CBI

Melanin Associated Antigen Induced Uveitis in Rats

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COMPARATIVE BIOSCIENCES, INC.
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

COMPARATIVE BIOSCIENCES, INC.

Premier Preclinical Contract Research Organization

- **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 **30 employees**
- **Highly experienced staff**
- **GLP, OECD, FDA, USDA, OLAW**
- **AAALAC Accreditation**

Melanin Associated Antigen Induced Uveitis in Rats

- CBI provides a validated model of MAA-induced anterior uveitis in rats. In this model, MAA in Freund's adjuvant is injected subcutaneously, leading to a consistent, strong immune-mediated anterior uveitis.
- MAA is chemically prepared from pigmented the iris of cattle by a proprietary method based upon the published procedure of Bora et al. Each lot is quality tested prior to use.
- CBI has conducted approximately 3-4 studies per year since 2008 with the use of over 1000 Lewis rats.
- Uveitis develops at about Day 17-18 and persists for about 2 weeks with subsequent resolution.
- Established positive control drugs include dexamethasone and systemic cyclosporin A. Topical cyclosporin A has some activity, also.

MAA Uveitis: Typical Study Design

- 10 Lewis rats per group, vehicle, test article at 2-3 dose levels, positive control of topical dexamethasone (DEX) or systemic cyclosporin A (CSA)
- Induce inflammation with MAA injection
- Slit lamp scoring of inflammation in anterior segment 3x weekly for 2 weeks beginning at 2 weeks post induction
- Weekly body weights
- Necropsy with histopathologic examination of eye
- Complete report

MAA Uveitis: Study Criteria

- The following are the major study criteria
  - Clinical ocular scores via slit lamp microscopy
  - Histopathologic scoring for inflammation
- The following are minor study criteria
  - General clinical observations
  - Body weights (generally there are no changes)
- Other criteria as requested by sponsor

Ocular Clinical Grading Scale

The following is the scoring system used for grading for MAA-Induced Inflammation by slit lamp biomicroscopy:

Grade	Criteria
0	Normal, eye, translucent and reflects light (red reflex)
0.5 (Trace)	Dilated vessels in iris
1	Engorged blood vessels in iris; abnormal pupil contraction
2	Hazy anterior chamber, decreased red reflex
3	Moderately opaque anterior chamber by pupil still visible; dull red reflex
4	Opaque anterior chamber and obscured pupil, red reflex absent, proptosis

Histopathology Procedures

- Sacrifice with collection and fixation of eyes at Day 21.
- Eye step-sectioned to present multiple levels of the anterior segment and stained with HE
- Other stains available
- Sections are evaluated and scored by ACVP pathologist
- Representative photomicrographs for report

Histopathology Grading

Grade	Criteria
0	Normal, no histopathologic lesions seen.
0.5 (Trace)	Trace multifocal infiltration of the iris and/or ciliary area with neutrophils and/or mononuclear cells. No to trace congestion.
1	Mild multifocal infiltration of the iris and/or ciliary area with neutrophils and/or mononuclear cells. Trace to mild congestion. No to trace extension of inflammation into cornea, anterior chamber and posterior chamber.
2	Mild to multifocal infiltration of the iris and/or ciliary area with neutrophils and/or mononuclear cells. Congestion and edema. No to mild extension of inflammation into cornea, anterior chamber and posterior chamber.
3	Moderate multifocal to diffuse, often expansile, infiltration of the iris and/or ciliary area with neutrophils and/or mononuclear cells. Hemorrhage, congestion and edema. No to moderate extension of inflammation into cornea, anterior chamber and posterior chamber.
4	Severe, expansile infiltration of the iris and/or ciliary area with neutrophils and/or mononuclear cells. Hemorrhage, congestion and edema. Inflammation may extend into cornea, anterior chamber and posterior chamber.

Sample Data

Ocular Clinical Scoring and Photos

Histopathology Scoring

Ocular Clinical Grading Scale

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4	Opaque anterior chamber and obscured pupil, red reflex absent, proptosis

Ocular Clinical Scoring: Sample Data

Group	Treatment	Day 0	Day 10	Day 18	Day 24	Day 27	Day 30
1	Vehicle	0 ± 0	3.2 ± 0.5	3.6 ± 0.5	3.2 ± 0.2	1.8 ± 0.4	1.0 ± 0.0
2	DEX (Top)	0 ± 0	1.9* ± 0.3	2.2* ± 0.4	1.9 ± 0.0*	1.5 ± 0.5	0 ± 0*
3	CSA (Top)	0 ± 0	2.0* ± 0.0	3.0 ± 0.0	3.0 ± 0.0	1.4 ± 0.5	0 ± 0*
4	Normal eye	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0 *
5	CSA (IM)	0± 0	2.0* ± 0.0	2.3* ± 0.5	1.8 ± 0.4*	1.5 ± 0.5	0 ± 0*

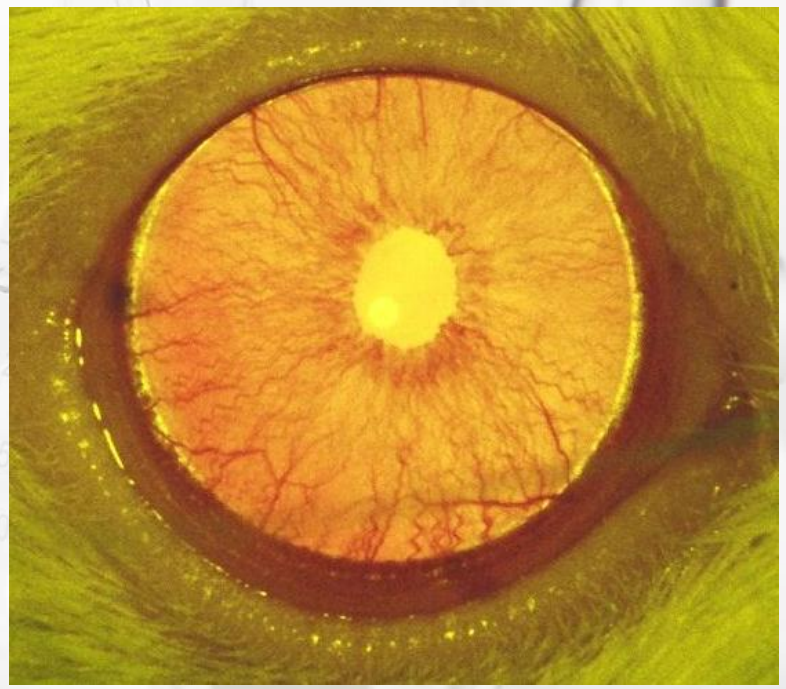
*Statistically significant . N=40/group

- Data is combined from validation study 2009 and three client studies, with an N of 40 animals per group
- There is statistically significant difference between vehicle-treated and dexamethasone, and between vehicle-treated and systemic cyclosporin, indicating that both drugs are suitable positive controls.
- Topical cyclosporin has some anti-inflammatory activity, but is not as active as topical dexamethasone or systemic cyclosporin.
- Maximum inflammation is present between Day 18 and 24
- Inflammation begins to resolve at about 4 weeks

Clinical Scoring

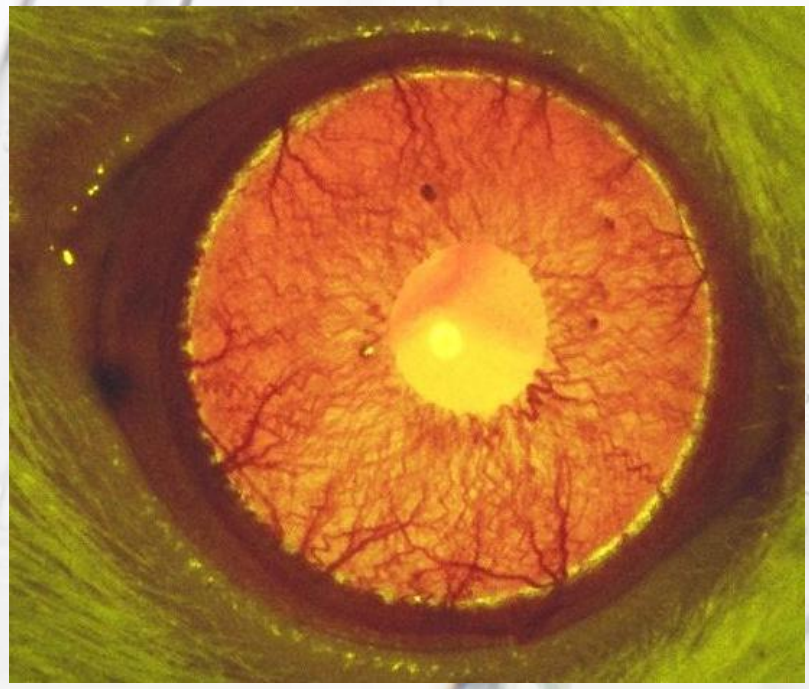
Normal Eye

Grade 0.5 Inflammation

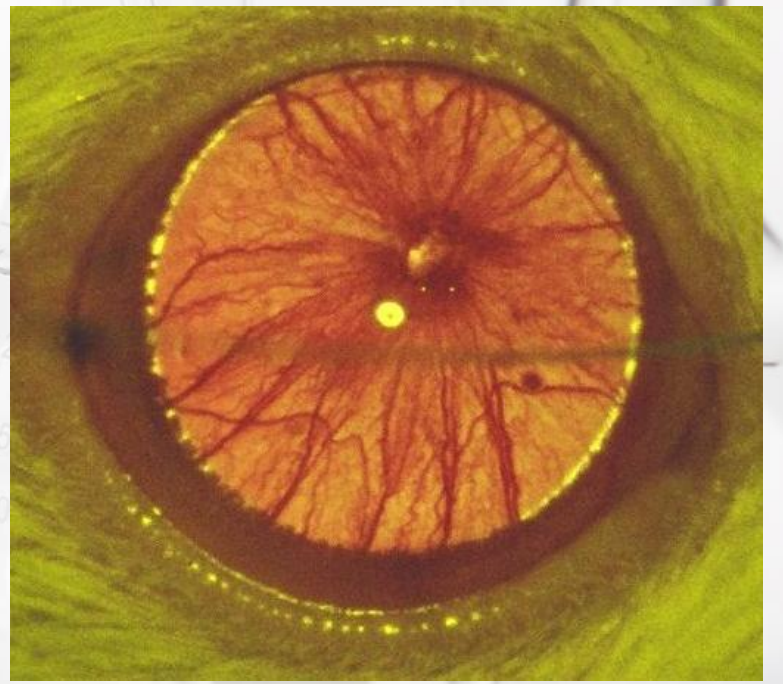


Clinical Scoring

Grade 2 Inflammation

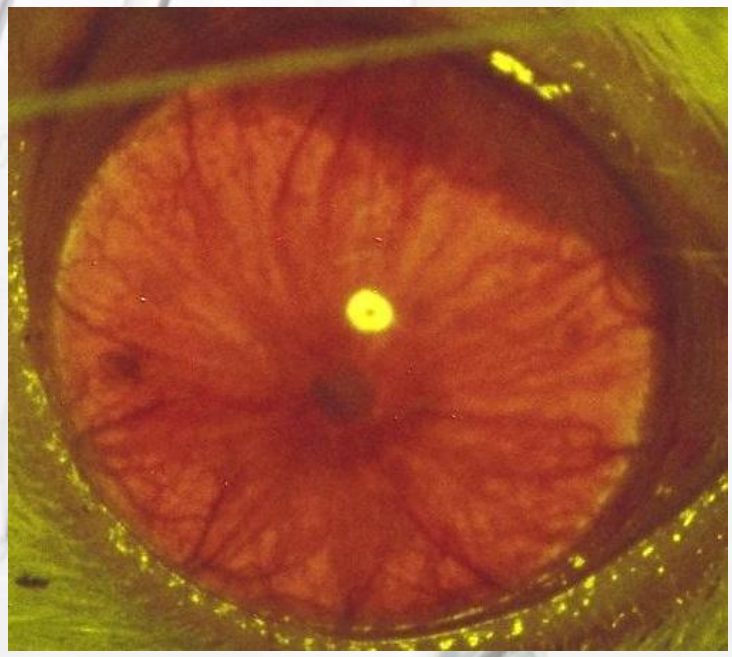


Grade 3 Inflammation

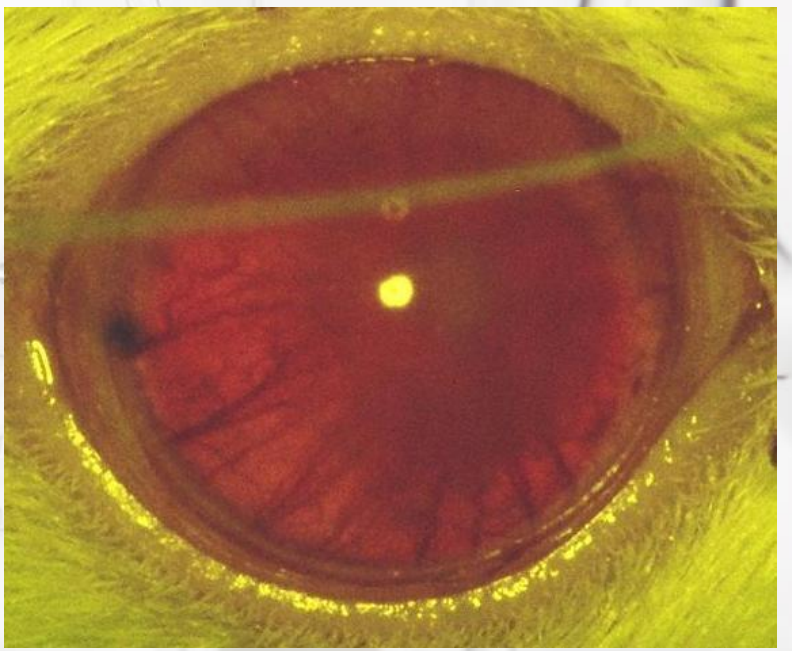


Clinical Scoring

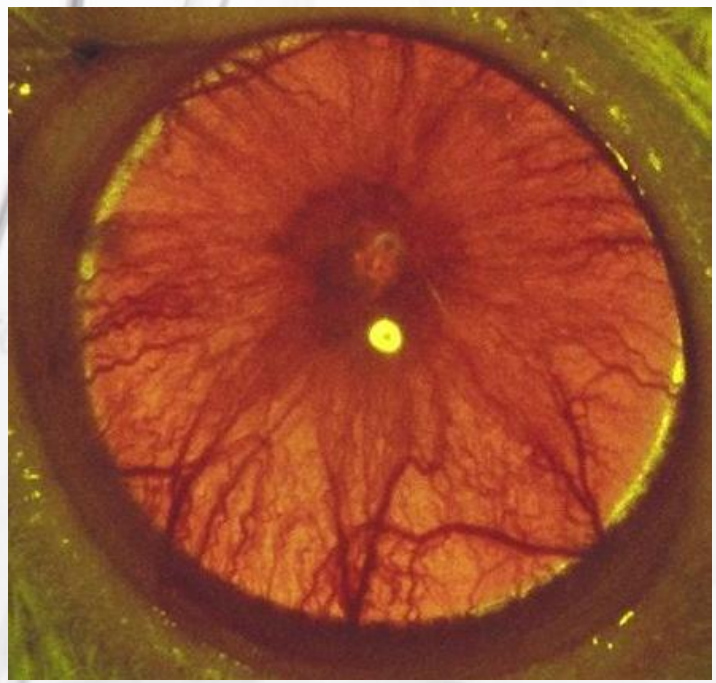
Grade 3 Inflammation



Grade 4 Inflammation



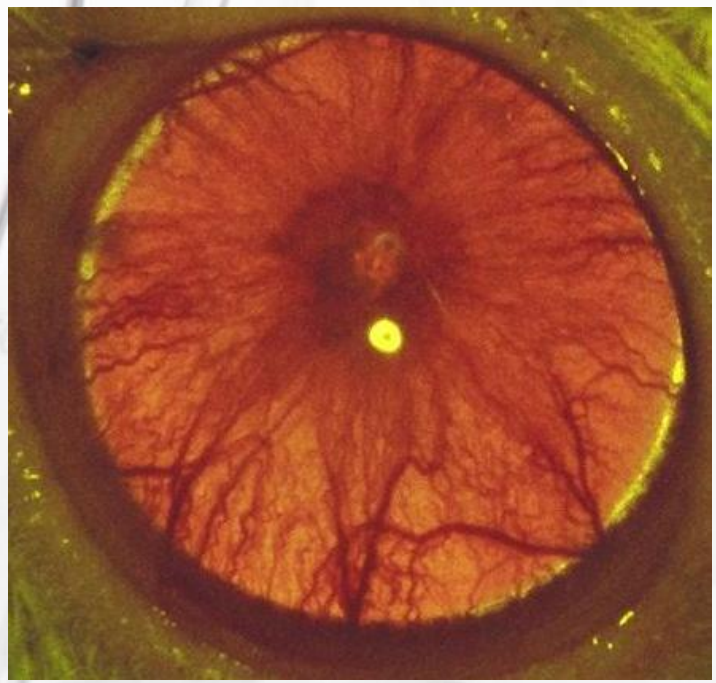
Clinical Ocular Photomicroscopy



MAA+vehicle. Severe inflammation with fibrin deposition, hyperemia, tortuosity of iral vessels, pupillary fixation. The anterior chamber is opaque and turbid.

MAA+dexamethasone. Mild hyperemia and tortuosity of iral vessels, normal pupil. The anterior chamber is clear

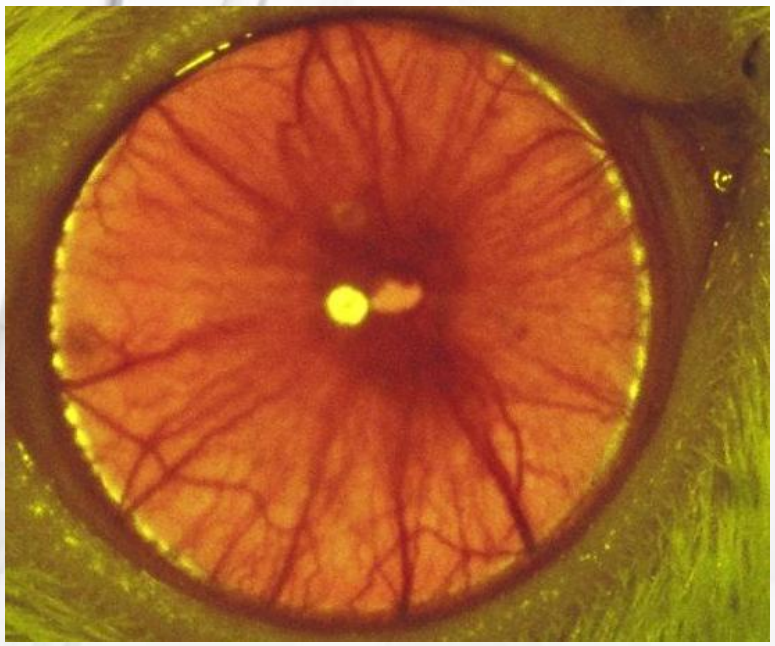
Clinical Ocular Photomicroscopy



MAA+vehicle. **Severe inflammation with fibrin deposition, hyperemia, tortuosity of iral vessels, pupillary fixation. The anterior chamber is opaque and turbid.**

MAA+minimally active test article. **Moderate inflammation with fibrin deposition, hyperemia, minimal tortuosity of iral vessels, pupillary fixation. The anterior chamber is slightly opaque and turbid**

Clinical Ocular Photomicroscopy



MAA+vehicle. **Severe inflammation with fibrin deposition, hyperemia, tortuosity of iral vessels, pupillary fixation. The anterior chamber is opaque and turbid**

MAA+IM cyclosporin. Mild hyperemia and tortuosity of iral vessels, normal pupil. The anterior chamber is clear.

Histopathology Grading Scale

Grade	Criteria
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4	Severe, expansile infiltration of the iris and/or ciliary area with neutrophils and/or mononuclear cells. Hemorrhage, congestion and edema. Inflammation may extend into cornea, anterior chamber and posterior chamber.

Histopathology Grading: Sample Data

Data from Day 18:

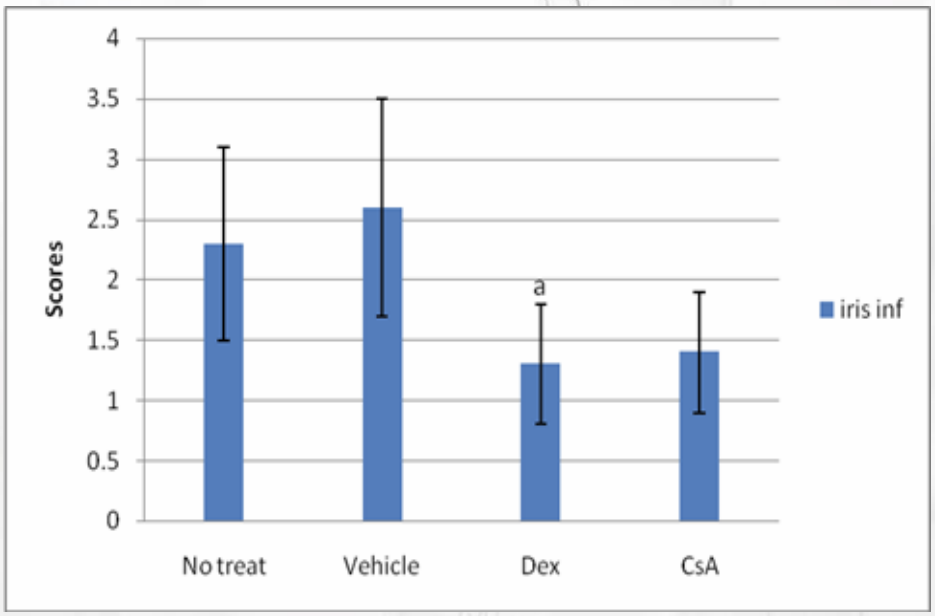
Group Day 18	Treatment**	Mean Score	Histopathologic findings
1	Vehicle	3.3 ± 0.8	Marked inflammation of the ciliary process, anterior chamber, cornea and vitreous was present
2	DEX (0.1%)	1.6* ± 0.4	Minimal to mild inflammation of primarily the ciliary process was present
3	CsA (15 mg/kg IP)	1.4* ± 0.5	Minimal to mild inflammation of primarily the ciliary process was present
4	Restasis (topical)	3.4 ± 0.7	Marked inflammation of the ciliary process, anterior chamber, cornea and vitreous was present
5	No treatment	3.4 ± 0.8	Marked inflammation of the ciliary process, anterior chamber, cornea and vitreous was present
6	Normal eye	0 ± 0	Normal eye, no lesions

Histopathology Grading: Sample Data

Data from Day 30:

Group Day 30	Treatment**	Mean Score	Histologic comment
1	Vehicle	2.3 ± 0.7	Moderate inflammation of the ciliary process, anterior chamber, cornea and vitreous was present
2	DEX (0.1%)	1.4* ± 0.4	Minimal to mild inflammation of primarily the ciliary process was present
3	CsA (15 mg/kg IP)	1.3* ± 0.5	Minimal to mild inflammation of primarily the ciliary process was present
4	No treatment	2.6 ± 0.8	Moderate inflammation of the ciliary process was present
5	Normal Eye	0 ± 0	Normal eye, no lesions

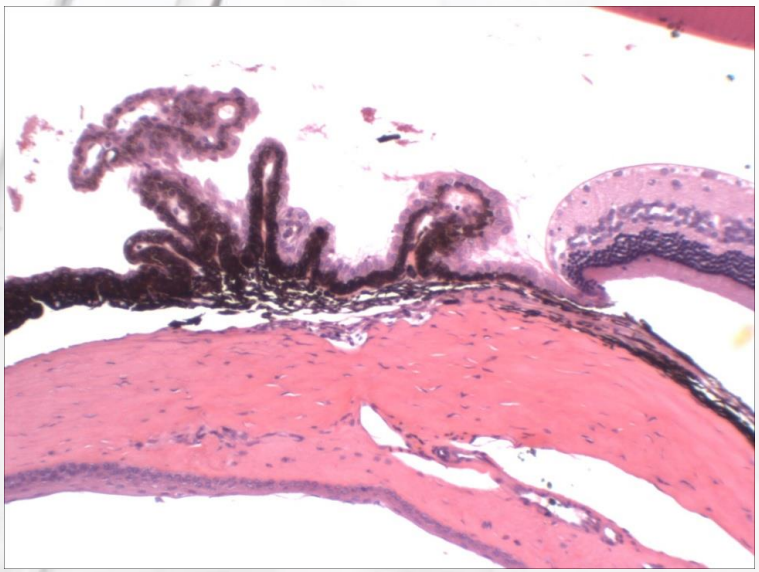
Histology Scoring



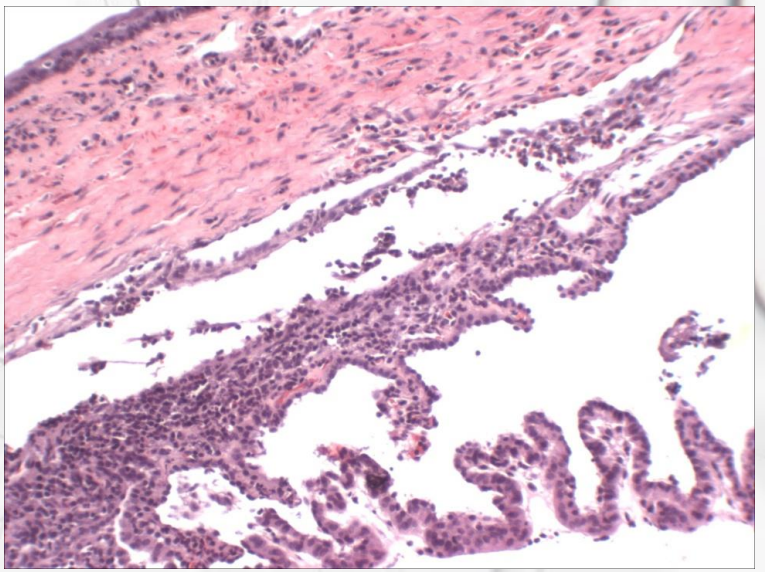
There is a statistically significant reduction in inflammation between MAA treated (no treatment and vehicle) vs dexamethasone. There is a downward trend in topical CSA.

a p-value < 0.05 relative to vehicle control group.

Histology Results



Normal anterior segment



MAA+Vehicle with severe inflammation of the anterior segment

Summary

- MAA-induced anterior uveitis is a robust, consistent model for assessment of immune-mediated inflammation of the anterior segment

- Treatment with dexamethasone and systemic cyclosporin produces significant inhibition of inflammation. Topical cyclosporin decreases inflammation to a lesser extent

Service and Quality

- ***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.
- ***We believe in sound science.*** Our ratio of scientists to non-scientists is one of the highest in the industry. Every study director is a PhD-level scientist.
- ***We believe in communication.*** Timely responses to your inquiries and frequent updates on your study are mandatory.
- ***We welcome visitors.*** You are always welcome at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.