, as described in Example 6.

15 The result of this example demonstrates that the presence of gedatolisib administered topically to the rabbit eye distributing to the posterior eye significantly reduces FA leakage associated with retinal neovascularization.

 E. Conclusion:

20 The study demonstrated that gedatolisib administered by topical “drops” for seven days achieved significant reduction in FA leakage of retinal neovascularization.

 It will be understood by those of skill in the art that numerous and various modifications
25 can be made without departing from the scope and spirit of the present disclosure. Therefore, it

should be understood that various embodiments of the invention described herein are illustrative only and not intended to limit the scope of the invention. All references cited herein are hereby incorporated by reference in their entirety.

We claim:

1. A method of treating an ocular disease in a human subject, wherein the method comprises:
 - (a) selecting a human subject in need of treatment of an ocular disease; and
 - (b) administering to the human subject a therapeutically effective amount of gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof.
2. A method for treating a human patient who has been determined to have an ocular disease, the method comprising administering to the patient gedatolisib, or a pharmaceutically acceptable salt, solvate, or ester thereof, in an amount and with a frequency sufficient to treat the ocular disease.
3. The method of claim 1 or 2, wherein gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered intravenously.
4. The method of claim 1 or 2, wherein gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered topically onto at least one eye of the subject.
5. The method of claim 1 or 2, wherein gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered intraocularly, intravitreally, suprachoroidally, or intravascularly.
6. The method of any one of claims 1-5, wherein gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, is administered at a dose of 0.001-5 mg/kg.
7. The method of any one of the preceding claims, wherein the ocular disease is selected from the group consisting of neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization, macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.

8. The method of claim 7, wherein the choroidal neovascularization disease is selected from the group consisting of myopic choroidal neovascularization (mCNV), angioid streaks, choroiditis (e.g., choroiditis secondary to ocular histoplasmosis), idiopathic degenerative myopia, retinal dystrophies, rubeosis iridis, pseudoxanthoma elasticum, and trauma.

9. The method of claim 7, wherein the ocular disease is neovascular age-related macular degeneration.

10. The method of claim 9, wherein the treatment results in an improvement of one or more symptoms selected from the group consisting of visual distortions, reduced central vision in one or both eyes, the need for brighter light when reading or doing close-up work, difficulty adjusting to low light levels, increased blurriness of printed words, difficulty recognizing faces, and a well-defined blurry spot or blind spot in the field of vision, compared to baseline.

11. The method of claim 9 or 10, wherein the treatment results in an improvement in vision as assessed by an ETDRS test via the “Number of Letters” scoring method.

12. The method of any one of claims 9-11, wherein the treatment results in an improvement in vision, as assessed by a “Best-Corrected Visual Acuity” test.

13. The method of claim 12, wherein the treatment results in a “doubling of the visual angle” in “Best-Corrected Visual Acuity” at 9 months after the start of treatment.

14. The method of any one of claims 10-13, wherein the improvement is assessed by diagnosed by eye dilation and visual examination, optical coherence tomography (OCT), an Amsler grid, indocyanine green angiography, and/or fluorescein angiography.

15. The method of claim 9, wherein the treatment results in a reduction in fluid, blood, and/or drusen compared to baseline.

16. The method of any one of the preceding claims, further comprising administering an additional therapy or therapeutic agent in combination with the gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof.

17. A kit for treating an ocular disease in a human patient, the kit comprising:
- (a) one or more doses of gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof; and
 - (b) instructions for using the gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof in the method of any one of the preceding claims.

18. The kit of claim 17, wherein the ocular disease is selected from the group consisting of neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization, macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.

19. Gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, for use in treating an ocular disease in a human patient.

20. Gedatolisib, or pharmaceutically acceptable salt, solvate, or ester thereof, for use according to claim 19, wherein the ocular disease is selected from the group consisting of neovascular age-related macular degeneration, diabetic macular edema, choroidal neovascularization, macular edema following retinal vein occlusion, central retinal vein occlusion, cystoid macular edema, glaucoma (*e.g.*, neovascular glaucoma), polypoidal choroidal vasculopathy, retinopathy of prematurity, ocular von Hippel Lindau disease lesions, diabetic retinopathy, and retinitis pigmentosa.