

Protocol Overview

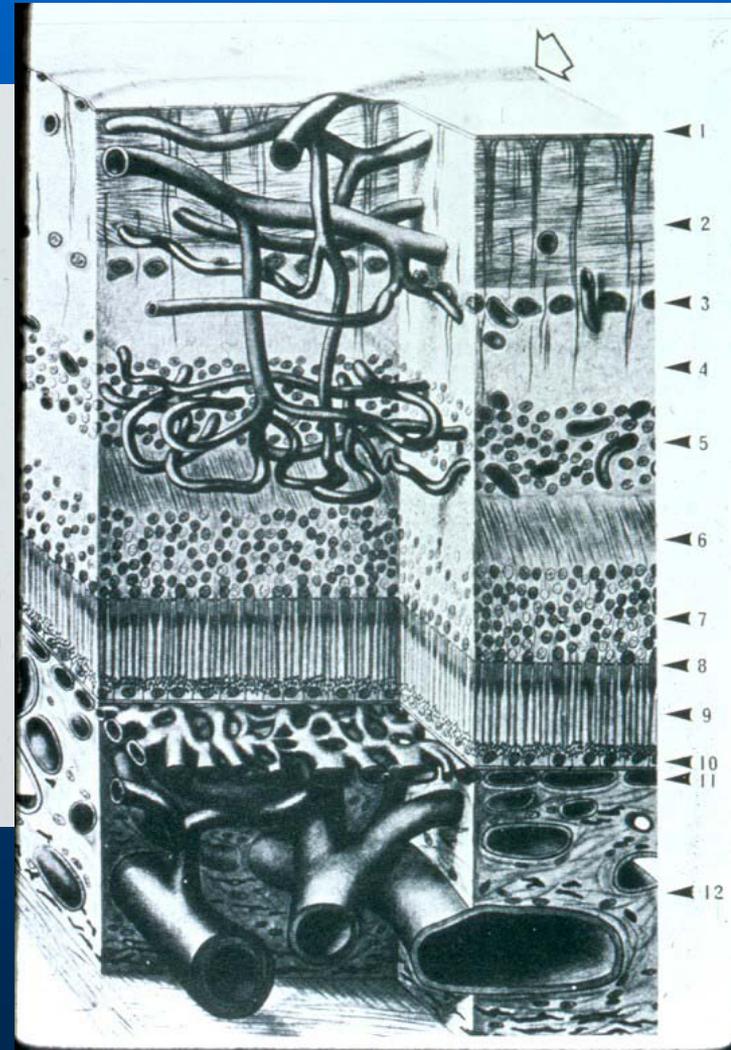
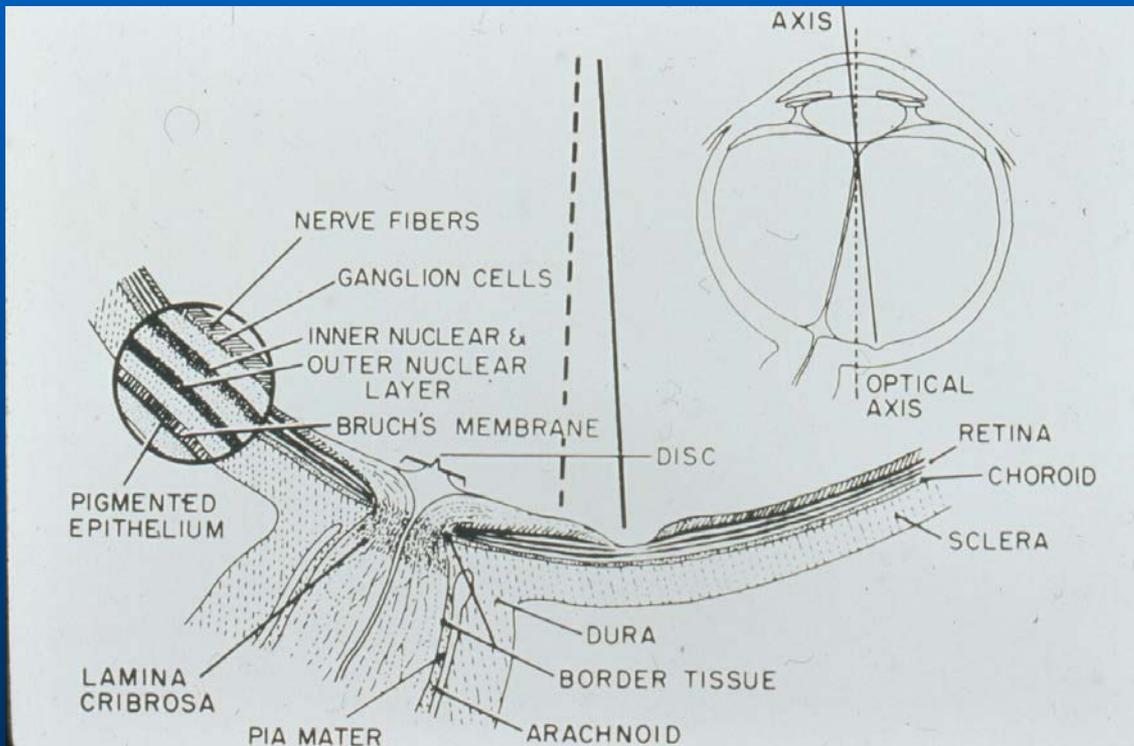
RetinoStat® Phase I Study

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Choroidal Neovascularization

Abnormal blood vessels are under the macula in subretinal space



Choroidal Neovascularization

The most common cause is age-related macular degeneration, a major public health problem

1.7 million people in the USA have visual impairment due to AMD

Major stimulus is vascular endothelial growth factor (VEGF)

Current treatment involves frequent intraocular injections of anti-VEGF antibodies

Alternative strategy

Delivery of a gene that expresses one or more anti-angiogenic proteins

Anti-angiogenic proteins

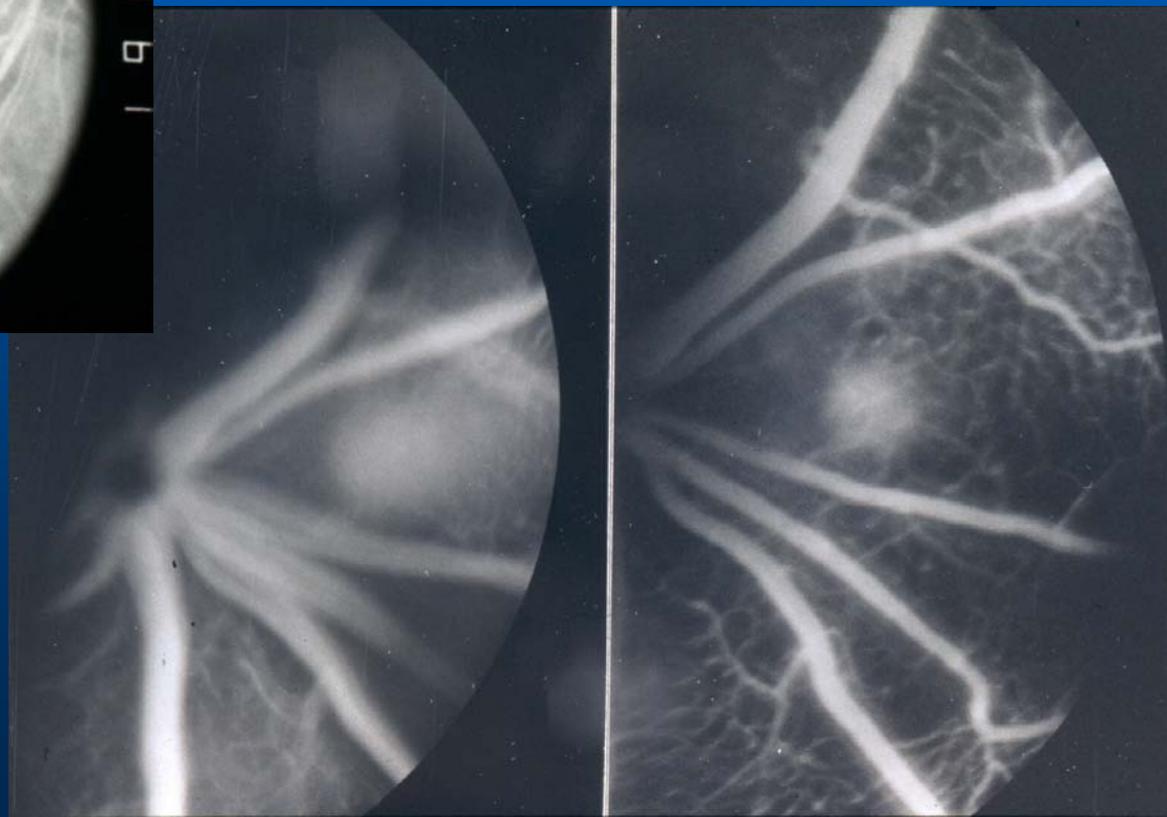
- Soluble VEGFR1
- Endostatin
- Angiostatin
- Pigment epithelium-derived factor (PEDF)

Fluorescein Angiography

Visualization of new vessels under retina



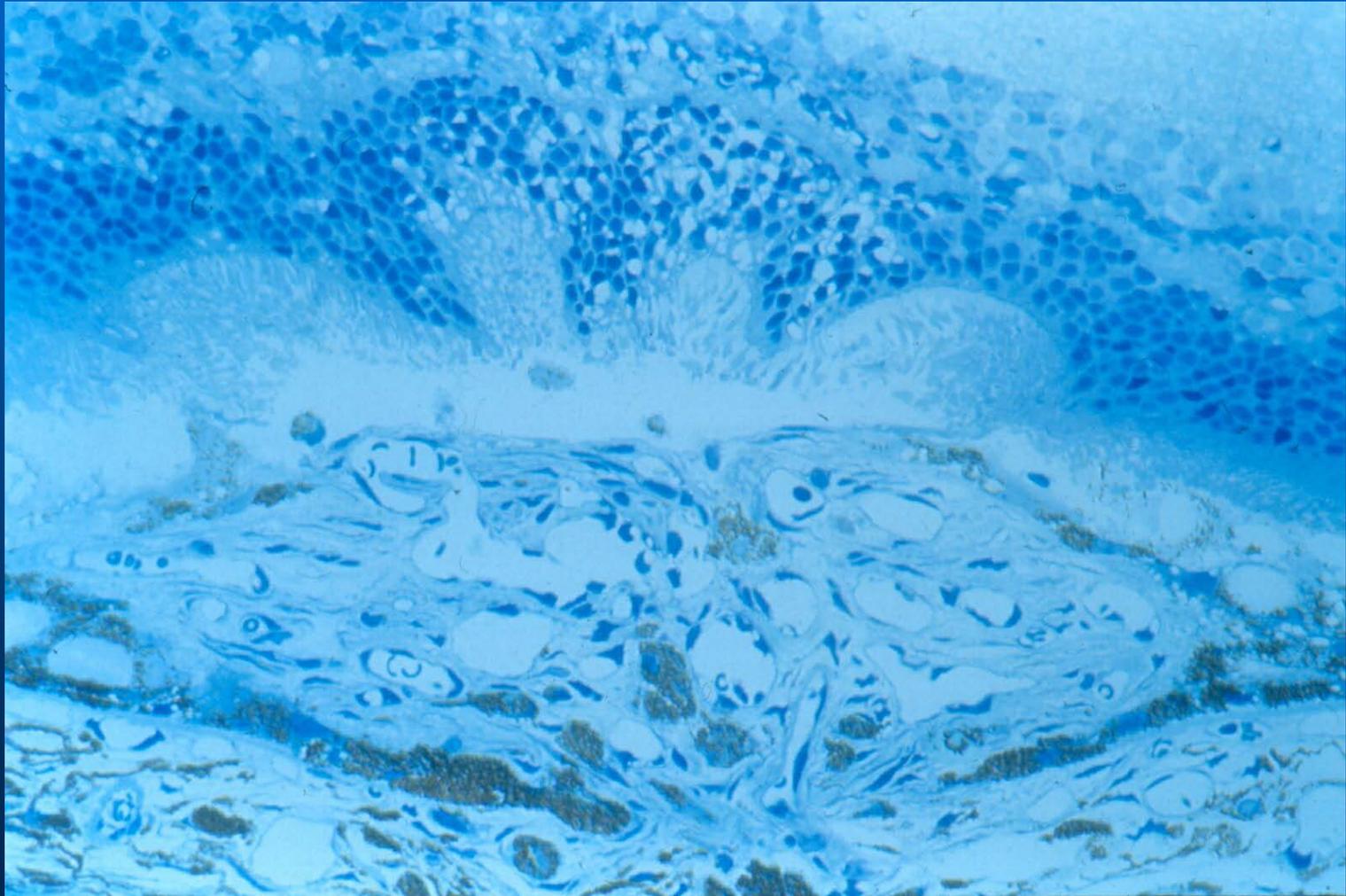
Mouse model of CNV



Patient with CNV due to AMD

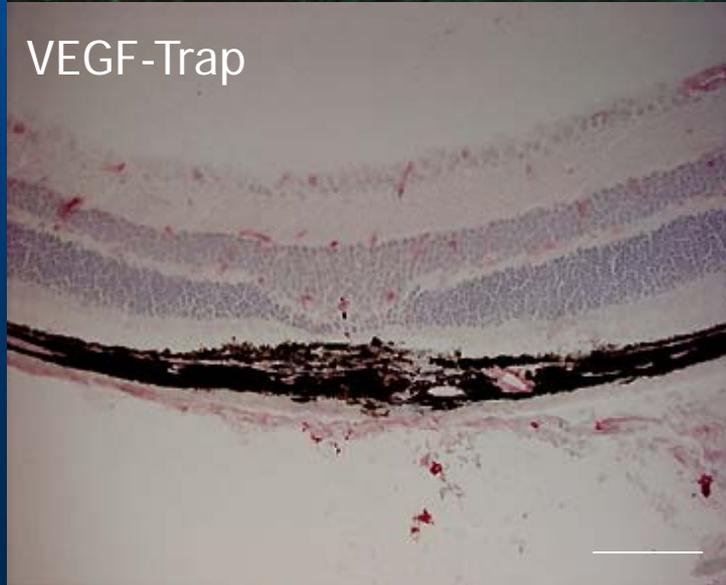
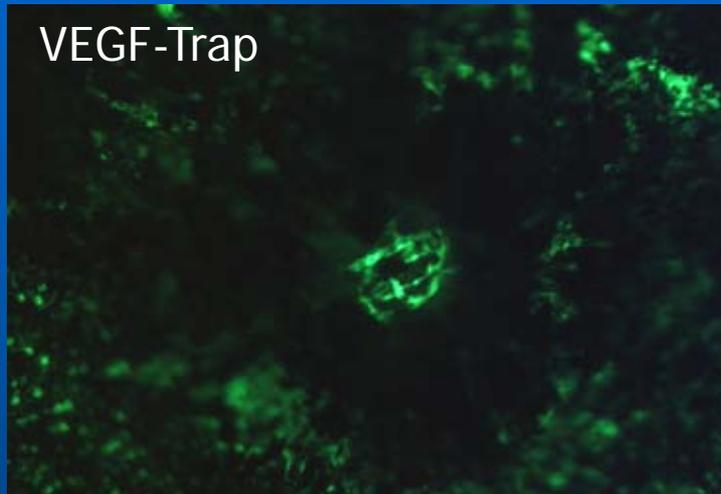
Laser-Induced CNV

2 weeks after laser

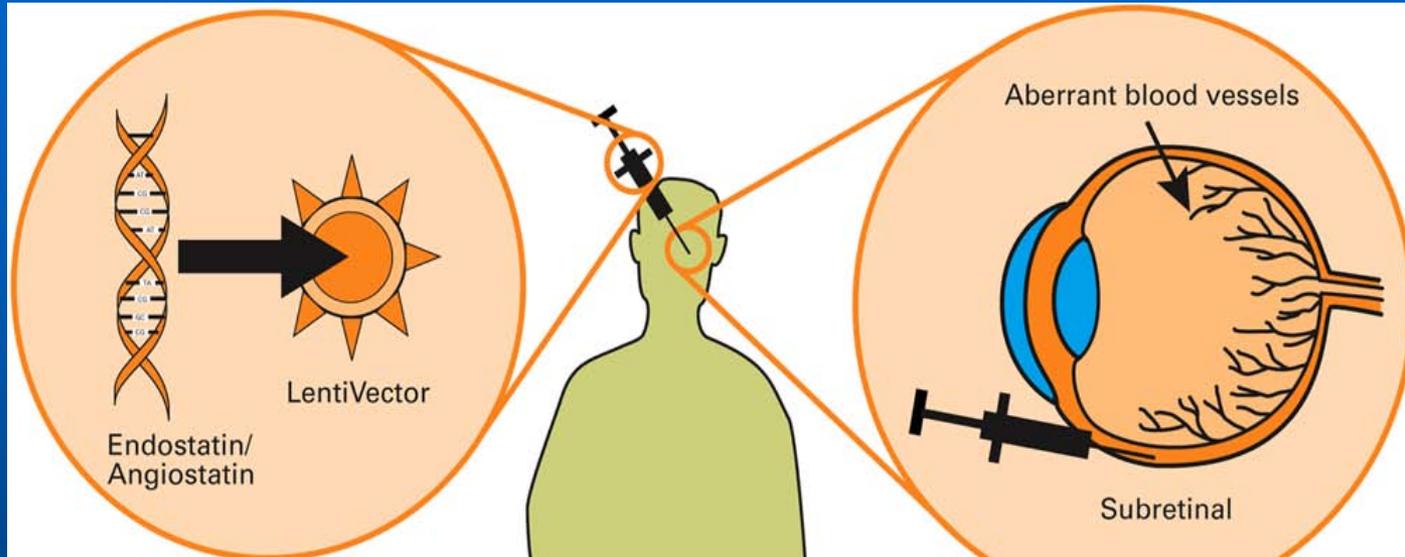


VEGF-Trap

Subcutaneous injections of 25 mg/kg prior to laser and on days 2, 5, 8, and 11

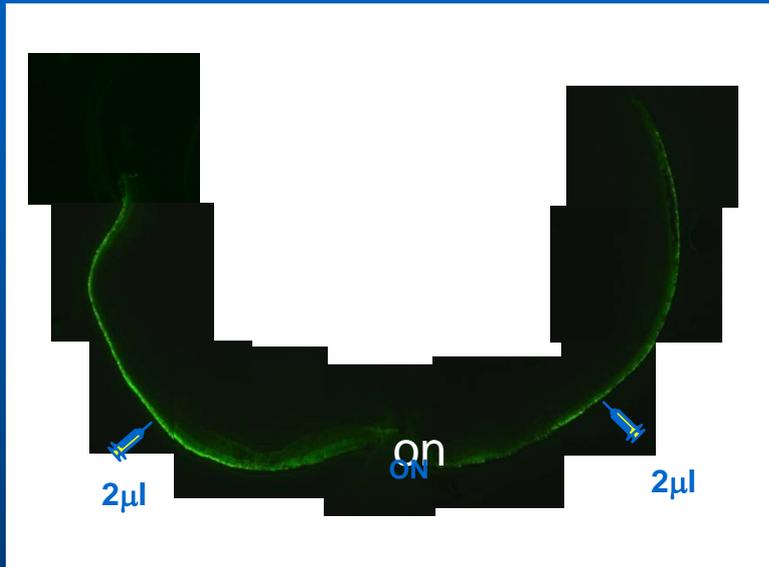


RetinoStat[®]

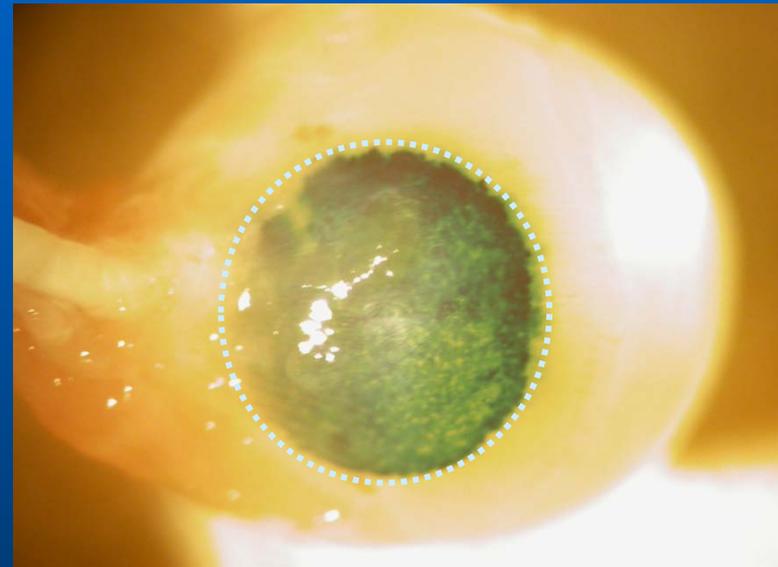


- **RetinoStat delivers two anti-angiogenic genes, endostatin and angiostatin**
CMV promoter
Packaged in non-primate lentiviral vector (Equine Infectious Anaemia Viral Vector)
- **Over 2 years safety data on the administration of related gene therapy product ProSavin[®]**

Widespread Expression Throughout the RPE



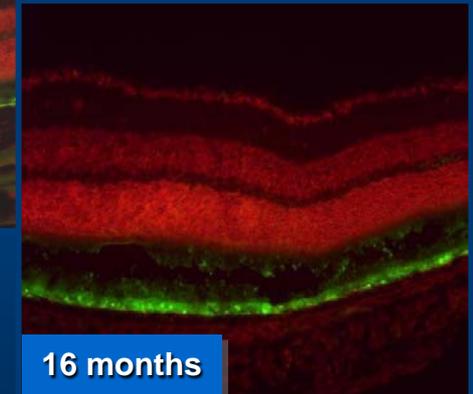
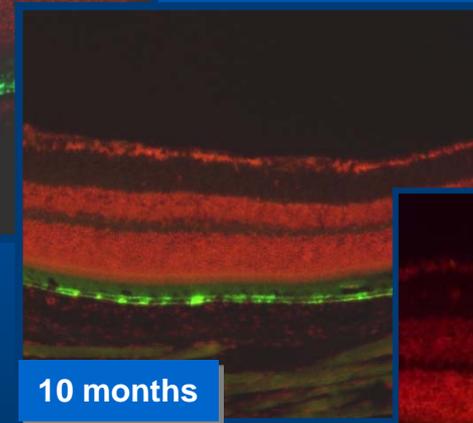
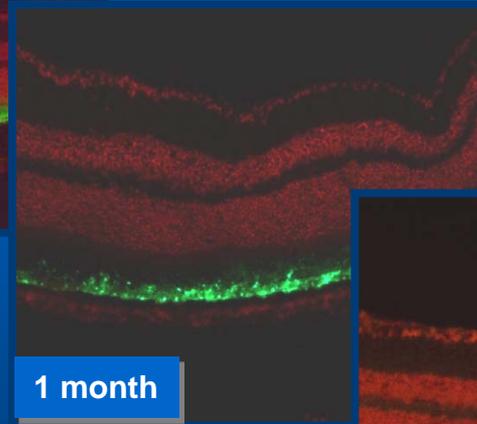
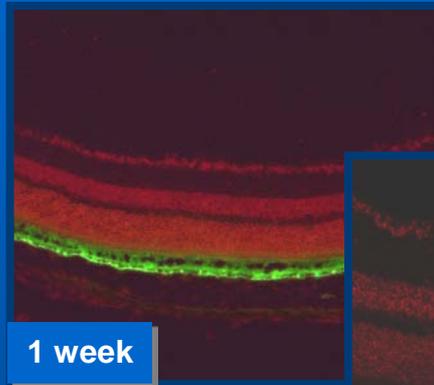
2 X 2μl LentiVector to the mouse retina results in full retinal coverage



1μl administration of LentiVector results in extensive expression in the retina (posterior retina of albino mouse)

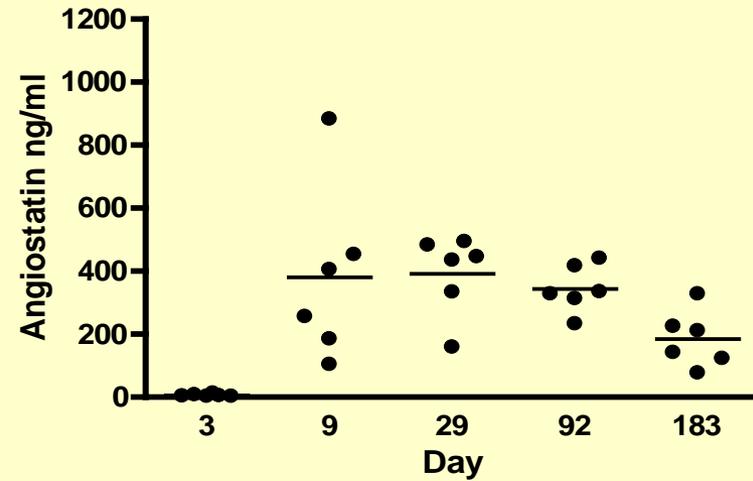
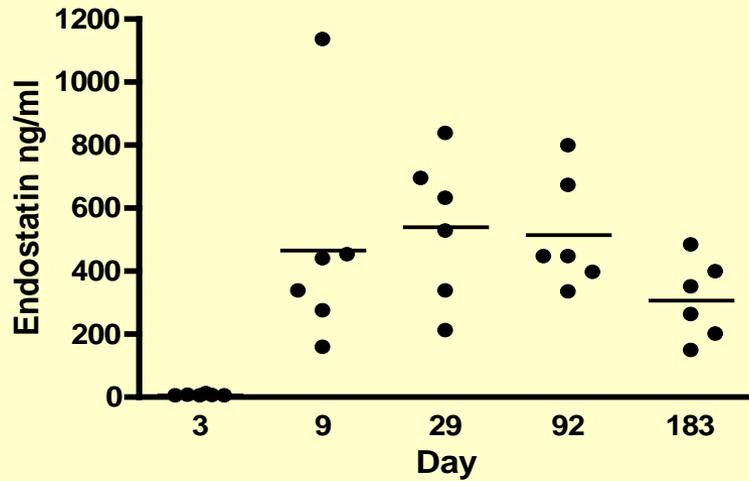
Long Term Gene Expression in the Retina

Inner Nuclear Layer |
Outer Nuclear Layer |
Retinal pigment epithelium —



6×10^6 TU CMV GFP administered via the subretinal route

Endostatin and Angiostatin Detected in Rabbit Vitreous



RetinoStat®

Endostatin

- C-terminal fragment of collagen XVIII
- Naturally occurring angiogenesis inhibitor, present in the ocular compartment
- Pleiotropic angiostatic activities: blocks VEGF signalling, reduces vascular permeability, decreases cell:matrix adhesion, promotes EC apoptosis
- Causes tumour regression in mice
- Proven efficacy in accepted models of ocular neovascularisation in the context of lentiviral¹, adenoviral², and AAV³ vector systems

1. Takahashi et al 2003
2. Mori et al 2001
3. Auricchio et al 2003

RetinoStat[®]

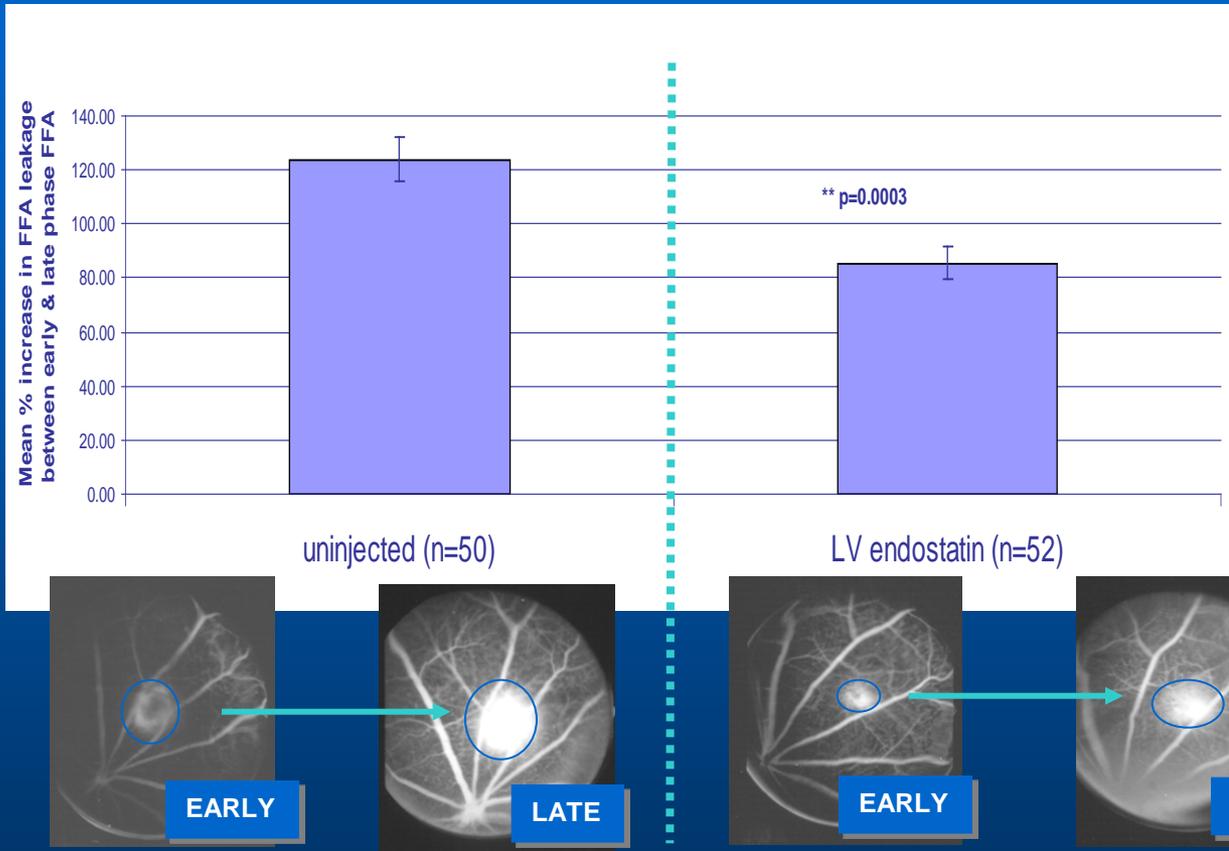
Angiostatin

- Proteolytic cleavage fragment of plasminogen
- Naturally occurring angiogenesis inhibitor, evident in the ocular compartment
- Prevents endothelial cell proliferation and migration
- Inhibits growth of primary carcinomas
- Proven efficacy in accepted models of ocular neovascularisation in the context of AAV^{1,3} and lentiviral² vector systems

1. Lai *et al* 2001
2. Igarashi *et al* 2003
3. Raisler *et al* 2002

RetinoStat®

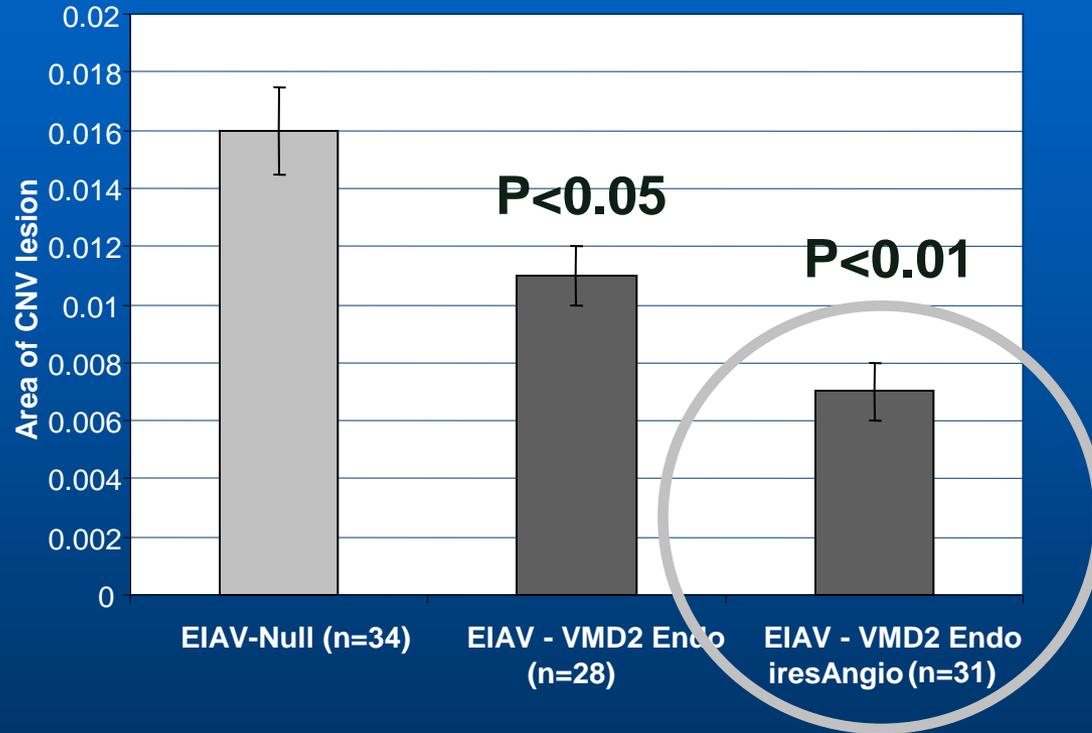
Efficacy *In Vivo* in mice



Reduced vessel leakage in the laser CNV model

RetinoStat

In vivo efficacy

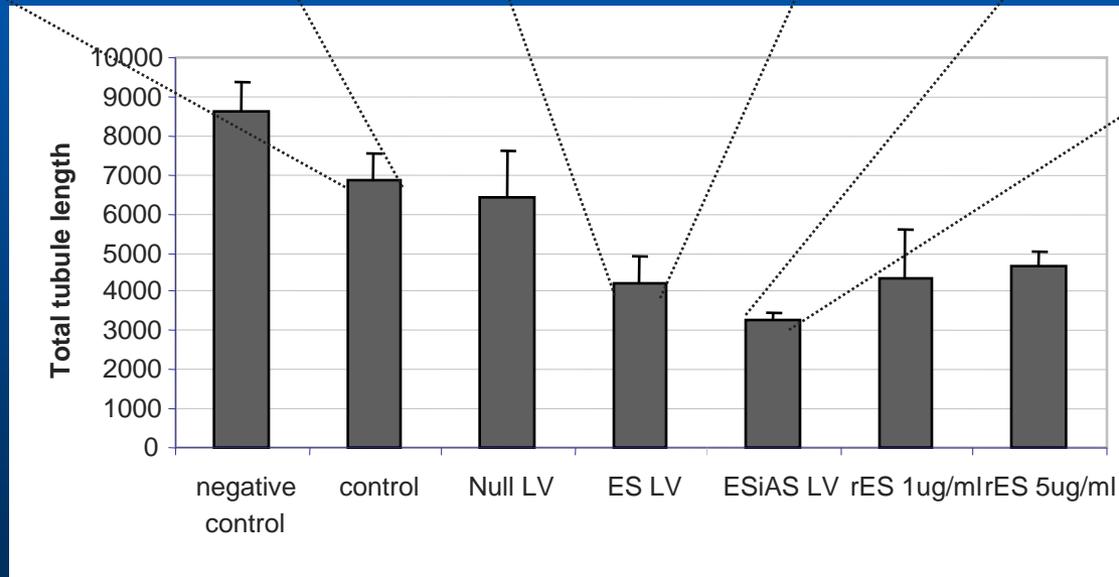
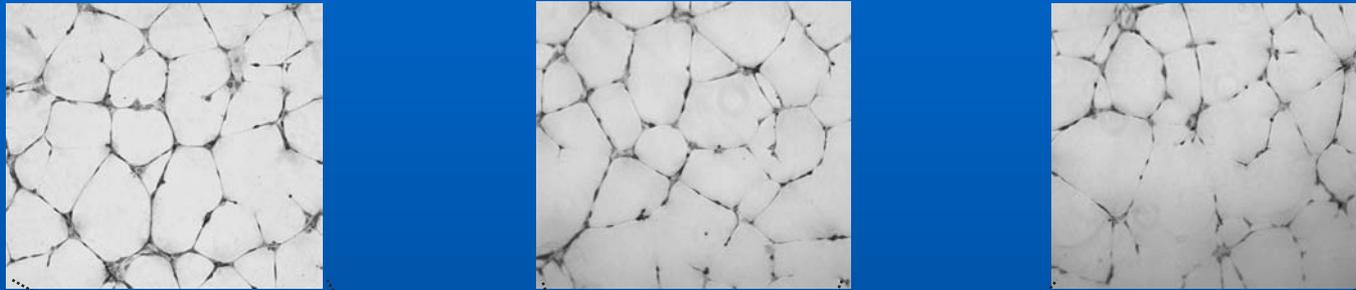


Double construct shows greatest efficacy

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Potency *in vitro*

Activity of supernatants derived from transduced cells in the matrigel tubule forming assay

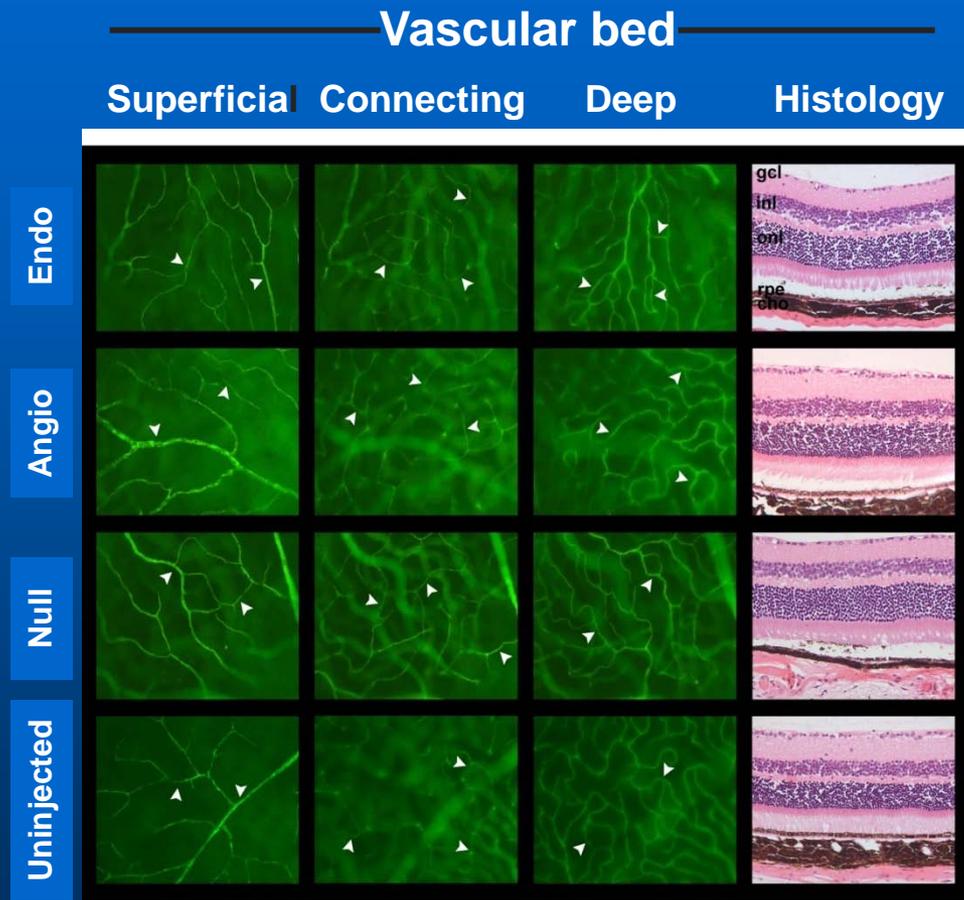


ES: Endostatin
AS: Angiostatin

- Endostatin and angiostatin act via different mechanisms to suppress angiogenesis hence both are incorporated into RetinoStat

RetinoStat[®]

Preservation of normal vasculature



- Status at one year
- Normal branching
- Normal reticular pattern
- Well perfused
- Normal retinal morphology

Balaggan et al
2006

Summary of Expression and Efficacy Data

- **Studies with reporter genes indicate RetinoStat vector enables delivery and expression primarily in RPE**
- **Expression over the long term with GFP (up to study termination, [16 months]) and RetinoStat (up to study termination, [6 months])**
- **Endostatin and angiostatin identified in vitreous over long term at therapeutic levels**
- **Long term expression of endostatin and angiostatin has no impact on normal 'resident' vasculature**
- **Efficacy: reduction in permeability and suppression of CNV**

RetinoStat[®] - Formal GLP Studies

Six month studies carried out in rabbit and NHP

- **Biodistribution/shedding (PK)**
- **Safety (Toxicology)**

Biodistribution Analysis

Large number of tissues and fluids were sampled (at days 2, 3, 5, 29, 92, 186) including: urine, CSF, ovary, testis, liver, heart, lung, spleen, kidney cortex, kidney medulla, right lacrimal gland, mandibular lymph node, right lower/upper eyelids, ventral rectus, optic chiasm, right optic nerve, white blood cells, superior oblique, nictitating membrane

Vector Shedding/dissemination (RNA)

- No vector shedding in urine, saliva, and contralateral eye tear swabs**
- RetinoStat vector particles found in vitreous fluid but gone by day 29**
- No vector dissemination in plasma or cerebrospinal fluid**

Integrated Vector Biodistribution (DNA)

- Long term RetinoStat found 100% in the eye**
- RetinoStat absent from other tissue or where present was below the lower level of quantification**

Summary of Nonclinical: Toxicology

RetinoStat is well tolerated in rabbit and NHPs (up to 6 months duration)

- **No adverse findings related to RetinoStat**
 - **No changes in retinal morphology, IOP or ERG**
 - **Transient observations associated with subretinal procedure**
- **Dose and time dependent ocular inflammation that is transient and mild at levels exceeding proposed clinical dose**
 - **No humoral response to vector components, endostatin or angiostatin**

Clinical Trial

A Phase I Dose Escalation Safety Study of Subretinally Injected RetinoStat, a Lentiviral Vector Expressing Endostatin and Angiostatin, in Patients with Advanced Neovascular Age-Related Macular Degeneration

Study Objectives

Primary Objective

To evaluate the safety and tolerability of subretinal injections of RetinoStat in subjects with advanced neovascular age-related macular degeneration

Secondary Objective

To evaluate the biological activity of RetinoStat

Study Endpoints

Primary Endpoint

- The incidence of adverse events following a single intraocular dose of RetinoStat
- Changes in best corrected visual acuity (BCVA)
- Evidence of ocular inflammation
- Intraocular pressure

Secondary Endpoints

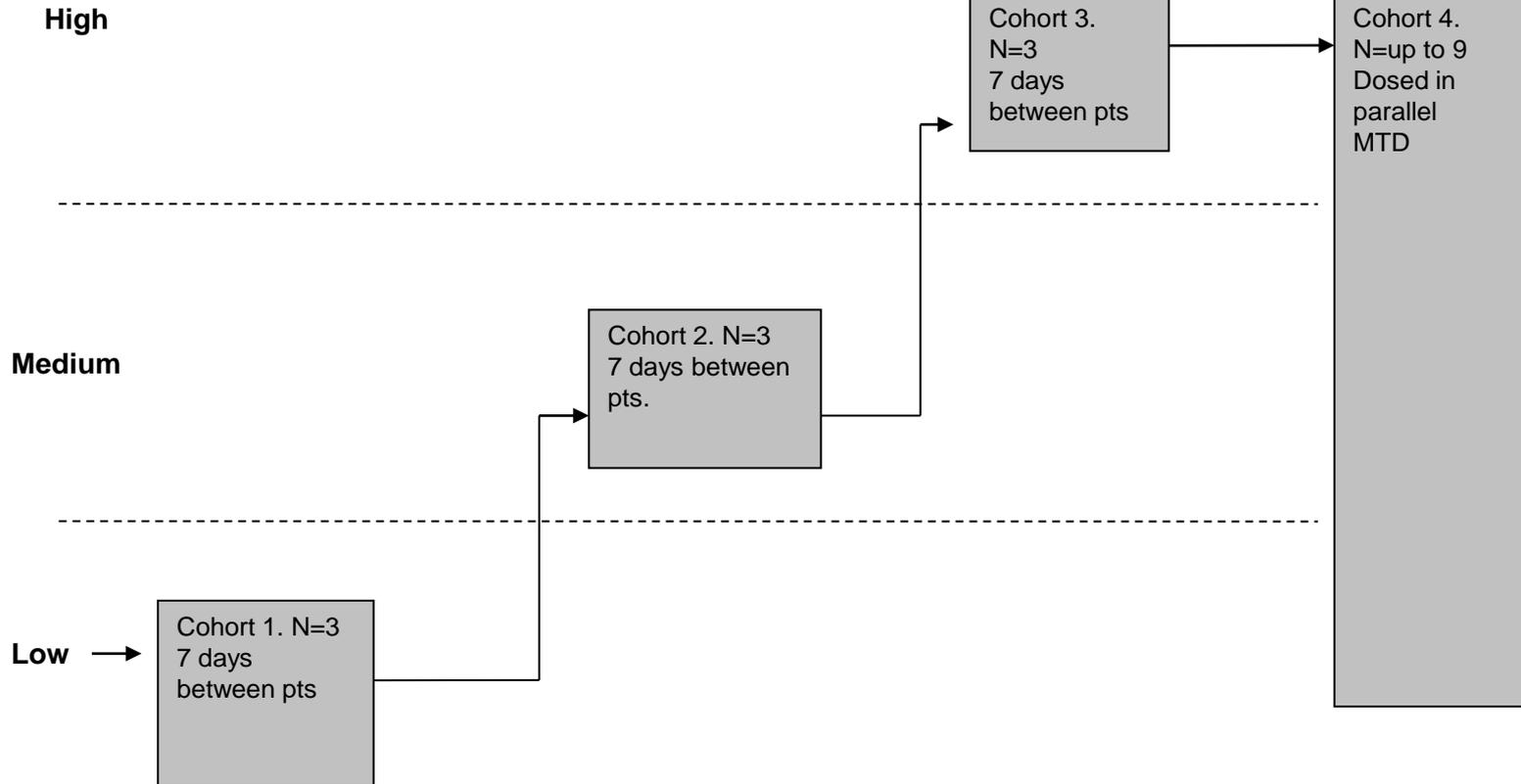
- The change from baseline in the amount of subretinal and intraretinal fluid measured by optical coherence tomography
- Change in size of active choroidal neovascularisation measured on fluorescein angiography
- The change from baseline in BCVA
- The change from baseline in levels of vascular endothelial growth factor (VEGF), endostatin and angiostatin in aqueous fluid

Study Population

- Up to 21 subjects with AMD, aged 50 years or more with active CNV that shows evidence of active leaking on fluorescein angiography
- BVCA less than or equal to 20/200 in the study eye
- BVCA in the fellow eye of 20/200 or better
- Subjects unlikely to benefit from standard of care – subfoveal fibrosis
- Women must be surgically sterile or post menopausal
- Men must be surgically sterile or agree to use two contraception methods if their partners are of childbearing capacity
- Partners of study participant should use barrier contraception for at least three months after RetinoStat administration

RetinoStat Study Design

DOSE



Minimum of 28 days between cohorts

Dosing Regimen

Cohort	Number of Patients	Dose Level	Total dose per eye (TU)	NOAEL (safety Factor)	MTD* (safety factor)
1	3	1	Low	1/13	1/100
2	3	2	Medium	1/1	1/10
3	3	3	High	2/1	1/4
4	9	1,2,3	Selected from the above doses	-	-

*Mild to moderate inflammation in 3 out of 4 animals
 Posterior synechia in one animal

Dosing Rationale

- Conservative definition of MTD used in pre-clinical models without any steroid prophylaxis
- Dose levels chosen based upon the MTD in two species
- FIM dose 1/100 of the MTD and 1/13 of the NOAEL in pre-clinical models
- Second dose level is a level that was well tolerated in pre-clinical model (approximately at the level of the NOAEL and 1/10 of the MTD).
- Highest dose level is just above NOAEL but 1/4 of the MTD (but will only be explored if the safety and tolerability of the two previous doses is satisfactory to the DSMB and the investigator).

Patient Follow Up

Post RetinoStat admin patients are followed up:

- Day 1 post administration
 - Weekly for the first month
 - Monthly for 1 year
 - Yearly for 15 years
-
- Safety follow up with full ophthalmic examination, physical, safety labs at each visit

DSMB

- Independent of the Sponsors and investigators
- Comprised of four eminent ophthalmologists and gene therapy experts
- Review data in real time
- Decide whether the dose can be escalated between cohorts
- Can modify the methodology, suspend or terminate the study for safety reasons
- Have defined safety rules by which the study is governed

Summary

- RetinoStat® is a gene therapy for the treatment of neovascular AMD
- Inserts the genes for endostatin and angiostatin into the RPE cells to establish local production
- Current treatments are effective in a large number of patients but involve multiple intraocular injections and have an attendant risk
- The patient population (subfoveal fibrosis), is known not to benefit from established treatments
- The patient population has advanced disease and benefit in terms of improvements in VA are likely to be minimal
- The FIM study is designed to ensure the safety of patients through a cautious dose escalation strategy
- Intensive post- administration and long term follow up using established safety measures used for monitoring progression, treatment and complications of AMD

Inclusion Criteria

- Males or females 50 years of age and older
- AMD with active CNV and evidence of leakage detected on FA and intraretinal or subretinal fluid detected by OCT.
- BCVA less than or equal to 20/200 in the study eye.
- BCVA in the fellow eye must be 20/200 or better.
- Poor visual prognosis in the study eye due to sub-retinal fibrosis beneath the fovea or other evidence of foveal damage.
- Patient must refuse or be considered unlikely to benefit from standard care
- Women must be surgically sterile or post menopausal
- Men must be surgically sterile or agree to use two contraception methods if their partners are of childbearing capacity

Exclusion Criteria

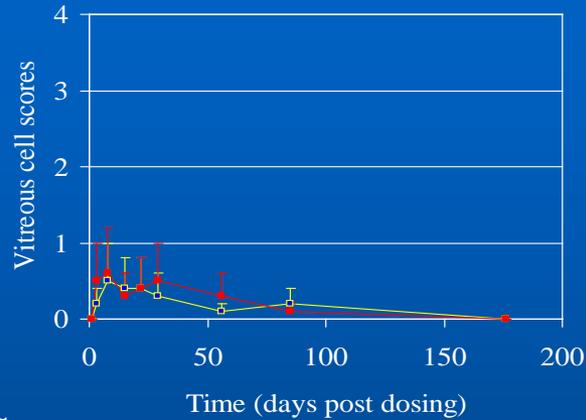
- Presence of significant ocular abnormalities in the study eye that prevent retinal assessment, including media opacities or cataract.
- Presence of vision loss from another ocular disease other than wet AMD.
- Severe clouding of the media precluding FA and OCT.
- Laser or photodynamic therapy within 12 weeks of screening.
- Any ocular surgery in the study eye within 12 weeks of screening.
- Treatment with intravitreal or periocular steroid within 3 months of enrolment.
- Treatment with anti-VEGF therapy within 1 month of enrolment.
- Any change in systemic steroid therapy within 3 months of screening.
- Life-threatening illness unrelated to AMD.
- Alcohol or other substance abuse.

Exclusion Criteria

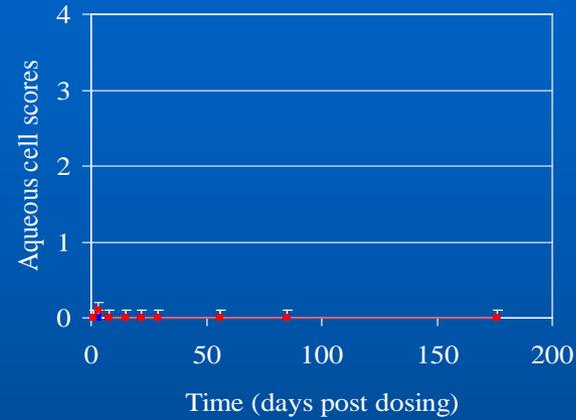
- Clinically significant laboratory test abnormalities, including full blood count, chemistry panel, liver function tests, electrocardiogram (ECG), Chest X rays.
- Intercurrent illness or infection 28 days prior to enrolment.
- Contraindications to use of anaesthesia (local or general, as appropriate).
- Concurrent anti-retroviral therapy that would inactivate the investigational agent.
- History of any investigational agent within 28 days prior to RetinoStat administration.
- Participation in a prior gene transfer therapy study.
- Enrolment in any other clinical study, for any condition, including those relating to AMD, throughout the duration of the RetinoStat study.
- Current or anticipated treatment with anticoagulant therapy or the use of anticoagulation therapy within the four weeks prior to surgery
- A medical history of HIV or Hepatitis A, B or C infection
- Inability to comply with the demands of the study

RetinoStat[®]: Rabbit inflammation scores

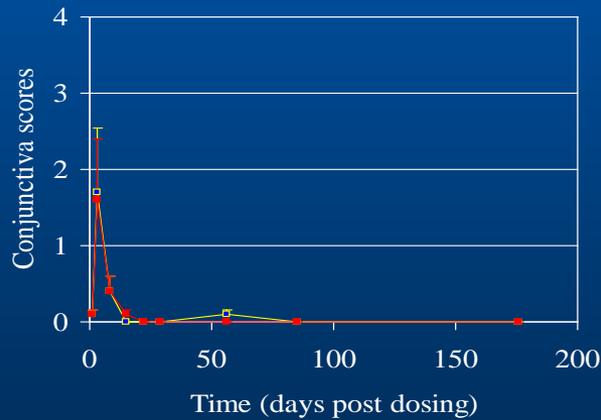
A



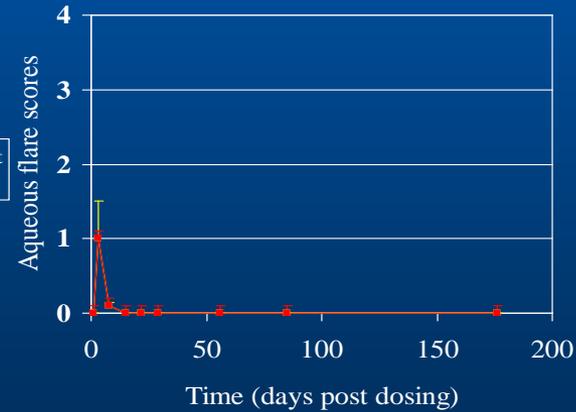
B



C

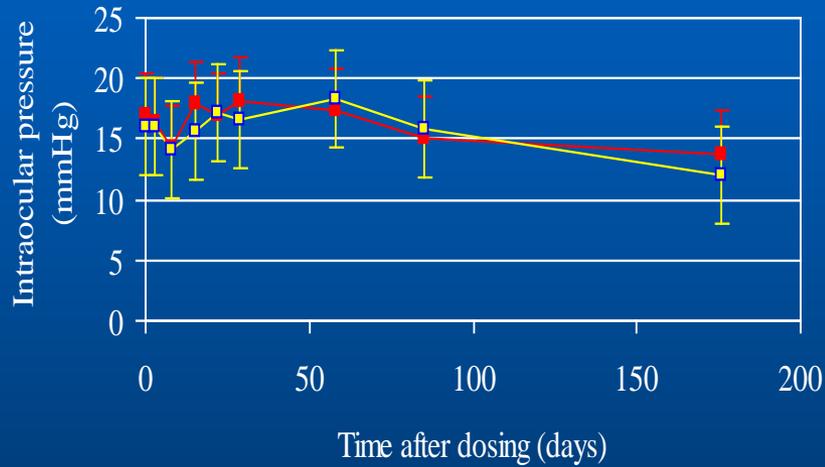


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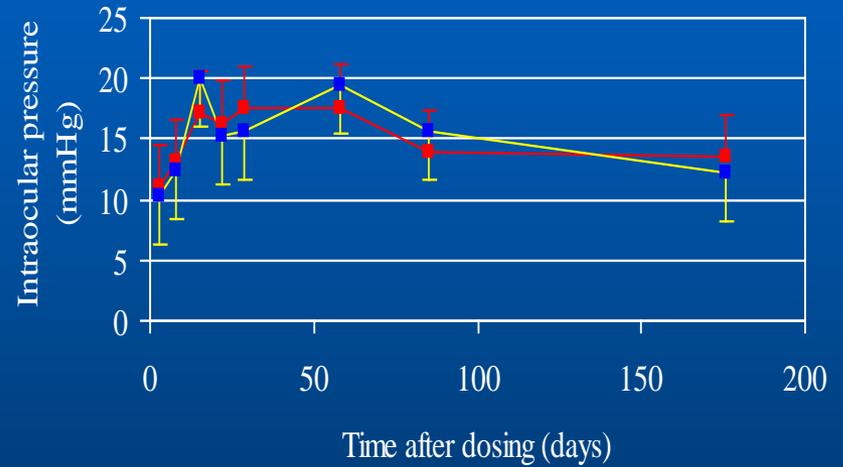


RetinoStat[®]: Rabbit Intraocular Pressure

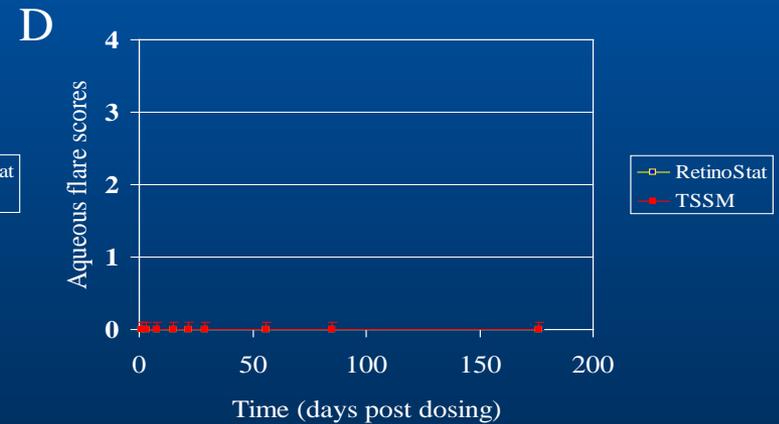
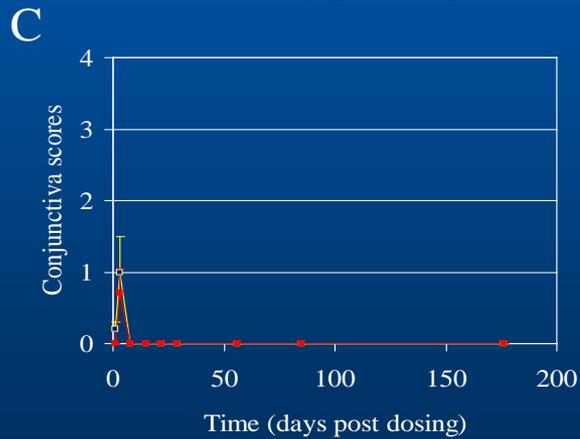
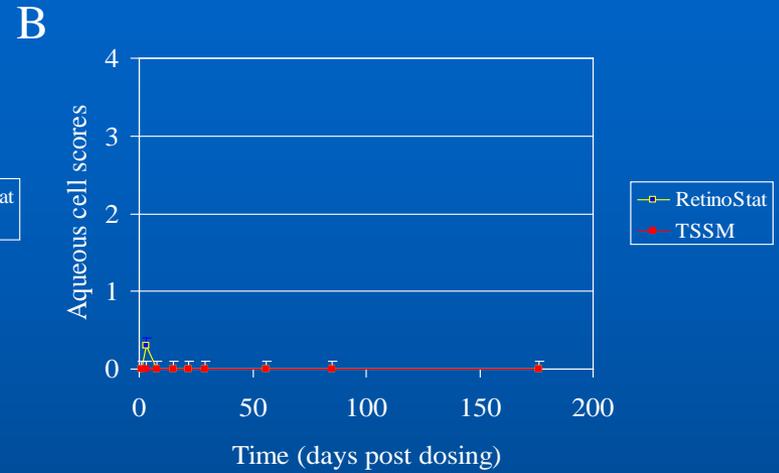
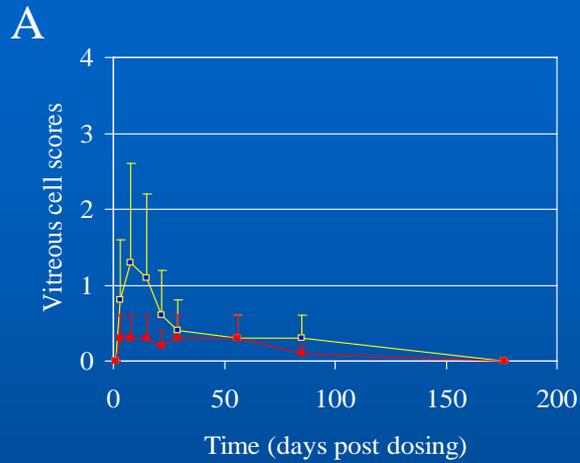
Left (untreated) eye



Right (treated) eye



RetinoStat[®]: NHP Inflammation Scores



RetinoStat[®]: NHP Intraocular Pressure

