(51) International Patent Classification: A61K 45/00
   A61P 27/00

(21) International Application Number: PCT/CA02/01859

(22) International Filing Date: 29 November 2002 (29.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
   60/335,287 30 November 2001 (30.11.2001) US

(71) Applicant (for all designated States except US): QLT, INC. [CA/CA]; 887 Great Northern Way, Vancouver, British Columbia V5T 4T5 (CA).

(72) Inventors; and
(75) Inventors/Applicants (for US only): MARGARON, Philippe, Maria, Clotaire [CA/CA]; 7128-18th Avenue, Suite 35, Burnaby, British Columbia V3N 1H1 (CA). TAO, Jing-Song [CA/CA]; 2450 West 18th Avenue, Vancouver, British Columbia V6L 1B1 (CA).

(74) Agents: ROBINSON, Christopher, J. et al.; Fetherstonehough & CO, 650 West Georgia Street, Suite 2200, Box 11560, Vancouver Centre, Vancouver, British Columbia V6B 4N8 (CA).


(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published: with international search report

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

(54) Title: TREATMENT OF NEOVASCULAR OPHTHALMIC DISEASE

(57) Abstract: Inhibitors of integrin-linked kinase (ILK) are used in the treatment of various eye diseases with underlining pathology of neovascularization of cornea, iris, retina or choroid.
TREATMENT OF NEOVASCULAR OPHTHALMIC DISEASE

TECHNICAL FIELD

The invention relates to the use of small molecule inhibitors of integrin-linked kinase (ILK) in the treatment of various eye diseases with underlying pathology of neovascularization of cornea, iris, retina or choroid.

BACKGROUND OF THE INVENTION

Vision is fundamentally important throughout life. However, the eye can be a fragile organ, and is susceptible to a number of hereditary and/or age related degenerative disorders. In the United States, a common cause of irreversible blindness or severe loss of vision is retinal dystrophies. The retina is the sensory tunic of the eye, containing light sensitive receptors, a complex of neurons, and pigmented epithelium, arranged in discrete layers. In humans, the macula is the portion of the retina that lies directly behind the lens. Cones, the photoreceptor cells responsible for central vision, are heavily concentrated in the macula. The peripheral retina is composed mainly of rods, which are responsible for side and night vision.

Neovascularization occurs in many eye diseases. Due to its significant epidemiology impact especially among aging population, it is becoming an important public health issue. Neovascularization, a pathological change characterized by an uncontrolled growth of vascular tissue, could occur in various parts of eye, including the cornea, iris, retina, and choroid. The consequences of neovascularization within these delicate ocular tissues are fibrosis, exudation, and/or hemorrhage that are responsible for vision loss in many common eye diseases.

Corneal neovascularization is characterized by invasion of vascular capillaries from the limbal vascular plexus into normally avascular cornea. In some cases, corneal neovascularization is associated with a decrease in visual acuity. The etiology of the corneal neovascularization is not fully understood. However, it is generally considered as a consequence of mechanical or chemical injury, or secondary to infection. Corneal neovascularization is associated with a variety of clinical conditions including contact lens wear, trauma and prior surgery (e.g., corneal transplant), viral, bacterial or protozoa infections, alkali burns and some immunologic diseases.

Iris neovascularization is characterized by the formation of leaky new blood vessels on the anterior surface of the iris and in the chamber angle recess. In the late stage of the disease, the vessels are enlarged and are accompanied by fibrous tissue, hence occluding the angle and causing the secondary neovascular glaucoma, a condition characterized by
high intraocular pressure, neovascularization of the iris and trabacular meshwork. Iris neovascularization and consequent neovascular glaucoma respond poorly to therapies and are frequent causes of blindness and enucleation. Iris neovascularization is associated with a variety of systemic and ocular diseases and secondary to trauma or therapies including surgery and radiation. Central retinal vein occlusion and diabetes mellitus are considered as leading causes of iris neovascularization.

Neovascularization of the retina involves the growth of new capillaries from the vessels that arise from the optic disk or inner retina. In the later stage, vision loss may occur due the development of various complications including scarring, tractional detachment of the retina, and hemorrhage.

Retinal neovascularization is associated with a variety of ocular and systemic diseases. Among those, diabetes mellitus, retinopathy of prematurity, central retinal vein occlusion, branch retinal vein occlusion and sickle cell disease are most frequently associated with retinal neovascularization.

Choroidal neovascularization (CNV) is characterized by an invasion of new blood vessels through Bruch's membrane. The consequence of CNV is severe and irreversible vision loss. CNV is associated with a variety of ocular diseases including degenerative conditions, inflammatory or infectious diseases and trauma. Age-related macular degeneration (AMD), angiod streaks, pathological myopia, ocular histoplasmosis syndrome, sarcoidosis and chronic uveitis are just a few examples of ocular conditions with choroidal neovascularization as a significant underlining pathological change.

The current treatment for many forms of ocular neovascularization involves photocoagulation or cryotherapy. Pan-retinal or focal photocoagulation is current standard therapy for diabetic retinopathy. It is partially effective in reducing the rate of vision loss in patients with diabetic retinopathy. Photocoagulation is also a destructive treatment with unwanted side effects, such as CNV, subretinal fibrosis, photocoagulation scar expansion, and inadvertent foveolar burns, that can cause loss of central visual acuity and scotoma formation. Patients with good visual acuity are less likely to recognize the benefits from this aggressive treatment and more likely to notice its side effects, which can include some loss of central and peripheral vision, and a reduction in color and night vision.

Visudyne™ therapy, an ocular application of photodynamic therapy (PDT) using photosensitizer verteporfin (Visudyne™, Novartis Ophthalmics) and red light (690 nm) is a treatment of choice for patients with predominantly classic lesions of the wet form of AMD. It is also effective in treating occult subfoveal choroidal neovascularization secondary to AMD. However, in many patients the treatment needs to be repeated at three-month intervals over the subsequent one to two years, due to recurring CNV. The causes of the
recurring vascular growth are not fully understood and may involve multiple factors including the induction of growth factors or inflammatory mediators following PDT.

The further development of treatments for neovascular ophthalmic disease is of great interest.

5

SUMMARY OF THE INVENTION

Methods and compositions are provided for a safe and effective pharmacologic treatment for all the eye diseases with an underlining pathology that is characteristic of ocular neovascularization. Treatment includes the administration of agents that interfere with the ILK signaling pathway, including integrin linked kinase (ILK) blocking agents; compounds that otherwise prevent the binding of natural ILK ligands to ILK; or compounds that prevent expression of, or signaling through, ILK. Such a treatment is used alone as single therapy or in combination with a second therapy as an adjunct to prevent, to reduce or to reverse the loss of visual acuity as well as loss of vision secondary to neovascularization of cornea, iris, retina or choroid.

In one aspect, the invention is directed to a method to prevent, to reduce or to reverse ocular neovascularization in an eye of an animal having a neovascular lesion, comprising the steps of identifying said lesion in the eye of the animal, administering to the animal an amount of small molecule inhibitor of ILK sufficient to allow said compound to localize in said lesion.

In another aspect, the invention is directed to a method of preventing, reducing or reversing loss of visual acuity or vision loss in a patient having corneal, iris, retinal or choroidal neovascularization lesion associated with various eye diseases, comprising the steps of administering an amount of a ILK inhibitor sufficient to allow said inhibitor to localize in said lesion. In a preferred embodiment, an ILK inhibitor will be administered alone as single therapy. In another preferred embodiment, an ILK inhibitor will be administered at an appropriate time, before, concurrent or after, in relation to a second therapy including but not limited to photodynamic therapy such as Visudyne™ therapy, photocoagulation or transpupillary thermotherapy as an adjunct treatment for ocular neovascularization.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D show HUVEC human endothelial cells grown in a Matrigel support in the presence or absence of ILK inhibitor MC-1 (figures 1A and 1C), MC-2 (figures 1B and D) or solvent control (dimethyl sulfoxonamide, DMSO)
DETAILED DESCRIPTION OF THE EMBODIMENT

In the subject methods, compounds that modulate the activity of integrin linked kinase (ILK) are administered systemically or locally to treat ophthalmic diseases with an underlying pathology that is characteristic of ocular neovascularization. Such a treatment is used alone as single therapy or in combination with a second therapy as an adjunct to prevent, to reduce or to reverse the loss of visual acuity as well as loss of vision secondary to neovascularization of cornea, iris, retina or choroid.

In one aspect, the invention is directed to a method to prevent, to reduce or to reverse ocular neovascularization in an eye of an animal having a neovascular lesion, comprising the steps of identifying said lesion in the eye of the animal, administering to the animal an amount of small molecule inhibitor of ILK sufficient to allow said compound to localize in said lesion. Methods utilizing local administration that provides for a prolonged localized concentration, which may utilize sustained release implants, viscous solutions, or other topical formulation, are of particular interest. An ILK inhibitor can be administered alone as single therapy, or in combination with a second therapy, for example at an appropriate time, before, concurrent or after, in relation to a second therapy including but not limited to photodynamic therapy, including but not limited to Visudyne™ therapy, photocoagulation or transpupillary thermotherapy as an adjunct treatment for ocular neovascularization.

Some examples of ocular disorders that may be treated by various embodiments of the present invention include, without limitation: retinal diseases (diabetic retinopathy, diabetic macular edema, chronic glaucoma, retinal detachment, sickle cell retinopathy, age related macular degeneration (AMD) due to subretinal neovascularization); rubeosis iritis; inflammatory diseases; chronic uveitis; neoplasms (retinoblastoma, pseudoglioma); Fuchs' heterochromic iridocyclitis; neovascular glaucoma; corneal neovascularization (inflammatory, transplantation, developmental hypoplasia of the iris); neovascularization resulting following a combined vitrectomy and lensectomy; vascular diseases (retinal ischemia, choroidal vascular insufficiency, choroidal thrombosis, carotid artery ischemia); neovascularization of the optic nerve; and neovascularization due to penetration of the eye or contusive ocular injury.

ILK MODULATING AGENTS

ILK is a 59 kDa serine/threonine kinase that associates with the cytoplasmic tails of β1 and β3 integrins. The enzymatic activity for ILK is modulated by the interaction of cells with the extracellular matrix component fibronectin, integrin clustering and a number of growth factors. Because of its intimate association with a wide variety of signaling
pathways that have been directly or indirectly implicated in various pathological processes, ILK may represent a therapeutic target for a variety clinical conditions including angiogenesis, cancer, inflammation and autoimmunity. The genetic sequence of human ILK is disclosed in U.S. Patent nos. 6,013,782; and 6,001,622, herein incorporated by reference.

Agents that block ILK activity provide a point of intervention in an important signaling pathway. Numerous agents are useful in reducing ILK activity, including agents that directly modulate ILK expression, e.g. expression vectors, anti-sense specific for ILK, ILK specific antibodies and analogs thereof, small organic molecules that block ILK catalytic activity, etc.; and agents that affect ILK activity through direct or indirect modulation of [PtdIns(3,4,5)P_3 ] levels in a cell. For example, small molecule inhibitors of integrin linked kinase are described in U.S. Patent Nos. 6,214,813 and 6,436,915, 6,420,400 and in the respective Examples. Antisense inhibitors of ILK are described in U.S. Patent no. 6,177,273, each herein incorporated by reference.

Agents that block ILK activity are used in the treatment of ocular disease relating to neovascularization. Numerous agents are useful in reducing ILK activity, including agents that directly modulate ILK expression, e.g. anti-sense specific for ILK, ILK specific antibodies and analogs thereof, small organic molecules that block ILK catalytic or binding activity, etc.; and agents that affect ILK activity through direct or indirect modulation of [PtdIns(3,4,5)P_3 ] levels in a cell. For example, small molecule inhibitors of integrin linked kinase are described in U.S. Patent No. 6,214,813, in co-pending application U.S. Serial Number 09/747,563, and in the co-pending application entitled "Hydrazonopyrazole derivatives and their use as anti-proliferative agents". Antisense inhibitors of ILK are described in U.S. Patent no. 6,177,273, each herein incorporated by reference.

Agents of interest for down-regulating ILK activity include direct blocking of [PtdIns(3,4,5)P_3 ] binding sites through competitive binding, steric hindrance, etc. Of particular interest are antibodies that bind to the PH domains, thereby blocking the site. Antibodies include fragments, e.g. F(Ab), F(Ab)\', and other mimetics of the binding site. Such antibodies can be raised by immunization with the protein or the specific domain. Mimetics are identified by screening methods. Analogs of [PtdIns(3,4,5)P_3 ] that compete for binding sites but do not result in activation of ILK are also of interest.

Because ILK activity is upregulated by the presence of the lipid [PtdIns(3,4,5)P_3 ], the activity of ILK can be manipulated by agents that affect cellular levels of [PtdIns(3,4,5)P_3 ], or that block the binding of [PtdIns(3,4,5)P_3 ] to ILK. The amino acid sequence of ILK contains a sequence motif found in pleckstrin homology (PH) domains, which are involved in the binding of phosphatidylinositol phosphates. The activity of ILK is also down-regulated
by inhibiting the activity of PI(3) kinase, thereby decreasing cellular levels of [PtdIns(3,4,5)P₃]. Agents of interest include inhibitors of PI(3) kinase, e.g. wortmannin, LY294002, etc. Physiologically effective levels of wortmannin range from about 10 to 1000 nM, usually from about 100 to 500 nM, and optimally at about 200 nM. Physiologically effective levels of LY294002 range from about 1 to 500 μM, usually from about 25 to 100 μM, and optimally at about 50 μM. The inhibitors are administered in vitro or in vitro at a dose sufficient to provide for these concentrations in the target tissue.

Drug screening can be used to identify agents that modulate ILK function. One can identify ligands or substrates that inhibit the action of ILK. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. Knowledge of the 3-dimensional structure of ILK, derived from crystallization of purified recombinant ILK protein, leads to the rational design of small drugs that specifically inhibit ILK activity. These drugs may be directed at specific domains of ILK, e.g. the kinase catalytic domain, ankyrin repeat domains, pleckstrin homology domains, etc. Among the agents of interest for drug screening are those that interfere with the binding of cytoplasmic integrin tails to ILK; the kinase activity of ILK; binding of [PtdIns(3,4,5)P₃] to the PH domains of ILK and agents that inhibit the production of [PtdIns(3,4,5)P₃] by PI(3) kinase.

The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering the physiological function of ILK. Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Assays of interest may detect agents that block ILK function, such as integrin binding, kinase activity, down regulation of E-cadherin, up regulation of LEF-1, binding properties, etc. For example, an expression construct comprising a ILK gene may be introduced into a cell line under conditions that allow expression. The level of ILK activity is determined by a functional assay, as previously described. In one screening assay,
candidate agents are added, and the formation of fibronectin matrix is detected. In another assay, the ability of candidate agents to enhance ILK function is determined.

METHODS OF TREATMENT

The subject methods are used for prophylactic or therapeutic purposes to treat ocular diseases to prevent, reduce or reverse the loss of visual acuity as well as loss of vision secondary to neovascularization of cornea, iris, retina or choroid. Use used herein, the term “treating” is used to refer to both prevention of disease, and treatment of pre-existing conditions. While treatment during early stages is desirable, the adverse symptoms of the disease may be at least partially alleviated by treatment during later stages.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of an ILK inhibitor is administered to a subject afflicted with a disease or disorder related to neovascularization, or to a tissue that has been neovascularized. The inhibitor may be administered in accordance with the method of the invention either alone of in combination with other known therapies for neovascularization. When co-administered with one or more other therapies, the inhibitor may be administered either simultaneously with the other treatment(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administration, which may be before or after a second therapy.


When photodynamic therapy is used in conjunction with ILK inhibitors in the treatment of ocular neovascular diseases, a wide range of photosensitizers may be used,
including, but not limited to, hematoporphyrin derivatives, pheophorbides, chlorins, bacteriochlorins, phthalocyanines, purpurins, merocyanines, texaphyrins and green porphyrins, as well as protoporphyrin precursors such as aminolevulinic acid (ALA) and derivatives thereof. Each photosensitizer can be activated with light containing a wavelength absorbed by the photosensitizer. The photosensitizers may be administered locally or systemically, preferably by injection. The dose of ILK inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patent has undergone. Ultimately, the attending physician will decide the dose with which to treat each individual patient. Initially, the attending physician may administer low doses and observe the patient's response. Larger doses may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further.

Some diseases lend themselves to acute treatment while others require to longer term therapy. Proliferative retinopathy can reach a threshold in a matter of days as seen in ROP, some cases of diabetic retinopathy, and neovascular glaucoma. Premature infants are at risk for neovascularization around what would be 35 weeks gestation, a few weeks after birth, and will remain at risk for a short period of time until the retina becomes vascularized.

Suitable animal models exist for determination of appropriate dosage, although the efficacy of a therapeutic effect for different mammals varies widely, for example doses typically are 20, 30 or even 40 times smaller (per unit body weight) in man than in the rat. Similarly the mode of administration can have a large effect on dosage. A murine model of oxygen-induced retinal neovascularization has been established which occurs in 100% of treated animals and is quantifiable (Smith et al. (1994) Invest. Ophthalmol. Vis. Sci. 35:101-111). Bioactivity can be determined by methods including the Miles vessel permeability assay (Miles and Miles (1952) J. Physiol. (Lond.) 118:228), which measures vessel permeability, and endothelial cell mitogenicity, which measures cell growth. Other suitable models are set forth in the Examples.

The compounds of this invention can be incorporated into a variety of formulations for therapeutic administration. Administration of an ILK inhibitor may be by delivery using any appropriate means including, but not limited to, systemic, local, or even direct application to the target tissue. Local delivery of an ILK inhibitor provides a high local concentration while reducing the likelihood of non-specific anti-angiogenic or other undesirable side effects that may follow systemic administration of an ILK inhibitor.
For local application, a range of about 0.05 to 0.2 or about 0.5 to 2.0 mg/ml of an ILK inhibitor in an appropriate formulation is administered either intra-ocularly (intra-vitreous, subretinal, intra-anterior chamber, intra-scleral), peri-ocularly (topically onto the cornea, subconjunctival, subtenon, transcleral). For systemic application, a range of 0.05 to 100 mg/kg body weight, preferably less than about 10 mg/kg is administered.

For intra- or peri-ocular administration, an ILK inhibitor in an injectable formulation can be administered by either an intra-ocular injection at above-described concentrations and at a frequency of once every 2-6 months or by an intra-ocular implantation of a device or a specific formulation of an ILK inhibitor allowing sustained release of the ILK inhibitor over a period of time. For corneal application, an ILK inhibitor in an appropriate formulation can be applied topically onto the cornea at a frequency of once very 4-6 hours. For systemic application, an ILK inhibitor in appropriate formulation can be administered orally 1-3 times a day.

The compounds of the present invention are formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, etc., administration. The ILK may be systemic after administration or may be localized by the use of an implant that acts to retain the active dose at the site of implantation.

The compounds of the present invention can be administered alone, in combination with each other, or they can be used in combination with other known compounds and therapies. In pharmaceutical dosage forms, the compounds may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

For oral preparations, the compounds can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.
The compounds can be formulated into preparations for injections by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Implants for sustained release formulations are well-known in the art. Implants are formulated as microspheres, slabs, etc. with biodegradable or non-biodegradable polymers. For example, polymers of lactic acid and/or glycolic acid form an erodible polymer that is well-tolerated by the host. The implant is placed in proximity to the site of infection, so that the local concentration of active agent is increased relative to the rest of the body.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Some of the specific compounds are more potent than others. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given compound.

It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, constructs, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which scope will be determined by the language in the claims.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a mouse" includes a plurality of such mice and reference to "the cytokine" includes reference to one or more cytokines and equivalents thereof known to those skilled in the art, and so forth.
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned herein are incorporated herein by reference for all relevant purposes, e.g., the purpose of describing and disclosing, for example, the cell lines, constructs, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

**EXPERIMENTAL**

**EXAMPLE 1**

Treatment of AMD using an ILK inhibitor as an adjunct to Visudyne™ therapy

Therapeutic effect of an ILK Inhibitor in AMD is evaluated using visual acuity as the primary clinical outcome. Patients with subfoveal CNV lesions caused by AMD are examined for the presence of lesions that meet the inclusion criteria. The inclusion criteria are defined as the presence of lesions measuring 5400 μm or less in greatest linear dimension with evidence of classic CNV and best-corrected visual acuity of approximately 20/40 to 20/200 based on fluorescein angiographic and visual acuity examination. Those determined as qualified for the treatment of AMD are randomly assigned to 4 groups. Group A, B, and C are treated with standard Visudyne™ therapy with an adjunct therapy using an ILK inhibitor. Patients of Group D are treated with standard Visudyne™ therapy in combination with a placebo of the ILK inhibitor.

For standard Visudyne™ therapy, patients are administered 30 ml of Visudyne™ (0.15 mg per kilogram of body weight). The active ingredient in Visudyne™ is verteporfin,
also known as BPD-MA (U.S. 5,095,030). The administration is by intravenous infusion over a period of 10 minutes. Fifteen minutes after the end of the infusion, the laser light is applied for 83 seconds to the CNV lesion through a fundus contact lens of known magnification to result in a light exposure of 50 J/cm². A circular spot of approximately 6000 microns encompassing the area of the lesion is exposed to the laser light.

For the adjunct therapy, patients of groups A, B, and C receive a daily oral administration of an ILK inhibitor described in U.S. Patent No. 6,214,813, in co-pending application U.S. Serial Number 09/747,563, or in the co-pending application entitled “Hydrazonopyrazole derivatives and their use as anti-proliferative agents” at the dose of 5, 10, 20 mg per kilogram body weight, respectively. The adjunct treatment commences three days after the patient receives the standard Visudyne™ therapy and continues for a period of one month.

As follow-up, patients are examined every three months. At each regularly scheduled follow-up visit, best-corrected visual acuity measurement, contrast threshold measurement, ophthalmoscopic examination, stereoscopic fundus photography, and fluorescein angiography are performed.

Alternatively, patients having subfoveal CNV lesions caused by AMD receive a daily oral dose of an ILK inhibitor as described above as a stand alone therapy, but do not receive Visudyne™ therapy.

Example 2

Treatment of diabetic retinopathy using an ILK inhibitor

Therapeutic effect of an ILK inhibitor in proliferative diabetic retinopathy is evaluated using visual acuity as the primary clinical outcome. Patients with proliferative diabetic retinopathy and visual acuity of 20/100 or better in each eye are included in the clinical evaluation. Patients are randomly assigned to 3 treatment groups and 1 placebo group. Group A, B, and C are treated with daily oral administration of an ILK inhibitor as described in Example 1 at the dose of 5, 10, 20 mg per kilogram body weight. Patients of Group D receive placebo. The treatment continues for a period of 24 months.

As follow-up, patents are examined every 4 months. At each regularly scheduled follow-up visit, best-corrected visual acuity measurement, contrast threshold measurement, indirect ophthalmoscopic examination, stereoscopic fundus photography, fluorescein angiography, and slit-lamp examination using 78- or 90-diopter lens are performed.
Example 3
Evaluation of ILK expression in ocular vascular tissue

This example documents the discovery that ILK is a therapeutic target for diseases with underlying pathology of ocular neovascularization.

Post mortem baboon eye samples were subjected to immunohistological analysis for the expression of ILK in ocular vasculature. Freshly obtained tissues were snap-frozen by immersing into a Dewar of liquid nitrogen. Cross sections of 5-10 microns were prepared and fixed in cold acetone (-20°C). Immunohistology was performed using a rabbit anti-ILK antibody (Upstate Biotechnology Institute, NY. Cat.# 06-550) and Zymed Histostatin™ Plus kit (Zymed, Cat.#85-9743).

Abundant expression of ILK was detected in choroidal and retinal endothelium in post mortem baboon eye samples. Under similar condition, no significant level of ILK expression was detected in retinal pigmented epithelial cells. In addition, no significant expression of ILK in neurons and photoreceptors was observed.

Example 4
ILK inhibitor MC-2 (KP-31861 as found in co-pending patent application serial no. 10/077,238) inhibits angiogenesis in an in vitro model of angiogenesis

The following model was used to evaluate anti-angiogenic activity of ILK inhibitors. This model provides a convenient short term quantifiable in vitro measurement of the anti-angiogenic activity of ILK inhibitor. HUVEC human endothelial cells were grown in a Matrigel support (VWR Catalog No. CACB 354234) in the presence or absence of ILK inhibitor MC-1 (Figure 1, panels A and C), MC-2 (figure X, panels B and D) or solvent control (dimethyl sulfonamide, DMSO). Tube formation was quantified by a computer-assisted image analysis method using Image Pro Plus (Media Cybernetics, ML) measuring total tube length as captured by the microscope in each well at 5 h after application of the ILK inhibitor. Cell viability was determined by measuring mitochondrial dehydrogenase activity in the culture supernatant at 8 h using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) proliferation assay (Promega Corporation, FL, USA., Pat. No. TB169). The MTS reagent was added to the culture at 5 h after application of the ILK inhibitor. The cells were incubated at 37°C for additional 3 h and culture supernatant was collected and absorbance at 490 nm was measured with an ELISA plate reader.

An inhibitory effect on endothelial tube formation was detected with MC-2. About 35, 60 or 70% inhibition of tube formation was observed at 5 h after the application of 50, 100 or 150 μM of MC-2, respectively. However, cell viability measured by metabolic activity
of the cells was not significantly affected by ILK inhibitor MC-2 based on all MTS measurement of supernatant collected at 8 h from the same culture. For MC-2, the metabolic activity was about 90, 80, and 75% of the control levels at these concentrations.

Example 5

Treatment of corneal neovascularization with an ILK inhibitor using a mouse model

The following model provides a quantifiable *in vivo* assay that can be used to evaluate anti-angiogenic activity of an ILK inhibitor. Corneal neovascularization is induced by a procedure known as silver nitrate cauterization. The procedure involves topical applications of silver nitrate onto the cornea by gently touching conjunctiva/limbus for one second followed by touching the central cornea of an anesthetized mouse for 8 seconds with a silver nitrate applicator (Graham-Field, NY, Item # 1590, 75% silver nitrate, 25% potassium nitrate). Immediately after, the eye is rinsed with 10 ml of saline followed by topical application of Gentak Ophthalmic Ointment (0.3%, Gentamicin sulfate) on the eye to prevent bacterial infections.

Corneal neovascularization is recorded and evaluated by examining and photographing the cornea daily using a stereo dissecting microscope connected to a color video camera and a computer. Angiogenesis is evaluated based on new blood vessel growth within previous avascular cornea using a scoring system (score of 0-4) that rates from no neovascularization to very severe neovascularization in cornea. In addition, upon completion of the experiment (day 5-7), corneal neovascularization is quantified using computer-assisted image analysis (Image Pro Plus, Media Cybernetics, ML) of dye-stained blood vessels in post mortem whole corneal mounts. Corneal vasculature is stained by IV injection of high molecular weight FITC-dextran into anesthetized mice before euthanasia.

Animals receive daily intra-peritoneal administration of an ILK inhibitor at the dose of 5, 25 or 50 mg/kg commencing on day-2 after the silver nitrate cauterization procedure until 24 h before the ending of the experiment. Corneal neovascularization of ILK inhibitor-treated animals is compared with that of vehicle-treated animals.

Example 6

Treatment of choroidal neovascularization with an ILK inhibitor using a monkey model of CNV

The following model provides an *in vivo* assay that can be used to evaluate therapeutic potential of ILK inhibitors for the treatment of CNV. CNV is induced by argon green laser burns that are placed in the maculae of cynomolgus monkeys using a modification of Ryan's model, as described in U.S. 5,798,349. The laser burn with size of
50 \mu m in diameter is induced by exposure to 350-450 mW laser light at 514 nm for 0.1 second using an argon laser (Coherent Argon Dye Laser #920, Coherent Medical Laser, Polo Alto, CA).

CNV is monitored by weekly examination with fundus photography and fluorescein angiography. At the termination of the experiment (2-3 months after the induction of CNV), eyes are enucleated under deep anesthesia and fixed in modified Kanovsky fixative. Bi-section is performed 20 min after fixation. Tissues are then embedded and sections are generated for histological and immunohistological analysis using antibodies against vasculature-specific markers including CD-31 and VE-Cadherin. The extent of neovascularization is quantified using a computer-assisted image analysis system with Image Pro Plus (Media Cybernetics, ML).

Animals receive daily oral administration of an ILK inhibitor at the dose of 10, 50 or 100 mg/kg for commencing after the onset of CNV (2-3 weeks after the laser treatment). As control, a group of monkeys receive daily oral treatment with vehicle only. CNV in ILK inhibitor-treated animals is compared with that of vehicle-treated animals for angiographic and immunohistological evidence of CNV.

Example 7

Treatment of retinal neovascularization with an ILK inhibitor using a mouse model of ischemia-induced retinopathy

The following model provides an in vivo assay that can be used to evaluate therapeutic potential of ILK inhibitors for the treatment of retinopathy. This is a mouse model of retinopathy of prematurity. Retinopathy in mice is induced by using dams and neonatal mice. Mice are exposed with their nursing dams to 75% oxygen/25% nitrogen from postnatal day 7 to day 12, then put back to room air.

At day 17, all pups are weighed, euthanised, and perfused with 1 ml fixative (4% paraformaldehyde/8% sucrose/sodium phosphate buffer, pH 7.2) through the left ventricle of heart. Eyes are enucleated and placed in fixative. The fixed tissues are paraffin-embedded and 4-\mu m sections are cut. Immunohistology procedure is performed to evaluate extent of retinal neovascularization using antibodies against endothelium-specific markers including CD-31 and VE-cadherin. The vascular specific staining is quantified using the computer-assisted image analysis method (Image Pro Plus, Media Cybernetics, ML).

The ILK inhibitor at the dose of 5, 25 or 50 mg/kg is administered daily through intra-peritoneal injection from day 12 through day 16. The control group receives daily injection of vehicle. The inhibitory effect of the ILK inhibitor on retinal neovascularization is determined.
by comparing the extent of vascular staining in mice treated with the ILK inhibitor and those treated with vehicle only.
WHAT IS CLAIMED IS:

1. A method for treating ocular neovascularization, the method comprising:
   administering an inhibitor of integrin linked kinase (ILK) to treat ocular neovascularization.

2. The method according to Claim 1, wherein said treatment reduces or reverses the loss of visual acuity secondary to neovascularization of cornea, iris, retina or choroid.

3. The method according to Claim 1, further comprising administering a second therapy for ocular neovascularization.

4. The method according to Claim 3, wherein second therapy is selected from the group consisting of Visudyne therapy, photocoagulation and transpupillary thermotherapy.

5. The method according to Claim 1, wherein said ocular neovascularization is selected from the group consisting of diabetic retinopathy, chronic glaucoma, retinal detachment, sickle cell retinopathy, age related macular degeneration (AMD) due to subretinal neovascularization; rubeosis iritis; inflammatory diseases; chronic uveitis; neoplasms; Fuchs' heterochronic iridocyclitis; neovascular glaucoma; corneal neovascularization; neovascularization resulting following a combined vitrectomy and lensectomy; retinal ischemia, choroidal vascular insufficiency, choroidal thrombosis, carotid artery ischemia; neovascularization of the optic nerve; and neovascularization due to penetration of the eye or contusive ocular injury.

6. The method according to Claim 1, wherein said ILK inhibitor is administered systemically.

7. The method according to Claim 1, wherein said ILK inhibitor is administered intra-ocularly.

8. The method according to Claim 1, wherein said ILK inhibitor is administered peri-ocularly.
9. The method according to Claim 1, wherein said ILK inhibitor is administered topically onto the cornea.

10. The method according to Claim 1, wherein said ILK inhibitor is administered by intra-ocular injection.

11. The method according to Claim 1, wherein said ILK inhibitor is administered by intra-ocular implantation.

12. The method according to Claim 1, wherein said ILK inhibitor is anti-sense specific for ILK.

13. The method according to Claim 1, wherein said ILK inhibitor is an ILK specific antibody and analog thereof.

14. The method according to Claim 1, wherein said ILK inhibitor is a small organic molecule that blocks ILK catalytic or binding activity.

15. The method according to Claim 1, wherein said ILK inhibitor is an agent that affects ILK activity through direct or indirect modulation of [PtdIns(3,4,5)P$_3$] levels.
Figure 1

A

Tube Formation at 5h (% of control)

MC-1 Expt. 1
MC-1 Expt. 2

0 25 50 100 150 DMSO

[MC-1] (µM)

B

Tube Formation at 5h (% of control)

MC-2 Expt. 1
MC-2 Expt. 2

0 25 50 100 150 DMSO

[MC-2] (µM)

C

Mitochondrial Activity at 8h (MTS, % of control)

MC-1 Expt. 1
MC-1 Expt. 2

0 25 50 100 150 DMSO

[MC-1] (µM)

D

Mitochondrial Activity at 8h (MTS, % of control)

MC-2 Expt. 1
MC-2 Expt. 2

0 25 50 100 150 DMSO

[MC-2] (µM)
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 A61K45/00 A61P27/00

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

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

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

WPI Data, BIOSIS, PAJ, EPO-Internal, MEDLINE, CHEM ABS Data, SCISEARCH, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

  *A* document defining the general state of the art which is not considered to be of particular relevance
  *E* earlier document but published on or after the international filing date
  *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another document or other special reason (as specified)
  *O* document referring to an oral disclosure, use, exhibition or other means
  *P* document published prior to the international filing date but later than the priority date claimed

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

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

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

* document member of the same patent family

**Date of the actual completion of the international search**

29 January 2003

**Date of mailing of the international search report**

04/02/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL–2280 HV Rijswijk
Tel. (+31-70) 340–2040, Tx 31 651 eps onl, Fax (+31-70) 340–3016

Authorized officer

Pilling, S
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>WO 0183481 A</td>
<td>08-11-2001</td>
<td>AU 5260901 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1277754 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0183481 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0177080 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1276723 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2002042501 A1</td>
</tr>
</tbody>
</table>