A Phase I Study in Glaucoma Subjects Receiving SCH 412499 (rAd-p21) Administered as a Single Injection into the Subconjunctival Space Prior to Primary Trabeculectomy

Protocol: #0307-589

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Sponsor: Schering-Plough Research Institute

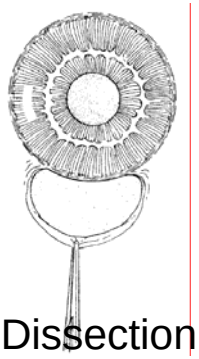
Agenda

- Medical need and current therapy
  - Paul L. Kaufman, M.D.
- Preclinical gene delivery, transgene expression and activity
  - Paul L. Kaufman, M.D.
- Non-clinical safety assessment
  - Robert Veneziale, Ph.D.
- Clinical protocol
  - Robert N. Weinreb, M.D.

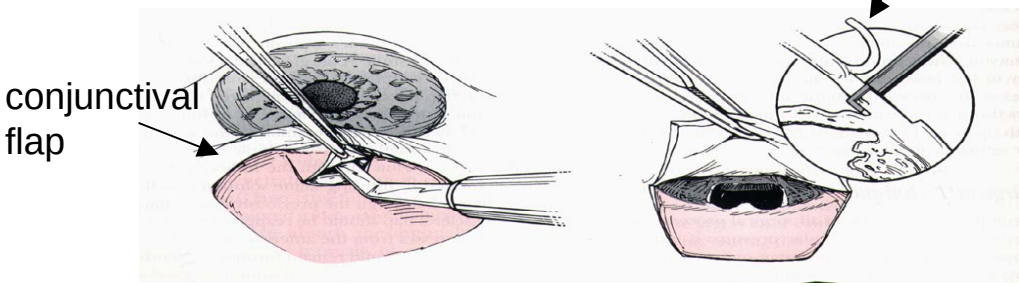
Glaucoma

- Progressive optic neuropathy
- One of two leading causes of blindness
- Current treatment:
  - medical therapy (topical)
  - laser trabeculoplasty
  - trabeculectomy (filtration surgery)
  - drainage tube/shunts
  - cyclodestructive procedures

Glaucoma Filtration Surgery: Trabeculectomy



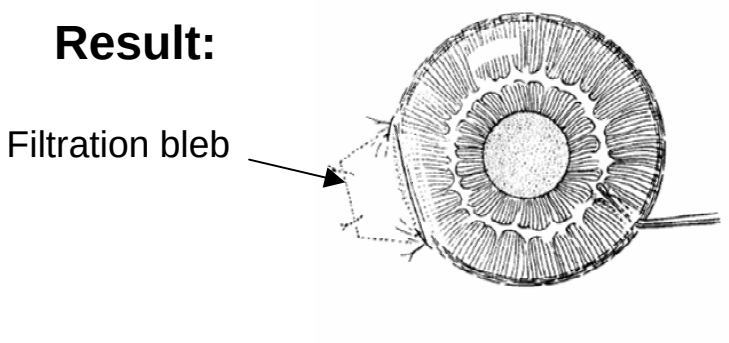
Dissection of Conjunctival Flap



Dissection of Scleral Flap

Scleral Punch

Result:



Problem: post surgical wound healing scars the bleb and IOP rises.

Limitations of Adjunctive Anti-proliferative Therapy in Glaucoma Surgery

- Non-specific cytotoxic agents result in conjunctival breakdown
  - mitomycin C (MMC)
  - 5-fluorouracil (5-FU)

- Clinical complications with MMC and 5-FU
  - endophthalmitis
  - hypotony
  - hypotony maculopathy

- What is needed?
  - *Block proliferation without complications of current anti-proliferative agents*

rAd-p21: A Novel Adjunct to Glaucoma Surgery

- $p21^{WAF1/Cip-1}$ is a natural inhibitor of the mammalian cell cycle
- rAd-p21 blocks proliferation of human ocular fibroblasts in vitro
- Single subconjunctival injection results in local p21 expression with minimal systemic exposure

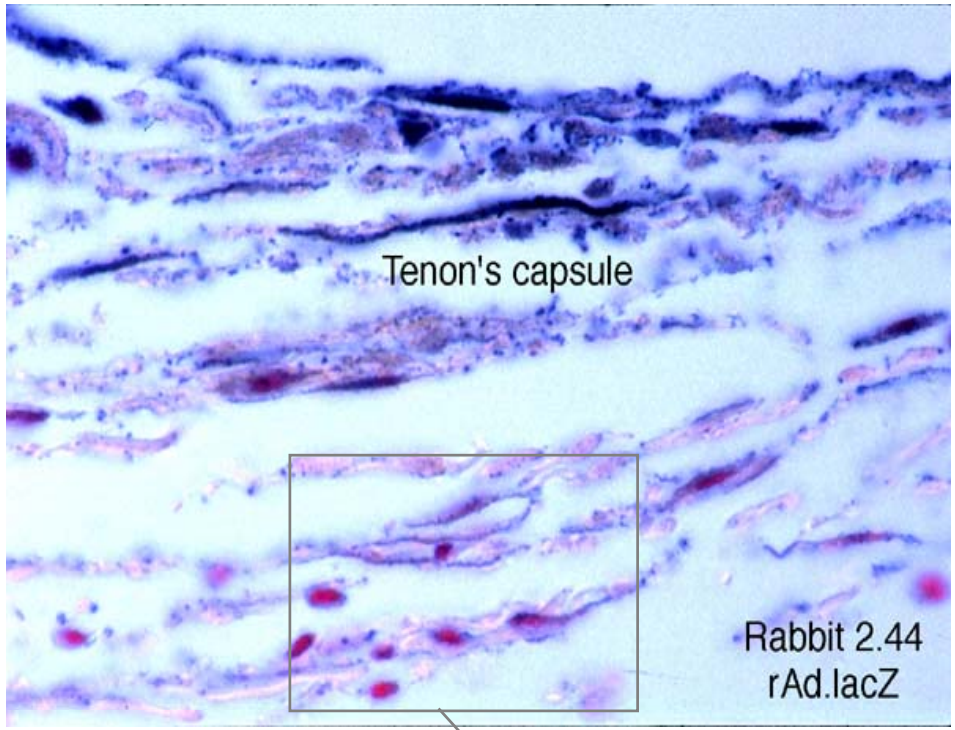
Preclinical Gene Delivery, Transgene Expression and Activity

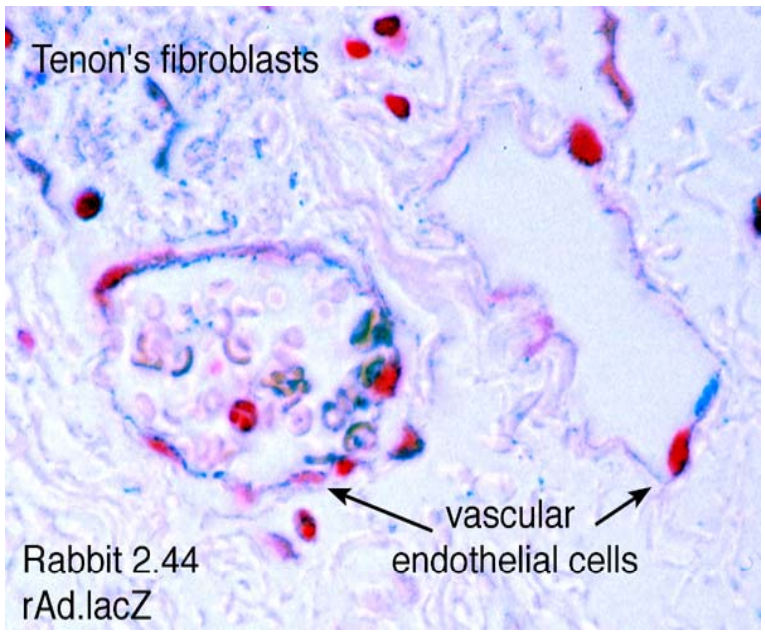
Adenovirus Gene Transfer and Expression after Subconjunctival Injection to Rabbits

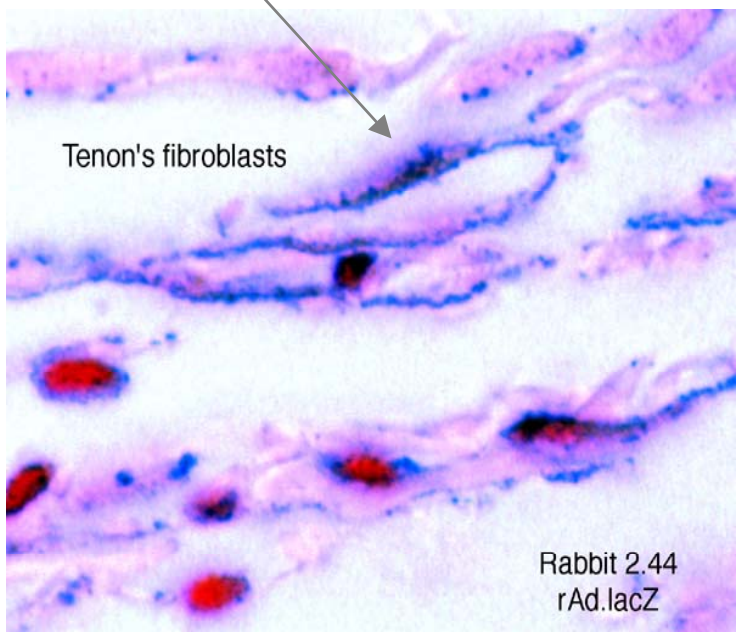
- Transduced cells include:
  - conjunctival and episcleral fibroblasts
  - vascular endothelial cells at injection site
  - occasional conjunctival epithelial cell at injection site

- Nucleic acid analyses of injection site:
  - detect rAd-p21 DNA and rAd-p21 mRNA for up to 75 days post injection
  - dose dependent delivery and expression

Rabbit rAd.lacZ





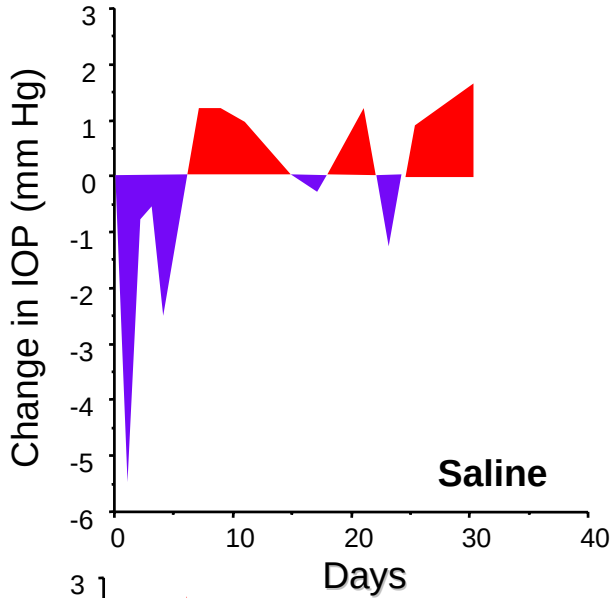


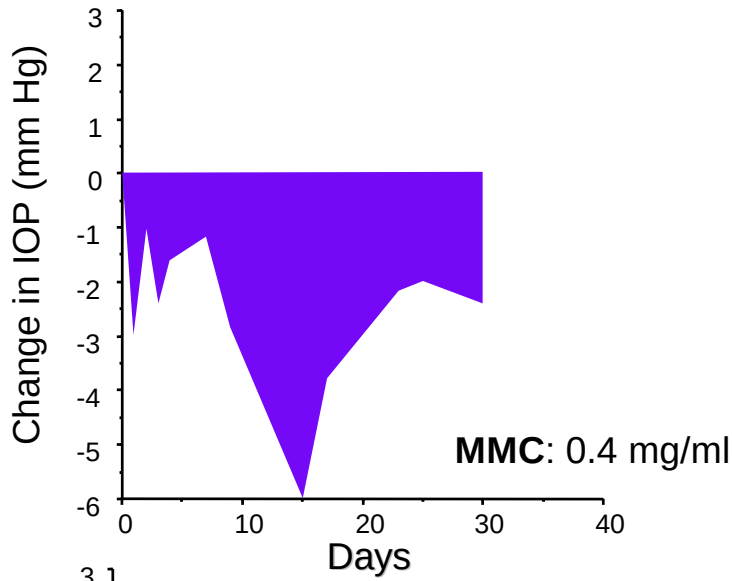
Rabbit Study

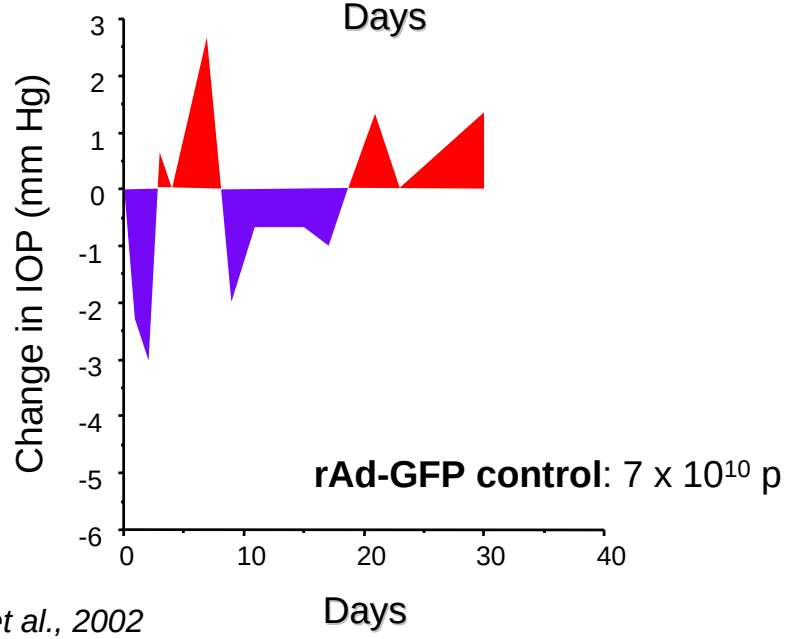
- Normal IOP in the surgical eye
- Reagent applied using soaked sponge
  - (BSS, MMC, rAd.p21, rAd.control)
- Full thickness sclerotomy
- Thirty (30) day post surgery follow-up

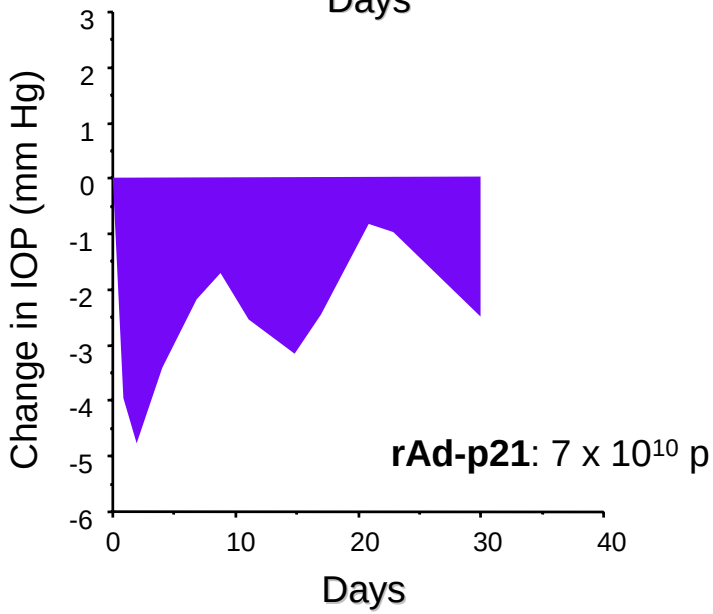
Perkins et al. 2002. Archives Ophthalmology. 120;941-949

rAd-p21 Treatment Results in Sustained IOP Reduction in a Rabbit Model



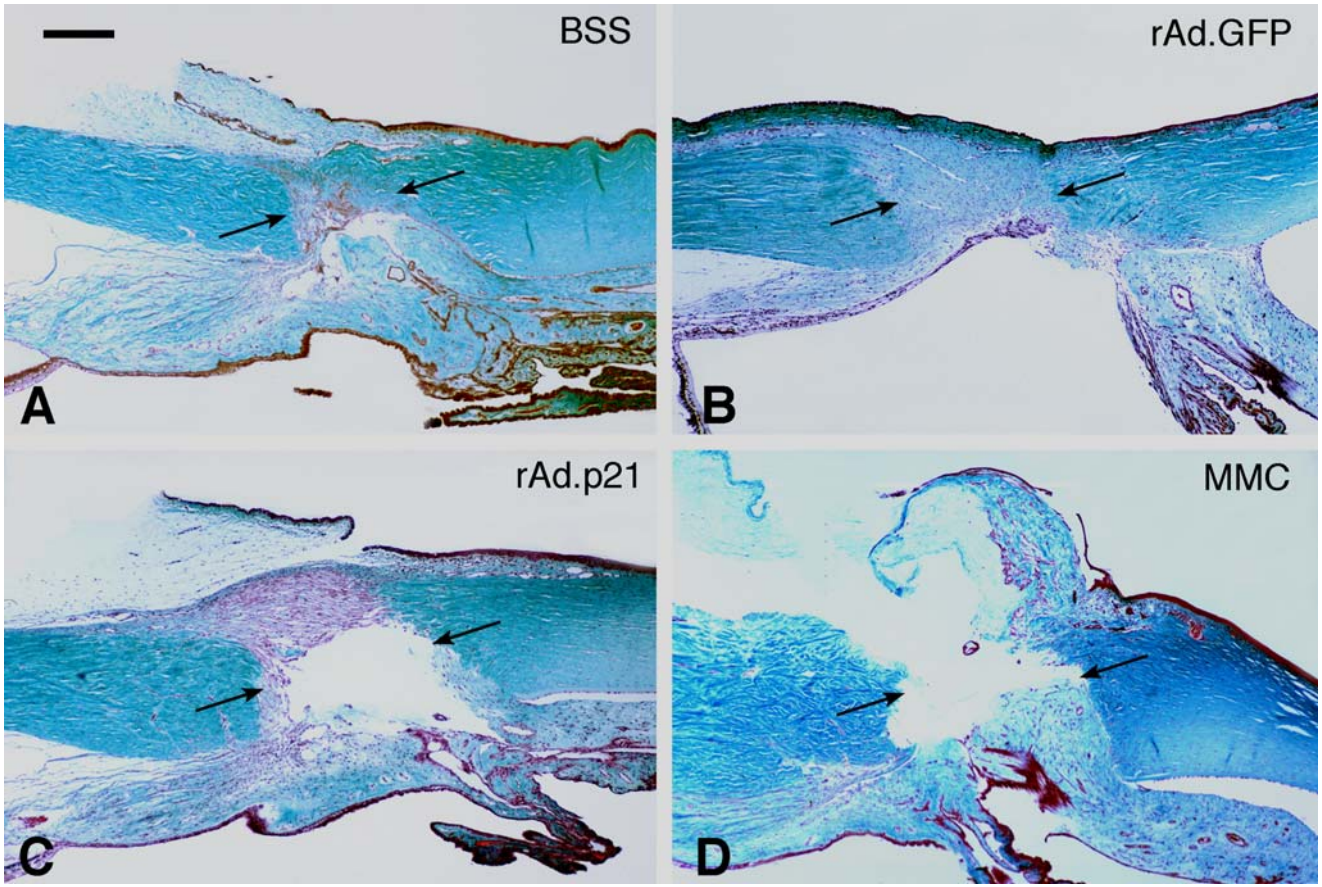






Perkins et al., 2002

rAd-p21 Inhibits Proliferation in Rabbit Sclerectomy Model: Histologic Evaluation of the Surgical Site



Glaucoma Disease Model in Primates

Objective: To evaluate the ability of rAd-p21 to prevent glaucoma surgery failure in a disease model.

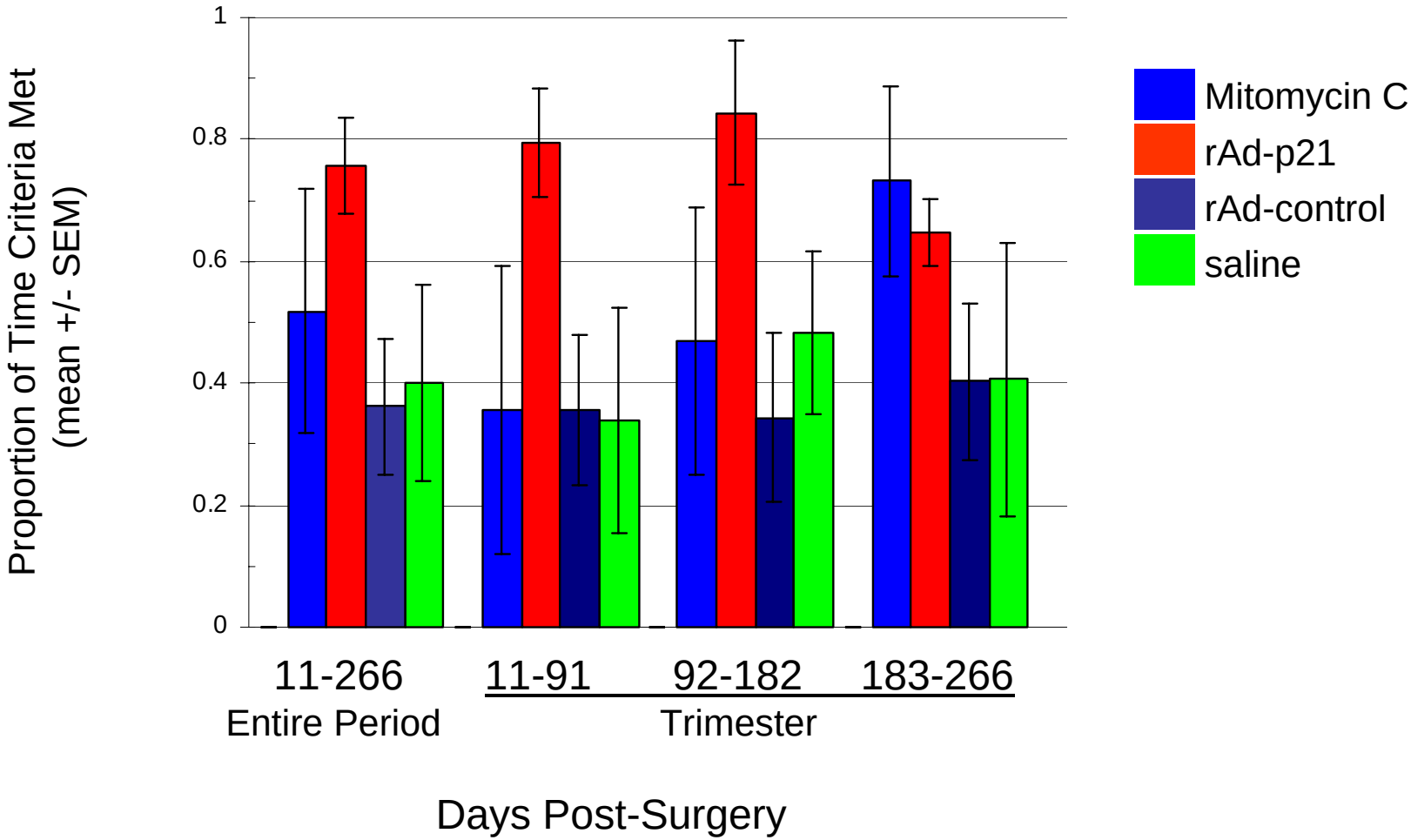
- Laser ablation of trabecular meshwork: induction of elevated IOP; unilateral
  - Avg pre-surgery range: 23 – 50 mm Hg
  - Normal range contralateral eye: 11 – 18 mm Hg

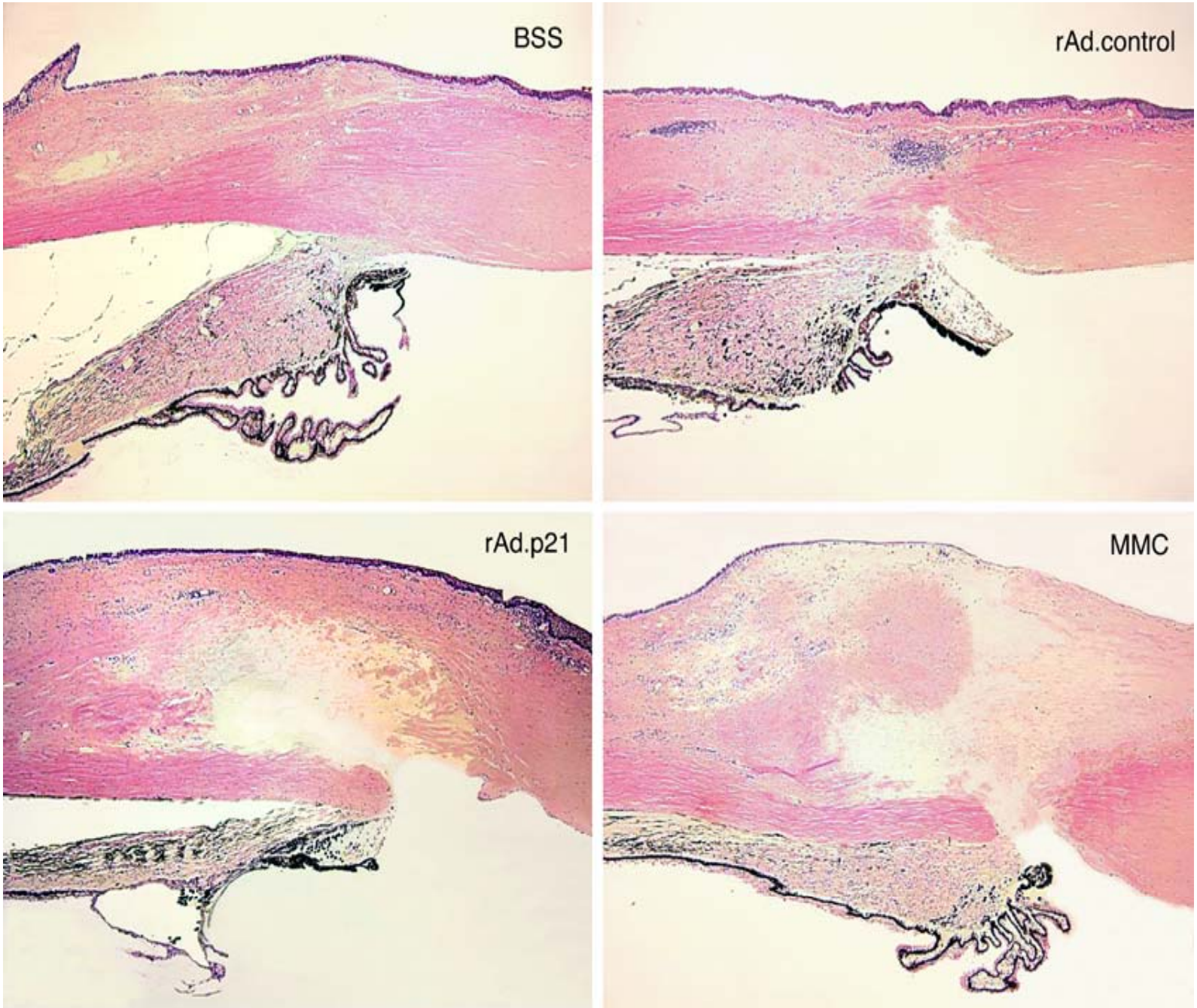
- Trabeculectomy: intraoperative topical application via drug soaked sponge; 5 minutes; n = 4/group; treatment groups include:
  - rAd-p21: 7 x 10^{10} total particles
  - rAd-control: 8 x 10^{10} total particles
  - Balanced salt solution (BSS)
  - Mitomycin C: 0.5 mg/ml

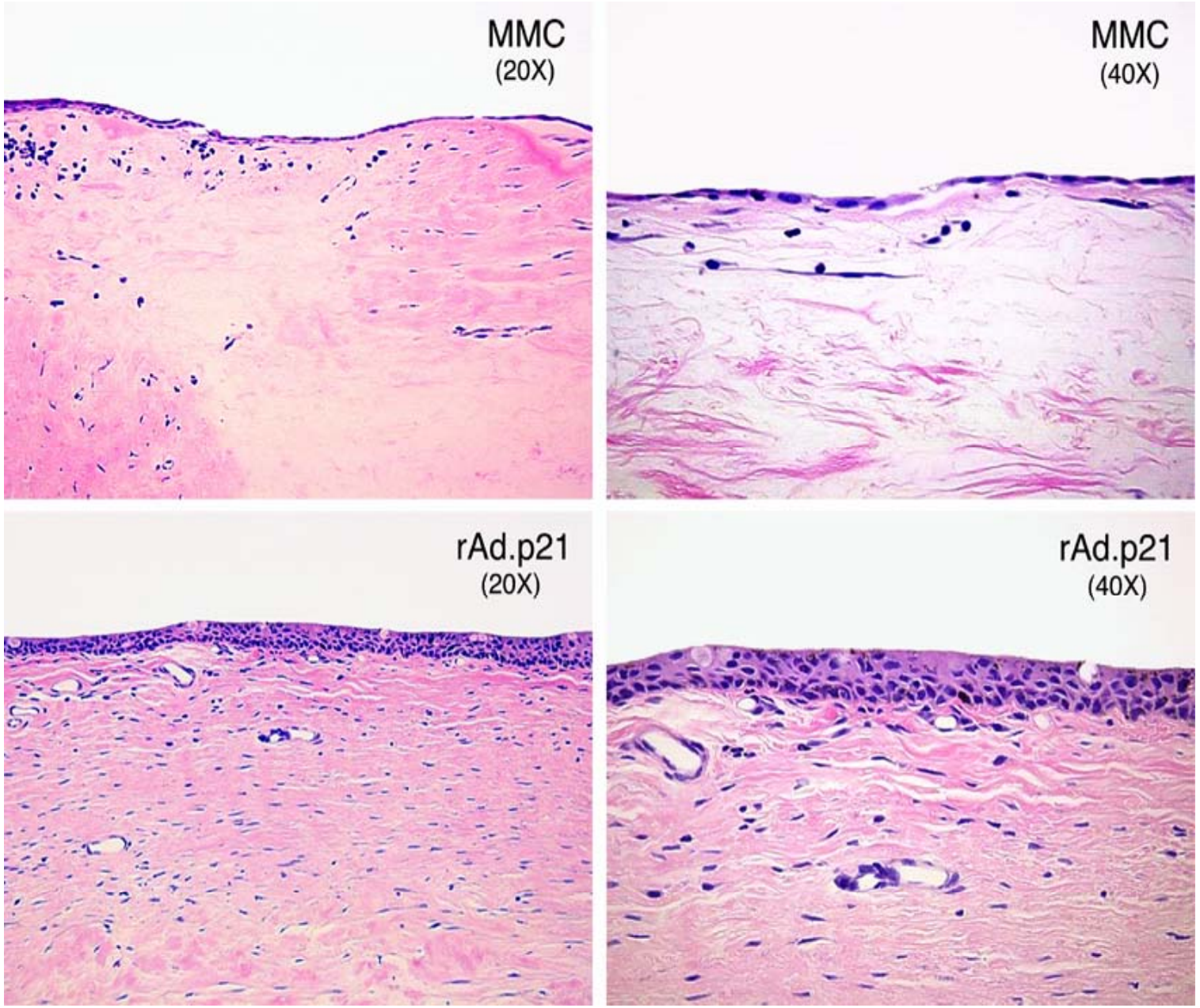
- 8 ½ months: sacrifice
  - IOP analyses

Heatley et al., submitted.

Evaluation of IOP
Success Criteria: ≤ Control Eye + 2 mm Hg




BSS
rAd.control
rAd.p21
MMC

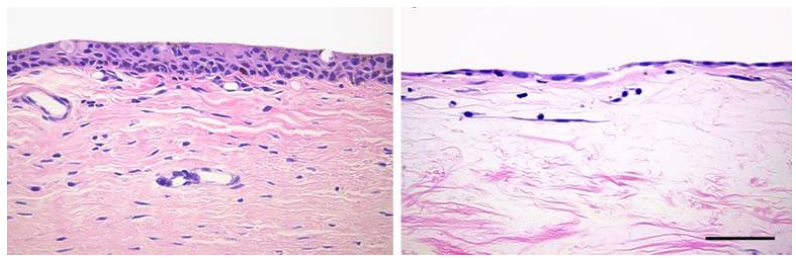

MMC
(20X)
MMC
(40X)
rAd.p21
(20X)
rAd.p21
(40X)

Serum Neutralizing Antibody and Cellular Inflammatory Responses to rAd-p21: non-GLP Studies

- **Serum anti-adenovirus neutralizing antibodies developed following a single subconjunctival injection of rAd-p21 to rabbits**
  - titers peaked at day 7; returned to baseline after ~3 weeks
  - did not affect subsequent rAd-p21 transduction of contralateral eye

- **Transient cellular inflammatory response in rabbit conjunctiva**
  - transient mononuclear cell infiltrate
  - resolved at 14 day
  - histology similar to eyes treated with placebo at day 14.

- **Tissue effects observed in MMC-treated eyes, but NOT rAd-p21-treated eyes (rabbits and primates)**
  - thin or absent conjunctival epithelium
  - hypocellular conjunctiva
  - disruption of normal tissue architecture



rAd-p21 **MMC**

Conclusions: Preclinical Evaluation of rAd-p21

- rAd-p21 inhibits proliferation in conjunctival fibroblasts
- Transient rAd transgene expression is coincident with the timing of the wound healing response after surgery
- In a rabbit filtration surgery model, rAd-p21 inhibits surgical failure (comparable to MMC)
- In an elevated IOP model in monkeys, treatment with rAd-p21 results in IOP control for > 8 months after trabeculectomy
- Transient conjunctival cellular inflammatory response to rAd-p21 was detected in rabbits without clinical manifestations
- Undesired tissue effects seen in MMC treated eyes are NOT observed in rAd-p21 treated rabbit or primate eyes.

Non-Clinical Safety Assessment

Robert Veneziale, Ph.D.
Schering-Plough Research Institute

Non-Clinical Safety Assessment: Protocols

- *Subconjunctival rAd-p21 Injection in Cynomolgus Monkeys*

Original Study

- $7.5x10^9$ or $7.5x10^{10}$ particles
- 100 μl per eye
- Bilateral injections - Day 1
- Surgery in one eye - Day 2
- Slit lamp and indirect exams
- Histology
- Biodistribution in blood and tissues

Follow Up Study

- $7.5x10^9$ or $7.5x10^{10}$ particles
- 100 μl per eye
- Bilateral injections - Day 1
- ✓ No surgery
- Slit lamp and indirect exams
- Histology of ocular tissues
- Biodistribution in blood
- ✓ Vision function assessment
  - Electroretinograms (ERG)
  - Visual evoked potential (VEP)

Table: Mononuclear Cell Infiltrate								
Tissue	Original Study				Follow Up Study			
	Low		High		Low		High	
Day =	6	29	6	29	7	29	7	29
Conjunctiva	+	+↓	++	+	++	++↓	++	+
Episclera					++	++↓	+++	++
Choroid	++		+++*				+++*	
Trab. meshwork							+	+↓
Ciliary body							++	
Sclera							+	

Severity = Minimal (+), Mild (++) or Moderate (+++)
↓ Lower incidence at the highest severity
* = Only one animal at the moderate severity

Biodistribution of Tissues with Measurable Levels of DNA on Day 6		
Tissue		Mean Copies/μg total DNA[a]
Conjunctiva/Sclera	Left	3.9×10^6
	Right	1.7×10^6
Optic Nerve	Left	1.1×10^3
	Right	5.1×10^2
Retina	Left	4.2×10^1
Brain - Lat. Gen. Nucleus	Right	2.5×10^1
Liver		4.9×10^2
Spleen		1.5×10^3
Kidney		1.6×10^1
Heart		8.7×10^1
Bone Marrow		1.8×10^1

a: Retina was per 0.1μg. Optic nerve and brain was up to 10 replicates of 0.1μg of DNA.

Note: DNA levels were below the quantitation limit for the lung, brain - occipital lobe and brain - optic chiasma. DNA was not detected in brain - lateral geniculate nucleus (left) and gonads.

Non-clinical Safety Assessment: Conclusions

- Clinical observations were transient.
  - Swollen and/or partially closed eye(s) at the high-dose only
  - Conjunctival congestion by slit lamp
- Histologic analyses revealed a transient mononuclear cell infiltrate in ocular tissues; independent of surgery.
  - Conjunctiva, episclera, choroid, trabecular meshwork, ciliary body and sclera
- ERG and VEP were normal; no effects on visual function.
- Biodistribution to non-target tissues was minimal and gonads were negative.

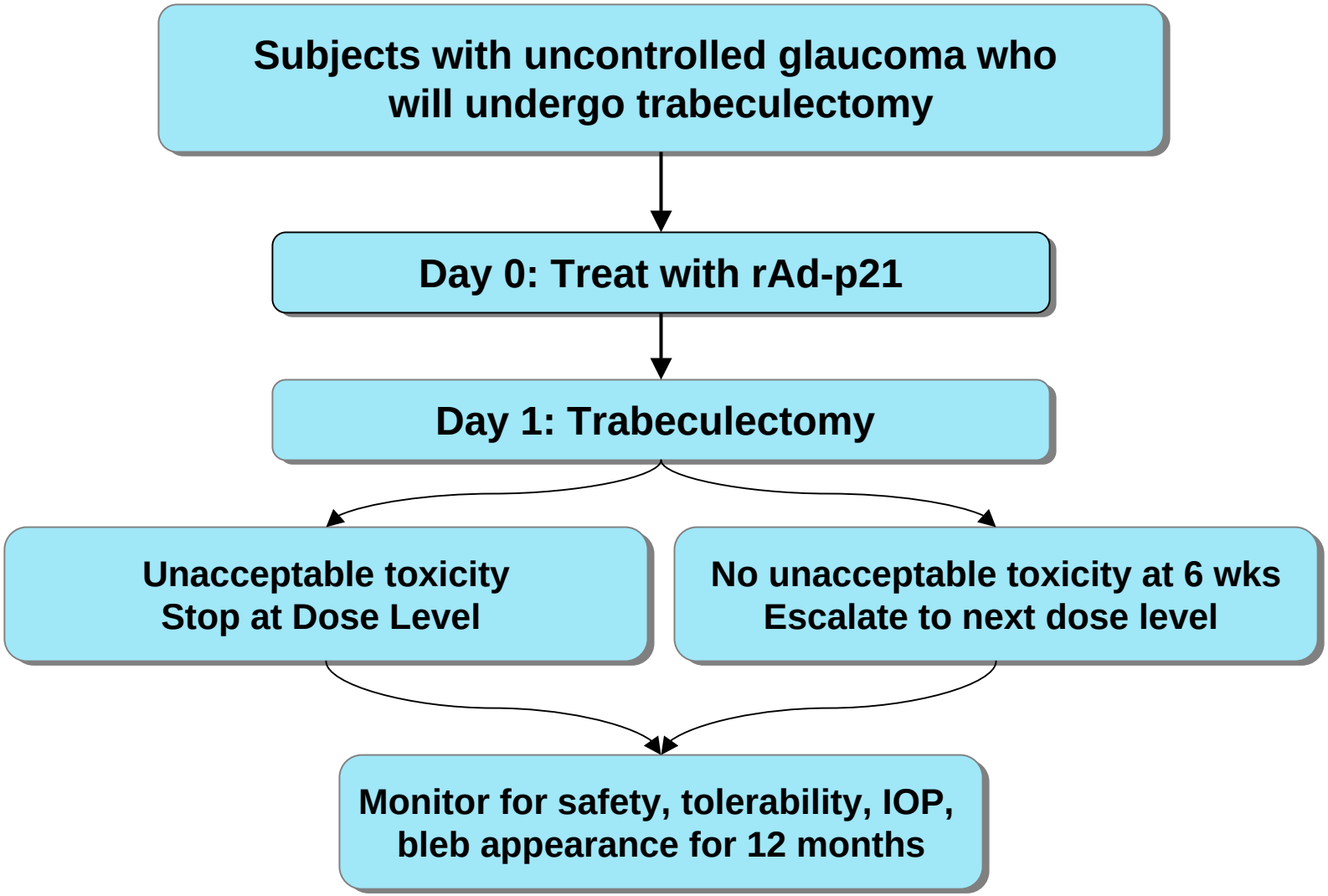
Phase I Clinical Protocol

Robert N. Weinreb, M.D.
University of California, San Diego

Proposed Clinical Protocol: #0307-589

- **Title:** A Phase I Study in Glaucoma Subjects Receiving SCH 412499 (rAd-p21) Administered as a Single Injection into the Subconjunctival Space Prior to Primary Trabeculectomy
- **Primary Objective:** To assess the safety and tolerance of rAd-p21 as adjunctive therapy consisting of a one-time preoperative injection in subjects scheduled for primary trabeculectomy.
- **Secondary Objective**: To monitor intraocular pressure and to study bleb appearance.
- **Study Design:**
  - Open-label, dose-escalation
  - 18 subjects expected in 3 dose cohorts
    - 7.5×10^9 particles
    - 2.5×10^{10} particles
    - 7.5×10^{10} particles
  - Single 100μl injection 18-24 hr prior to surgery

Study Design



Inclusion Criteria

- Subjects 40-75 years, inclusive of either sex, and any race
- Glaucoma inadequately controlled by maximal medical or laser therapy
  - primary open angle
  - pigmentary
  - pseudoexfoliation
- No previous conjunctival incisional ocular surgery
- Phakic or pseudophakic (IOL with clear cornea cataract surgery)
- Clinical laboratory tests within normal limits
- Free of any clinically significant disease that would interfere with study evaluation
- Use of birth control by male and female subjects prior to study and for at least 3 months post treatment

Major Exclusion Criteria

- No light perception; monocular
- Visual field with mean deviation of 15 db or worse; split fixation
- Active ocular diseases other than glaucoma:
  - surface disease
  - ocular herpes or cytomegalovirus; active or history of
  - iris neovascularization
  - diabetic proliferative retinopathy; active or history of
  - uveitis; blepharitis; chronic or recurrent
- Aphakia
- Previous cyclodestructive procedure
- Poorly controlled diabetes or hypertension
- Currently on antiviral therapy
- Need for combined procedure
- Active upper respiratory tract infection
- Unwilling to discontinue contact lens use
- Subjects not able to tolerate applanation tonometry
- Iridocorneal endothelial syndrome