Preclinical Models of Ocular Glaucoma at CBI
Focus on Eyes
Over 20 years of experience
Conveniently located in the heart of Silicon Valley, amidst many biotech companies
State of the art, purpose-built facility
Approximately 40 employees
Highly experienced staff
GLP, OECD, FDA, USDA, OLAW
AAALAC Accreditation
Glaucoma in Humans

• Common group of diseases resulting in increased intraocular pressure and damage to the retina and optic nerve
  – Most common: Wide or open angle slow exit of aqueous humor through trabecular meshwork
  – Narrow Angle-acute, iris blocks the trabecular meshwork
    • Normal Angle glaucoma-not common
Causes in Patients

- Heredity
- Age
- Ocular trauma
- Ocular inflammation
- Cataracts

Clinical presentation of untreated glaucoma
Current Clinical Medical Treatments

• Drugs
  – Prostaglandin analogs (lanoprost)
  – Parasympathomimetic or mitotic agents
  – Carbonic anhydrase inhibitors
  – Adenergics – Beta 1 antagonists
  – Alpha 2 agonists
  – Hyperosmotic agents
Clinical Surgery Treatments

• Trabeculectomy
• Canalplasty
• Glaucoma drainage implants
• Laser assisted non-penetrating deep sclerectomy
Preclinical Glaucoma Models at CBI

- Numerous models available:
  - Transgenic models in rodents
  - Episcleral vein cauterization in rodents
  - Surgical and device models
  - Steroid-induced in rabbits
  - Chymotrypsin-induced in rabbits
  - IOP reduction in normal rabbits and dogs
  - Other models or custom models upon request
Rat models of Glaucoma: Normal Eye IOP in Rats

- Albino and Brown Norway – typical species used
- Episceral cauterization is a useful and effective model to induce increased IOP
- In rats, IOP is consistent in normal animals
- No meaningful differences between left and right eyes, between sexes or between strains
Murine/Transgenic Models

• DBA/2J inbred mice (aged) spontaneously develop glaucoma

• Tg-MYOC^{Y437H} mice - Mouse model of primary open angle glaucoma (POAG)-express human transgene, at >40 weeks (Zode, Sheffield, 2015)

• Mutations in the myocilin (MYOC) gene, which encodes a protein expressed abundantly in the trabecular meshwork, are the most common genetically defined cause of glaucoma

• CBI working with transgenic provider to be first non-academic CRO with this model
Episcleral vein cauterization glaucoma model in rats

- Assess IOP changes following unilateral laser cauterization of episcleral veins in rats
- Sprague Dawley or Brown Norway rats
- Laser photocoagulation of limbus on Day 0 on right eye, left eye untreated
- IOP measurements at prescribed intervals
- Intraocular pressure in the operated eyes clearly increased dramatically within 15 minutes of cautery and remained fairly stable, and statistically significantly increased for days to weeks.
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

Episcleral vein cauterization rat glaucoma model-surgical procedure

- Under general anesthesia and aseptic conditions
- Episcleral vessels are identified
- Vessels are cauterized with a laser to permanently occlude the vessels
- “Touch ups” may be needed
Episcleral vein cauterization rat glaucoma model

Over 7 days, there is a sustained increase in the IOP in the cauterized eye.

Table 1: Summary Data of IOP in Sprague Dawley vs. Brown Norway Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>SD rats</th>
<th>BN rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD Operated eye</td>
<td>OS Normal Eye</td>
</tr>
<tr>
<td>-2D</td>
<td>11.8±4 (mm Hg)</td>
<td>10±7</td>
</tr>
<tr>
<td>-1D</td>
<td>14±4</td>
<td>13±3</td>
</tr>
<tr>
<td>-30 min</td>
<td>14±8</td>
<td>11±2</td>
</tr>
<tr>
<td>-15 min</td>
<td>24±8</td>
<td>12±4</td>
</tr>
<tr>
<td>0 (prior to surg)</td>
<td>22±8</td>
<td>14±3</td>
</tr>
<tr>
<td>15 min post op</td>
<td>31±3</td>
<td>13±4</td>
</tr>
<tr>
<td>1h</td>
<td>29±2</td>
<td>13±2</td>
</tr>
<tr>
<td>2h</td>
<td>24±3</td>
<td>16±3</td>
</tr>
<tr>
<td>3h</td>
<td>28±4</td>
<td>14±4</td>
</tr>
<tr>
<td>4h</td>
<td>29±2</td>
<td>15±2</td>
</tr>
<tr>
<td>5h</td>
<td>31±3</td>
<td>15±3</td>
</tr>
<tr>
<td>6h</td>
<td>28±5</td>
<td>13±4</td>
</tr>
<tr>
<td>Day 1</td>
<td>27±2</td>
<td>16±6</td>
</tr>
<tr>
<td>Day 2</td>
<td>29±2</td>
<td>12±5</td>
</tr>
<tr>
<td>Day 3</td>
<td>27±4</td>
<td>10±8</td>
</tr>
<tr>
<td>Day 7</td>
<td>29±3</td>
<td>12±4</td>
</tr>
</tbody>
</table>
Episcleral vein cauterization rat glaucoma model

Over **7 weeks**, there is a sustained increase the IOP in the cauterized eye
Episcleral vein cauterization rat glaucoma model

Over 6 weeks, there is a sustained increase the IOP in the cauterized eye. There was a repeat procedure at 1 week.
Episcleral vein cauterization rat glaucoma model

Over several weeks, there is an increase in the number of TUNEL positive apoptotic RPE cells in the retinal

<table>
<thead>
<tr>
<th>TUNEL staining for apoptosis of damaged RPE cells (cell counts/hpf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal retina</td>
</tr>
<tr>
<td>Glaucomatous eye (Day 13, n=4)</td>
</tr>
<tr>
<td>Glaucomatous eye (Day 35, n=4)</td>
</tr>
</tbody>
</table>
Rabbit Models of Glaucoma

- Chymotrypsin-induced glaucoma in rabbits - longest and most reliable IOP elevation
- Steroid-induced - 3-5 week administration of cortico-steroid will increase IOP
- Laser-induced - short term, not very reliable, structure of the irido-corneal angle, which is different from that of humans.
Rabbit: Increases in IOP in chymotrypsin-treated eyes

- Chymotrypsin injected unilaterally resulting in obstruction of the outflow and increases in the IOP.
- Right eyes - Chymotrypsin treated. There is a significant increase in IOP over a 3 week period. (N=8)
- Left eyes: Normal
IOP in Chymotrypsin treated eyes: Vehicle vs Test Article

Group 1-Chymotrypsin-treated and vehicle-treated. There is a significant increase in IOP over a 3 week period with a return towards normal at 3 weeks (n=8).

Group 2: Chymotrypsin-treated and test article-treated. The test article does not reduce the IOP in chymotrypsin-treated rabbits, and may increase the IOP.
Steroid Induction

• Rat model induced by topical application of dexamethasone
  • Rats share similar anatomical and developmental characteristics of the anterior chamber, especially in aqueous outflow pathway with humans
  • Reasonable IOP elevation as retinal and ON changes are similar to humans
• Rabbit model induced via betamethasone subconjunctival injection
• Mimics human chronic open-angle glaucoma
Custom Surgical and Device Models

- Glaucoma filtration devices and drainage implant surgery - custom surgery
- Rabbits preferred species
- Study duration - days to months
- Parameters - local tolerability, IOP, funduscropy, histopathology,
IOP effects in normal animals

• The IOP lowering effects as well as local tolerability of test compounds may be assessed in normal animals effective
• Rodents, rabbits, dogs are suitable, particularly dogs
• Cost effective and large numbers of compounds are easily screened for days to weeks
• Lanoprost, Timolol are suitable positive controls
Normal Eye IOP in Rats

- Albino and Brown Norway - typical
- IOP consistent in normal animals
- No meaningful differences between left and right eyes, between sexes or between strains
Normal Eye IOP in Albino vs Dutch Belted Rabbits

- Dutch belted and Albino rabbits-No meaningful differences between left and right eyes, between sexes or between strains
- Below-comparison of normal values in Dutch belted vs Albino rabbits
Normal Eye IOP Reduction in Dogs

- Beagle Dogs
- No meaningful differences between left and right eyes, between sexes
- Below Left - IOP values in normal dogs.
- Below Right - left eye of patient dog with unilateral spontaneous glaucoma.
Typical Endpoint Assessments for Ocular Studies at CBI

- Slit lamp biomicroscopy
- Tonometry
- Pachymetry
- ERG
- OCT
- Goniscopy
- Funduscopy
- Angiography (photo at right)
- Histopathology and Immunohistochemistry
- Pathology
- Photomicroscopy and Histomorphometry
Typical Routes of Administration for Ocular Studies at CBI

• Topical
• Intra vitreal
• Subretinal
• Subconjunctival
• Intracameral
• Subtenon
• Transcleral
• Device implantation
CBI offers ocular toxicology, pharmacokinetic and pharmacology capabilities

• Acute, subacute and chronic toxicology studies
• Discovery and investigative toxicology studies
• Ocular and other routes of delivery
• Special ocular assessments
• Complete, prompt reports
CBI offers ocular toxicology, pharmacokinetic and pharmacology capabilities

- Ocular and other routes of delivery
- Special ocular assessments
- Tissue and fluid collection from different subparts of the eye
- Ex. aqueous, vitreous, anterior segment, retina, sclera
- Complete, prompt reports
CBI offers ocular toxicology, pharmacokinetic and pharmacology capabilities

- Wide range of ocular pharmacology and efficacy studies
- Inflammation
- Oxygen-induced retinopathy
- Choroidal neovascularization
- Glaucoma
- Diabetes-induced retinopathy
- Cataract and lens
- Ocular surgery
- Corneal injury and transplant
- Ocular implants
- Dry Eye
- Custom studies
Ocular Histopathology

CBI offers ocular histopathology, immunohistochemistry and histomorphometry

- Routine ocular histopathology in all species
- Immunohistochemistry
- Ocular histomorphometry
- Complete, prompt reports, GLP, nonGLP
Service and Quality

- **The people at CBI**—from the executive team to the study directors to the research associates expect to have to earn your trust and business.
- **Our ratio of scientists to non-scientists** is one of the highest in the industry. We believe in sound science and every study director is a PhD-level scientist.
- **Thoroughness in planning and execution** is key to a successful study. All protocols are vetted and approved by multiple personnel. Our QAU has a rigorous training program. All non-GLP studies are conducted in the spirit of GLP with the same SOPs.
- **We believe in communication**: timely responses to your inquiries and frequent updates on your study are mandatory.
- **Rapid initiation and adjustments**; with the collective expertise of must larger organizations but the flexibility of a smaller more nimble group.
- **You are always welcome** at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.
Our Staff

• **Study Directors**
  – PhD level scientists
  – Appointed by management for each job
  – Serves as single point of control and is responsible and accountable for study conduct and scientific interpretation
  – Experienced attentive and communicative
  – Rapid study initiation and report preparation

• **Research Associates**
  – Bachelor Level Scientists
  – Extensive technical training

• **Quality Assurance**
  – Full time, dedicated
  – Rigorous training program

• **CBI Management**
  – Experienced senior scientific management-with large and small pharma experience
Summary

• With a focus on quality, CBI provides state of the art:
  – Toxicology
  – Pharmacokinetics
  – Efficacy
  – Pharmacology
  – In house histopathology

• Experienced attentive and communicative study directors

• Rapid study initiation and report preparation

• Established, stable business

• Regulatory compliance

• Favorable pricing structure