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14. ABSTRACT

Retinal detachment (RD) is a prevalent cause of blindness that is common after ocular injury to military personnel. Permanent vision loss occurs due to death of photoreceptors and formation of excessive scar tissue, known as proliferative vitreoretinopathy (PVR). There are no effective pharmaceuticals to prevent these problems. The inflammatory protein, macrophage migration inhibitory factor (MIF), is produced at high levels in RD and PVR, as well as in excitotoxic (NMDA-mediated) damage, which is important in blast injury. The proposed research will test the ability of different clinically-relevant MIF inhibitors to block photoreceptor death and abnormal healing after RD or NMDA damage in different animal models. One of these drugs, ibudilast, has already been approved for human use in Japan as an anti-inflammatory agent and is currently undergoing clinical trials in the US as a neuroprotective agent for several neurologic diseases. This research will have considerable promise for treating ocular disease triggered by military injuries. We hope it will provide ground work for a clinical trial in patients, which could one day lead to therapeutics that could prevent vision loss.

15. SUBJECT TERMS

Retinal detachment (RD), proliferative vitreoretinopathy (PVR), N-methyl-D-aspartate (NMDA), macrophage migration inhibitory factor (MIF), MIF inhibitor, chick, rabbit, retinal pigment epithelium (RPE), ARPE-19, ibudilast, AV411, CPSI-1306, AV1013, scratch assay, MTT assay, contraction assay, epithelial mesenchymal transition (EMT)

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1. INTRODUCTION:

Our project pertains to the FY17 VRP Technology/Therapeutic Development Award Focus Areas: "Preclinical animal studies to evaluate safety and/or efficacy of treatments or technologies returning form and function after traumatic injury to: (1) orbit and ocular tissues (optic nerve, retina, and uvea), (2) eyelid, and/or (3) adnexal structures" and "Preclinical animal studies to evaluate safety and/or efficacy of treatments or technologies to reduce/control scarring and/or pathological healing response(s) after military-relevant ocular/visual system injury." Since MIF promotes photoreceptor apoptosis and retinal gliosis and it antagonizes the anti-inflammatory effects of glucocorticoids, which are the standard pharmacologic treatment used in traumatized eyes, our hypothesis is that clinically relevant MIF inhibitors that target the inflammatory response, will yield benefit in retinal neuroprotection and scar prevention in traumatic retinal damage. Our hypothesis will be tested with the following specific aims. Aim 1: Test the hypothesis that clinically-relevant MIF inhibitors block neuronal apoptosis in our in vivo RD and NMDA damage models. We will evaluate neuroprotection using electrophysiology, spectral domain OCT (SD-OCT), fundus imaging, and histology. Any potential toxicity will be evaluated. Aim 2: Test the hypothesis that clinically-relevant MIF inhibitors block gliosis and pathologic wound healing in traumatic RD. Studies with cell lines (retinal pigment epithelium and Müller glia) and our in vivo RD, RD-PVR, and NMDA models will be performed. Retinal fibrosis will be evaluated with SD-OCT and histology. Aim 3: Evaluate the effects on intraocular pressure (IOP) and the ocular pharmacokinetics of clinically relevant MIF inhibitors. Rabbits will be used to determine the effects of the drugs on IOP and the pharmacokinetics of ocular delivery of MIF inhibitors.

2. KEYWORDS:

Retinal detachment (RD), proliferative vitreoretinopathy (PVR), N-methyl-D-aspartate (NMDA), macrophage migration inhibitory factor (MIF), MIF inhibitor, chick, rabbit, retinal pigment epithelium (RPE), ARPE-19, ibudilast, AV411, CPSI-1306, AV1013, scratch assay, MTT assay, contraction assay, epithelial mesenchymal transition (EMT)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Task 1. Evaluate the clinically relevant MIF inhibitors, CPSI-1306, ibudilast/AV1013 in our in vivo retinal damage models (RD and NMDA) for neuroprotection of photoreceptors.
This task will utilize electrophysiology, SD-OCT, fundus imaging, and TUNEL/IHC to assess the ability of MIF inhibitors to protect the retinal neurons in RD- and NMDA-induced retinal damage in chicks and find the appropriate dosage. This will include dose escalation studies in untreated animals with a single intravitreal injection in chicks to find a toxic and maximal therapeutic dose (1a). Dose ranging studies will be conducted in retinal damage models to find the optimal dose for neuroprotection (1b). Finally, drug timing studies will be performed to test the impact of timing on the neuroprotective benefit (1c).
Timeframe: Months 1-18

Subtask 1a. Perform dose escalation studies in untreated animals to find toxic and maximal therapeutic doses. This task will involve weighing, ocular examination with Nussenblat scale grading, IOP measurements, ERG, SD-OCT, and fundus imaging. This will be carried out on untreated chick eyes on a limited number of chicks (n=4).
Timeframe: Months 1-6

Y1Q1 Progress: We have acquired the equipment necessary to complete these measurements. Specifically, the AVIA Tonopen for IOP measurements, the fundus camera with foot pedals, the Vetflo isoflurane anesthesia machine, the Leica datastation for processing SD-OCT data, and the micro-injector with nanofil syringe. Additionally, we have established a protocol for ERG data acquisition for chicks and collected a normative data set for untreated chick eyes at post-hatch day 8 (P8) and P15 for ERG measurements.

Y1Q2 Progress: We have established and refined protocols for clinical examination of the chicks, intraocular injections, and SD-OCT imaging. We identified the maximal solubility of ibudilast in appropriate diluents at 7pH for intravitreal drug delivery in humans (4.5mg/mL in sterile water). We have tested 12 chicks with intravitreal ibudilast at this maximal dose. Four of these chicks were sacrificed and stained for cell death with a TUNEL assay at day 1 post injection, when apoptosis should be maximal. We demonstrated no increase in TUNEL staining in ibudilast and control, indicating no neurotoxicity at this maximal achievable dose. Eight birds for a longer timecourse were injected as per our established protocol. At day 3 (three days post injection), we tested SD-OCT imaging in one chick to establish a protocol for future testing. We acquired good image captures of each eye using a standardized protocol. Additional SD-OCT images were acquired at D7 and D16 and ERG captures were taken at D10 and D14.
We have also undergone training with ULAR staff for chick intubation in the case that extended-length isoflurane anesthesia would be needed for the approved procedures. In addition, we have purchased four chick brooders (3 model 0534 for 1-2 week old chicks and 1 model CQB for older chicks) to house the chicks in our building vivarium. We have purchased a heat lamp so that chicks will be under the heat lamp and on top of a heating pad to maintain their body temperature with continuous temperature monitoring during experimental procedures.

Y1Q3 Progress: We made progress finding the maximum soluble concentration of CPSI-1306 (1.25mg/mL in sterile water). This concentration has been used as the maximal dose for the CPSI-1306 toxic/maximal therapeutic dose studies and we are analyzing the results. We have purchased the chicks for the CPSI-1306 maximal dose studies. We have also added more birds to establish a better sample size for ibudilast maximal dose studies and these are in process. We have received the AV1013 ibudilast analog as well. We have been expanding the normative databases for OCT and ERG and have obtained more chicks to add data for the day 21 and day 28 timepoints.

Y1Q4 Progress: We completed the maximal dose toxicity study for CPSI-1306 at 1.25mg/ml. This includes the TUNEL data at D1 and D3, the INL thickness data at D28, the ERG/OCT data at D7, D14, D21, and D28. The additional older chicks for the ERG and OCT normative databases have also been completed.

Remaining Tasks: We need to finish final histology analysis to evaluate neuroprotective effect on INL thickness for the max dose ibudilast timecourse.
Completion: 27 chicks (27/27) + 18 ERG Tests (18/18) + 19 OCT Tests (19/19) + 22 normative ERG dataset chick measurements (22/22) + 12 normative OCT dataset chick measurements (12/12) + 13 TUNEL stains/analysis (12/13) + 12 DAPI INL thickness measurements (4/12) + Training & Protocol Generation (ERG, OCT, Injection, Intubation, Slit Lamp, Fundus Imaging) (14) + Equipment (Tonopen, OCT Datastation, Fundus Camera, Injector, Isoflurane, Brooders x4, Intubation Tubes) (10) = 147 pts: 138/147 = 93.9%

Subtask 1b. Perform dose ranging studies for MIF inhibitors in retinal damage models. This task will be performed on a limited number of chicks (n=4) that have undergone retinal damage (RD or NMDA). They will be treated to find the optimal dose for neuroprotection with the MIF inhibitors. TUNEL analysis will be carried out on day 3 for RDs and day 1 for NMDA damaged retina. Following this study a larger study (n=10) will be carried out on day 14 (RD) or day 7 (NMDA) chicks with IHC, SD-OCT, fundus imaging, and ERG.
Timeframe: Months 1-18

Y1Q1 Progress: In addition to collecting the normative dataset, we have also begun tests on NMDA damaged chicks to establish differences between healthy and damaged eyes. Data has been collected on P8, 1 day post NMDA injection and P15, 8 days post NMDA injection. The protocol generation, training, and equipment will all be used for this task as well.

Y1Q2 Progress: Progress in this task is described in subtask 1a in regards to equipment, training, and procedural refinement.

Y1Q3 Progress: We completed an initial ibudilast dose finding study: We have completed initial day 1 TUNEL experiments to determine the effectiveness of 5 doses of ibudilast on NMDA damaged chicks. We are also evaluating “NMDA +vehicle” treated chicks to add additional numbers to our previous experiment to evaluate for statistical significance.

Y1Q4 Progress: We added another 4 chicks to our original 5 doses of ibudilast to increase our statistical power. We have also completed additional lower dosages at n=6 per dose for the ibudilast drug dosage study (10^{-4}, 10^{-5}, and 10^{-6} mg/ml) since we found a neuroprotective effect at very low doses. Interestingly, we have yet to observe a decrease in overall effect at these lower doses. The optimal dose found at this time is 10^{-5} mg/ml.

Remaining Tasks: Complete day 3 RD TUNEL experiments on three doses of ibudilast as determined from the NMDA studies. Complete expanded study (n=10) on optimal dose for RD and NMDA, D3 and D1 respectively. Complete extended timecourse study on optimal dose for RD and NMDA, D14 and D10 respectively. Complete all experiments for CPSI-1306 and AV1013. ERG and OCT tests should be performed on a subset of 3 chicks per group (4 groups for RD, 3 groups for NMDA) at multiple timepoints. If one of the drugs is significantly more effective, we will eliminate the more in-depth analyses of the less effective drugs.
Completion: ~252 chicks (62/252) + 42 ERG Tests (0/42) + 42 OCT Tests (0/42) + 182 TUNEL stains/analysis (61/182) + 70 DAPI INL thickness measurements (0/70) + 14 NMDA Normative chicks (14/14) + Training & Protocol Generation (ERG, OCT, Injection, Intubation, Slit Lamp, Fundus Imaging) (14) + Equipment (Tonopen, OCT Datastation, Fundus Camera, Injector, Isoflurane, Brooders x4, Intubation Tubes) (10) = 626: 161/626 = 25.7%

Subtask 1c. Drug timing studies in retinal damage models to test the impact of timing on the neuroprotective benefit of MIF inhibitors.
RD and NMDA damage models will be treated with optimized MIF inhibitor dose 2-6 hours after damage initiation. TUNEL staining and retinal thickness measurements will be taken to evaluate the impact of MIF inhibitors on retinal neuroprotection following injury.
Timeframe: Months 4-18

Y1Q1 Progress: We are trained on intravitreal injections in chicks thanks to Dr. Fischer's lab. Equipment required for this task has arrived.

Y1Q2 Progress: Progress in this task is described in subtask 1a in regards to equipment, training, and procedural refinement.

Y1Q3 Progress: No significant progress.

Y1Q4 Progress: No significant progress.

Remaining Tasks: Experimental groups have not yet been started.
Completion: ~90 chicks (90) + 70 TUNEL stains/analysis (0/70) + 20 DAPI INL thickness measurements (0/20) Training & Protocol Generation (Injection, Slit Lamp, Fundus Imaging) (3) + Equipment (Tonopen, Injector, Isoflurane, Brooders x4) (7) = 94: 10/190 = 5.3%

Task 2. Evaluate the ability of clinically relevant MIF inhibitors, CPSI-1306 and ibudilast/AV1013 to block gliosis and pathological wound healing and to protect against PVR in traumatic RD. *In vitro* studies with ARPE19 (retinal pigment epithelium) and MIO-M1 (Müller glia) cell lines and *in vivo* RD & NMDA models will be used to assess MIF inhibitor's anti-gliotic and anti-fibrotic activity.
Timeframe: Months 3-36

Subtask 2a. Test the hypothesis that MIF inhibitors block gliosis and pathological wound healing responses *in vitro*. Human RPE (ARPE-19) and Müller glia (MIO-M1) cell lines will be incubated with TGFβ to mimic PVR conditions. The effect of clinically relevant MIF inhibitors will be tested through scratch assays to assess migration, MTT assays to assess proliferation, contraction assays, and epithelial mesenchymal transition (EMT) assays to assess which aspects of pathological wound healing may be impacted by the MIF inhibitor treatment. Additionally, the ibudilast/AV1013 combination will allow for elucidation to the role phosphodiesterase in wound healing.
Timeframe: Months 6-30

Y1Q1 Progress: The MIO-M1 cell line has been acquired and passaging has already begun. Preliminary scratch assays on ARPE-19 cells comparing multiple doses of ibudilast and ISO-1 have been completed.

Y1Q2 Progress: We are working on validating our cell line resources prior to completing our *in vitro* experiments. This will include STR analysis of the cell lines (ARPE-19 and MIO-M1) as well as confirming the appropriate expression markers in these cell lines.

Y1Q3 Progress: DNA for both cell lines has been collected and submitted to the OSU Genomics Core Facility for STR analysis to authenticate the cell lines. This showed ARPE-19 had the expected STR profile, but MIO-M1 had a mismatch with the karyotype (XY instead of expected XX). These studies show MIO-M1 is not a suitable line for this research. Additional scratch assays with ARPE-19 have been completed.

Y1Q4 Progress: MTT assays were performed on the three drugs at different concentrations. CPSI-1306 had no observed effect on cell proliferation. However, the higher concentrations of ibudilast and AV1013 displayed decreased proliferation compared to controls. Both ibudilast and AV1013 were then tested on TGFβ treated cells, and results show AV1013 is more effective. Gel contraction assays were also performed with three conditions: no cells (control), ARPE-19 with TGFβ, and ARPE-19 without TGFβ. Both cell conditions had significant contraction, and cells treated with TGFβ showed more contraction. EMT staining is underway.

Remaining Tasks: Perform drug treated contraction assays. Perform drug treated EMT staining.
Completion: Cell line Verification (1/1) + 1 cell line * With & Without TGFβ (2) * 4 tests * 3 drugs (not including vehicle) = 26: 14/26 = 53.8%

Subtask 2b. Test the hypothesis that MIF inhibitors block gliosis and pathological wound healing responses *in vivo*. Doses of MIF inhibitors shown to block TUNEL staining will be used to evaluate the effect on retinal gliosis in our RD and NMDA damage models. Retinas will be evaluated with immunostaining for GFAP to detect gliosis, aquaporin 4 to detect Müller glia and astrocyte changes, CD45 to detect microglia accumulation, and PCNA to evaluate cell proliferation of Müller progenitor cells at day 14 (RD) and day 7/10 (NMDA). Studies will be carried out in an *in vivo* rabbit PVR model to determine if intravitreal drug administration blocks PVR formation. Retinas will be graded with the Fastenburg grading scale.
Timeframe: Months 12-36

Y1Q1 Progress: Equipment has arrived that is necessary for this task.

Y1Q2 Progress: Progress in this task is described in subtask 1a in regards to equipment, training, and procedural refinement.

Y1Q3 Progress: No significant progress.

Y1Q4 Progress: No significant progress.

Remaining Tasks: All experimental groups have not yet been started.
Completion: ~70 chicks (0/70) + 350 stains (GFAP, AQP4, CD45, PCNA, Sox2/9) (0/350) + ~70 Rabbits (0/70) + 50 ONL Thickness measurements (0/50) + 64 ERG tests (0/64) + 68 OCT measurements (0/68) + Training & Protocol Generation (Chick & Rabbit Injection, Chick & Rabbit Slit Lamp, Chick & Rabbit Fundus Imaging) (6/12) + Equipment (Tonopen, Fundus Camera, Injector, Isoflurane, Brooders x4) (8/8) = 692: 14/692 = 2.0%

Task 3. Determine the ocular pharmacokinetics of clinically relevant MIF inhibitors and their effects on IOP in rabbits. In vivo studies in untreated chicks will be performed in parallel to Aim 1 to evaluate retinal drug levels after a single injection. The MIF inhibitor that shows the greatest promise in Aims 1/2 will be further evaluated in rabbits for pharmacokinetic properties. An assay will be developed by the Pharmacoanalytical Core Facility at OSU to analyze the plasma, vitreous, and retinal drug levels. ERG and SD-OCT will be used to assess potential retinal toxicity. Systemic organ toxicity will be evaluated through necropsy by the Veterinary Core facility.
Timeframe: Months 1-36

Task 3a. Pharmacokinetic study – A screening pilot study using chick, performed in parallel to Aim 1, will be performed to evaluate drug levels in the retina. One untreated chick will be treated with one (or two) intravitreal drug injections at multiple doses and retinal drug levels will be evaluated after 1 day using the assay developed by the Pharmacoanalytical Core facility.
Timeframe: Months 1-6

Y1Q1 Progress: Equipment has arrived that is necessary for this task.
Y1Q2 Progress: Progress in this task is described in subtask 1a in regards to equipment, training, and procedural refinement.

Y1Q3 Progress: No significant progress.

Y1Q4 Progress: No significant progress.

Remaining Tasks: Experimental groups have not yet been started.
Completion: ~18 chicks (18) + Assay Generation (4) + Equipment (Tonopen, Injector, Isoflurane, Brooders x4) (7) = 29: 7/29 = 24.1%

Task 3b. Pharmacokinetic study – An extended pilot study using chick, performed in parallel to Aim 1, will be performed to evaluate retinal drug levels after one (or two) intravitreal treatments of MIF inhibitors in untreated animals using an extended pilot study.
Untreated chicks (n=3) will be treated with one (or two) drug injections at 1-2 doses. Retinal and vitreous drug levels will be evaluated at day 1 and day 3.
Timeframe: Months 3-12

Y1Q1 Progress: Equipment has arrived that is necessary for this task.

Y1Q2 Progress: Progress in this task is described in subtask 1a in regards to equipment, training, and procedural refinement.

Y1Q3 Progress: No significant progress.

Y1Q4 Progress: No significant progress.

Remaining Tasks: Experimental groups have not yet been started.
Completion: ~36 chicks (36) + Assay Generation (4) + Equipment (Tonopen, Injector, Isoflurane, Brooders x4) (7) = 47: 7/47 = 14.9%

Task 3c. Rabbit pharmacokinetic study – The MIF inhibitor drug showing the most promise in Aims 1/2 will be further evaluated in rabbits for pharmacokinetic properties.
Untreated rabbit eyes (n=3) will be intravitreally injected with the MIF inhibitor dosing scheme established in Aim 1. Fellow eyes will remain untreated. Clinical examination and IOP measurements will be performed prior to harvest for drug level evaluation. The plasma, vitreous, and retinal drug levels will be analyzed at different timepoints with assays developed by the Pharmacoanalytical Core facility at OSU. ERG, SD-OCT, and fundus imaging will be used to assess potential retinal toxicity. Systemic organ toxicity will be evaluated by necropsy at the 168 hour timepoint by the Veterinary Core facility.
Timeframe: Months 24-36

Y1Q1 Progress: Equipment has arrived that is necessary for this task.

Y1Q2 Progress: No significant progress.

Y1Q3 Progress: No significant progress.

Y1Q4 Progress: No significant progress.

Remaining Tasks: Experimental groups have not yet been started.
Completion: ~45 Rabbits (45) + ~15 ERG Tests (15) + ~15 OCT Tests (15) + Training & Protocol Generation (Rabbit ERG, Rabbit OCT, Rabbit Injection, Rabbit Slit Lamp, Rabbit Fundus Imaging) (10) + Equipment (Tonopen, OCT Datastation, Fundus Camera, Injector, Isoflurane) (5) = 90: 5/90 = 5.6%

What was accomplished under these goals?

A. Subtask 1A

a. **Preparation:** Particularly in the 1st and 2nd quarters we prepared numerous standardized forms and SOPs for our experimental procedures. Additionally, we had to order a number of supplies for our experiments including chick brooders, a tonopen, a fundus imaging camera, a micro-injector, the Leica SD-OCT datastation, and an isoflurane anesthesia machine.

b. **ERG Normative Database:** During the 1st fiscal year we created a normative database of untreated control chicks tested on the ERG. This included data from 1 week post-hatch to 5 weeks post-hatch. With this database, we can compare damaged and drug-treated results to normal untreated chicks (**Figs 1, 2, & 3**).

c. **Clinical Examinations:** With the start of the long extended timepoint studies to assess for potential retinal toxicity, we began performing clinical examinations on the chicks including IOP measurements, weight, slit lamp exams, and fundus imaging (**Fig 4**). The drugs did not cause any clinical side effects compared to control in the slit-lamp or fundus examinations (data not shown). There were no changes in weight between control and any of the treatment groups (data not shown). There was significant variability in the IOP measurements (data not shown) as discussed in section 5. After consultation with our collaborators, the variability appears to be due to the style of tonometer. Therefore, we will purchase a new style of tonometer (Icare) which has a finer tip and should yield more consistent measurements.

d. **SD-OCT Normative Database:** We acquired a normative database of SD-OCT measurements similar to the normative ERG database to use in the comparison of retinal thicknesses of NMDA and drug-treated eyes. Measurements were acquired every week from post-hatch week 1 to post-hatch week 5 (Untreated, **Fig 5**). A standardized guideline for layer identification was also created (**Fig 6**).

e. **Ibudilast Max Dose Toxicity Experiment:** We established that the maximum soluble concentration of ibudilast in sterile water is 4.5 mg/ml. Sterile water was used since it is an acceptable diluent for human intraocular medications. The experimental design was injection of 20ul max dose ibudilast in the left eye and 20ul vehicle injection in the right eye. If this dose showed no toxicity, no further dose toxicity studies would be necessary. After establishing our experimental methods in one test chick, we assigned four chicks for analysis D1 after injection and eight chicks for analysis D21 after injection. The D1 chick retinas were stained with TUNEL to detect any cell death. At D1 no significant difference was found between the ibudilast-treated and vehicle-treated eyes (9 vs. 3 cells/mm^2, p=0.55) (**Fig 7**). The D21 chicks were stained with DAPI for INL thickness measurements and analysis is in process. The D21 chicks were also measured with the ERG system and SD-OCT system. The ERG measurements were acquired at D10 and D14 and analyzed with two-way ANOVA with Tukey post-hoc testing using GraphPad Prism. No significant differences were found between the drug-treated, vehicle-treated, and age-matched untreated groups. Rarely, significant difference points were found within the different datasets, but none of these differences were consistent across all flash intensity ranges and appeared to be due to noise (**Figs 1, 2, & 3**). SD-OCT thickness measurements were acquired on D7 and D16 and showed no significant differences between the max dose ibudilast-treated, vehicle-treated, or age-matched untreated groups (**Fig 5**). Statistics were calculated using two-way ANOVA with Tukey post-hoc testing in JMP. We concluded that the maximum soluble concentration was non-toxic and no further doses would be required for this subtask.

f. **CPSI-1306 Max Dose Toxicity Experiment:** We established that the maximum soluble concentration of CPSI-1306 in sterile water is 1.25 mg/ml. As with ibudilast, if the maximum dose was non-toxic, then no further doses would be tested for toxicity. We assigned four chicks for D1 after injection, four chicks for D3 after injection, and six chicks for D28 after injection. SD-OCT and ERG data was obtained from chicks from the D28 group at four timepoints. Of the six

chicks assigned to the D28 timepoint, two died before reaching the endpoint. This did not affect our ability to successfully collect the required SD-OCT and ERG data. The D1 and D3 chicks were stained with TUNEL to detect any cell death. Neither timepoint displayed a significant difference between the max dose CPSI-1306-treated and vehicle-treated eyes (D1: 1.15 v 0 cells/mm^2, p=0.391; D3: 39.30 v 0 cells/mm^2, p=0.368) (**Fig 7**). The four remaining chicks were stained with DAPI for INL thickness evaluation. No significant difference was detected between the CPSI-1306-treated and vehicle-treated eyes (Total: 123.34±12.75 v 123.41±8.87; INL: 39.37±3.99 v 39.61±3.82, respectively) (**Fig 7**). ERG measurements were taken on three chicks at D8, D14, D21, and D28 post-injection (**Figs 1, 2, & 3**) and were analyzed with two-way ANOVA with Tukey post-hoc testing using GraphPad Prism. We noticed a general trend of decreasing amplitude over time in untreated chicks (with age and time under anesthesia), as well as some electrical noise in oscillatory potentials for the CPSI-1306 D21 timepoint. The trend of decreasing ERG amplitudes with age and between first eye and second eye tested is of unclear significance and will be investigated further. We suspect that the time under anesthesia may influence the ERG amplitudes. We will conduct studies with a new ERG system in which both eyes can be recorded simultaneously and significantly reduce the recording time (Celeris, Diagnosys). No other major differences were noted between the max dose CPSI-1306 treated eyes and controls. SD-OCT measurements were taken on three chicks at D8, D14, D21 and D28 post-injection (**Fig 5**). Statistics were calculated using two-way ANOVA with Tukey post-hoc testing in JMP. No significant differences between CPSI-1306-treated, vehicle-treated, or untreated eyes were detected. We found no evidence of toxicity for the maximum soluble CPSI-1306 concentration of 1.25 mg/ml and concluded no further doses needed testing for this subtask.

B. Subtask 1B

a. **NMDA Damage Normative Database:** We created an NMDA damage normative database to have NMDA control data for comparison against max dose drug toxicity data for ERG/SD-OCT and against NMDA+Drug data for TUNEL analysis. For ERG, we measured at D1, D6, and D13 post-injection (**Figs 1, 2, & 3**) and were analyzed with two-way ANOVA with Tukey post-hoc testing using GraphPad Prism. D1 and D6 NMDA conditions were found to be significantly different from almost all other conditions at almost all flash intensities. D13 NMDA trends lower in amplitude than the untreated and vehicle conditions, but the differences are not significant in most cases. We took SD-OCT measurements at D1 and D14 to measure the retinal layer thicknesses (**Fig 5**). The D1 NMDA inner retinal thicknesses were significantly thicker than the other conditions due to swelling, while the D14 NMDA thicknesses were significantly thinner than the other conditions due loss of inner retinal thickness. NMDA treated histologic studies are in the final stages of completion.

b. **Ibudilast Dose Ranging Study:** We performed a study to find the optimal neuroprotective dose of ibudilast in the NMDA damage model (**Fig 8**). We initially tested the maximal dose of 4.5 mg/ml, as well as a range of doses from 1.0 mg/ml decreased by factors of 10. We repeated the experiment twice (n=7-8) to 0.001mg/ml and added additional doses tested down to 10^{-6}mg/ml (n=6). The optimal dose was found to be 10^{-5}mg/ml (759.44 v 3624.94 TUNEL+ cells/mm^2 retina, p<0.0001).

c. **Retinal Detachment:** We began creating chick retinal detachments (RD) to optimize our technique.

C. Subtask 2A

a. **Cell Line Verification:** We initially proposed working on two cell lines: ARPE-19 and MIO-M1, which model human retinal pigment epithelium cells and human Müller glia, respectively. To verify these cell lines, we ran STR profiling to compare with published results in the literature and confirm the identity of the cell types. Our ARPE-19 cell line matched the established profile perfectly (**Table 1**). While the MIO-M1 cell line does not have a published STR profile, the characterizing literature reports the cell line originated from a female patient with an XX karyotype. However, the STR profile results showed the presence of both X and Y chromosomes, suggesting a male source (**Fig 9**). After discussion with several collaborators, we discontinued all further work with the MIO-M1 cell line.

b. **Scratch Assay:** We conducted a series of scratch assays with the ARPE-19 cell line testing the effects of ibudilast and CPSI-1306 on wound closure and migration in the presence of TGFβ

with and without exogenous MIF (**Fig 10**). We found no statistical significance between the conditions, implying the drugs do not impede ARPE-19 migration.

c. **MTT Assay:** We performed MTT assays on the ARPE-19 cell line, treated with TGFβ to mimic PVR-like conditions. MTT measures cell proliferation, an important aspect of PVR development. Cells were treated with different concentrations of CPSI-1306, ibudilast, and AV1013, the ibudilast analogue that lacks phosphodiesterase inhibitor activity (**Fig 11**). CPSI-1306 showed no significant effect on cell proliferation ($p \geq 0.475$), while ibudilast and AV1013 significantly reduced the cell proliferation (ibudilast: 0.45mg/ml v control, $p=0.046$, AV1013: 0.45mg/ml v control, $p<0.0001$) (**Fig 12**). Ibudilast decreased proliferation moderately at the highest dose of 1mg/ml (0.636 v 0.999 AU, $p=0.0002$, ibudilast and control respectively), while AV1013 significantly blocked cell proliferation at lower doses in a dose-dependent manner (0.076 v 0.999 AU, $p<0.0001$, AV1013 1mg/ml and control respectively). Statistics were calculated with Tukey HSD post-hoc testing in JMP.

d. **Gel Contraction Assay:** Another aspect of wound healing and PVR is cell contraction. We performed gel contraction assays with three-dimensional collagen gels. Using a Cell Biolabs CytoSelect 24-well Cell Contraction Assay kit, we measured the rate of contraction over a period of 72 hours. We calculated a contraction ratio by measuring the area of the gel and dividing it by the initial area at time 0. This initial experiment was performed on ARPE-19 cells with and without TGFβ to confirm that the experimental model is working (no cell control ratio: 1.0 after 72hrs, cells control ratio: 0.283 after 72hrs, cells+TGFβ ratio: 0.189 after 72hrs) (**Fig 13**).

e. **EMT Staining Assay:** A hallmark of PVR is epithelial mesenchymal transition (EMT). We are in the initial stages of processing cells in 8-well transwell slides to stain for EMT markers and test the ability of the MIF inhibitors to block EMT in the *in vitro* PVR model in ARPE-19 cells treated with TGFβ.

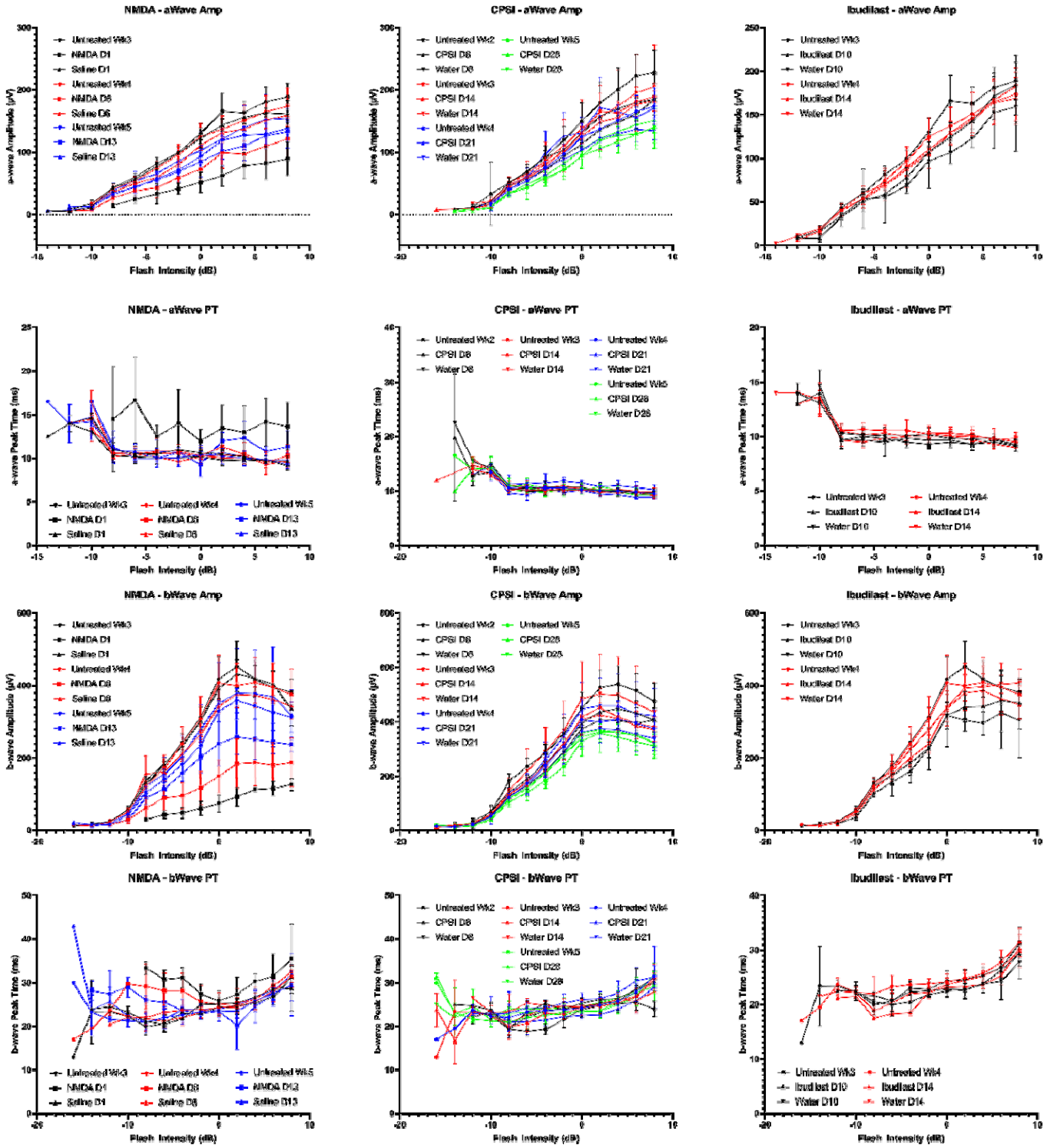


Figure 1. ERG A-wave and B-wave amplitudes and peak times. NMDA damaged (left column), max dose CPSI-1306 undamaged (middle column), and max dose ibudilast undamaged (right column) treated chick ERG measurements were collected on at least three chicks per group. NMDA treated chicks were measured at D1, D6, and D13 post-injection and compared to their vehicle treated eye (sterile saline) and age-matched untreated counterparts. CPSI-1306 treated chicks were measured at D8, D14, D21, and D28 and compared to their vehicle treated eye (sterile water) and age-matched untreated counterparts. Ibudilast treated chicks were measured at D10 and D14 and compared to their vehicle treated eye (sterile water) and age-matched untreated counterparts. A-wave amplitudes (row 1), peak times (row 2), B-wave amplitudes (row 3), and peak times (row 4).

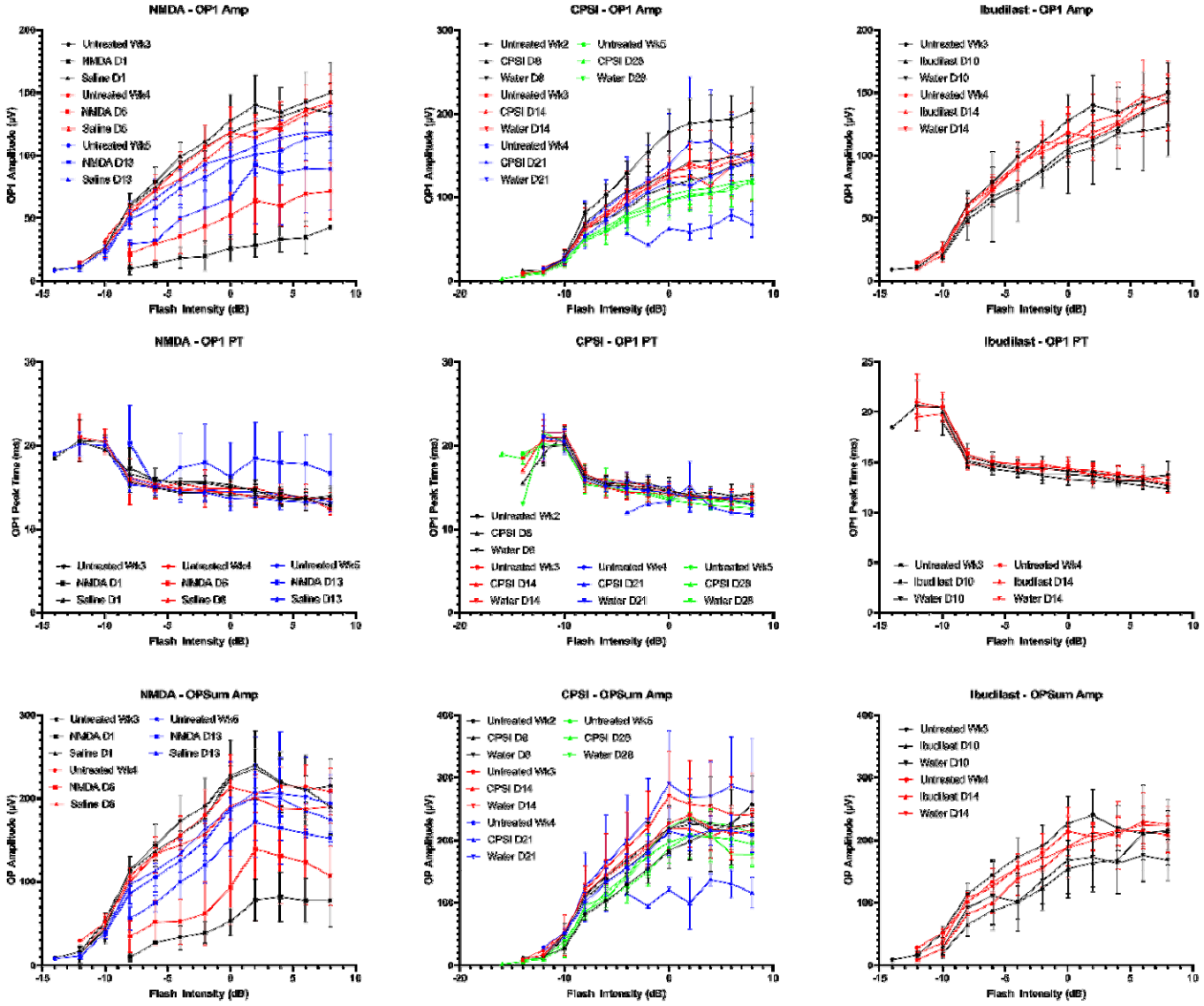


Figure 2. ERG Oscillatory potential (OP) measurements. NMDA damaged (left column), max dose CPSI-1306 undamaged (middle column), and max dose ibudilast undamaged (right column) chick ERG measurements were collected on at least three chicks per group. NMDA treated chicks were measured at D1, D6, and D13 post-injection and compared to their vehicle-treated eye (sterile saline) and age-matched untreated counterparts. CPSI-1306 treated chicks were measured at D8, D14, D21, and D28 and compared to their vehicle-treated eye (sterile water) and age-matched untreated counterparts. Ibudilast treated chicks were measured at D10 and D14 and compared to their vehicle-treated eye (sterile water) and age-matched untreated counterparts. First OP wave amplitudes (row 1) and peak times (row 2) and the overall sum of the measure OP waves (row 3) are shown.

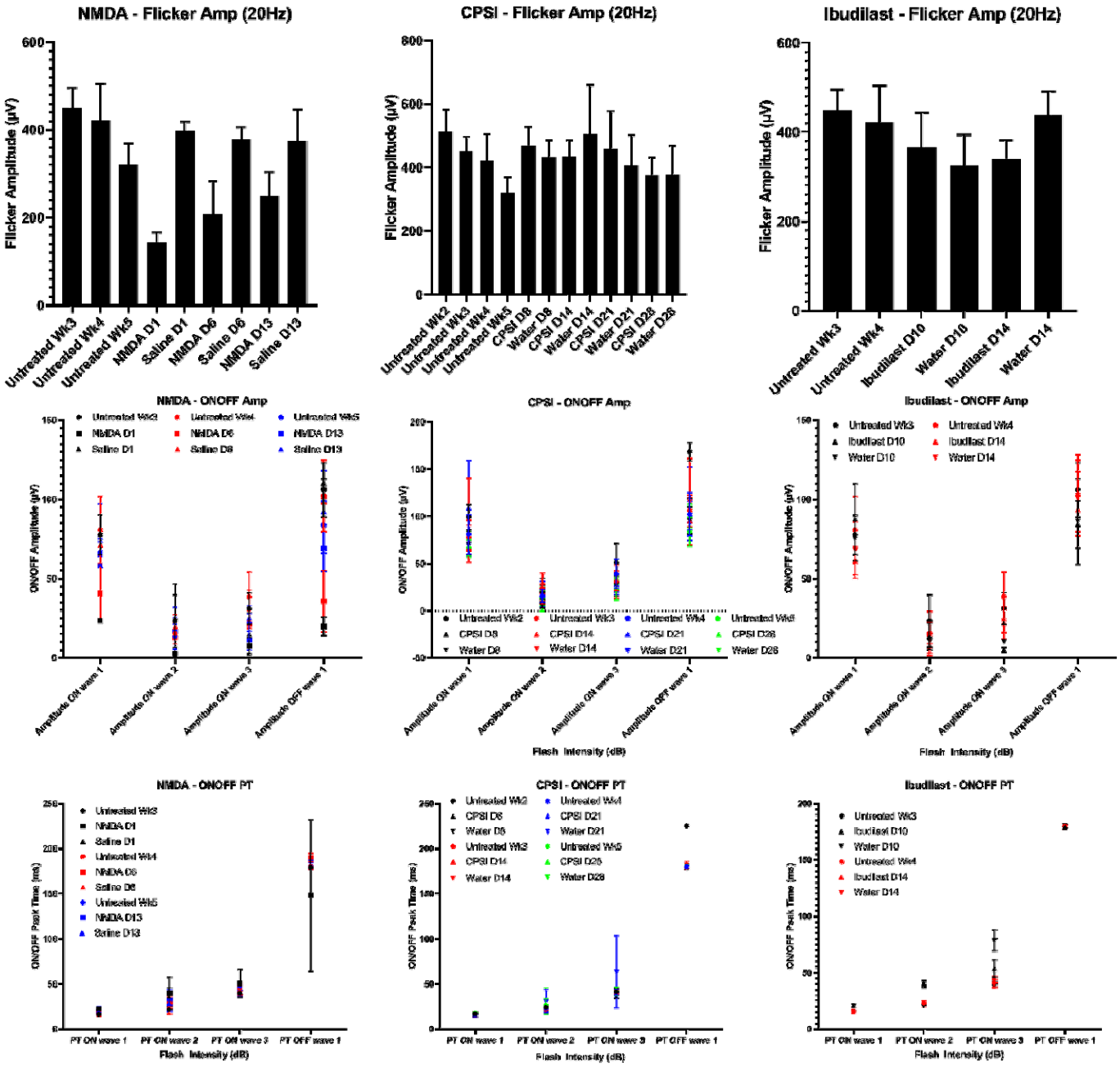


Figure 3. ERG Flicker and ON/OFF measurements. NMDA damaged (left column), max dose CPSI-1306 undamaged (middle column), and max dose ibudilast undamaged (right column) treated chick ERG measurements were collected on at least three chicks per group. NMDA treated chicks were measured at D1, D6, and D13 post-injection and compared to their vehicle treated eye (sterile saline) and age-matched untreated counterparts. CPSI-1306 treated chicks were measured at D8, D14, D21, and D28 and compared to their vehicle treated eye (sterile water) and age-matched untreated counterparts. Ibudilast treated chicks were measured at D10 and D14 and compared to their vehicle treated eye (sterile water) and age-matched untreated counterparts. Flicker amplitudes at 20Hz (row 1), ON/OFF wave amplitudes (row 2), and ON/OFF wave peak times (row 3). Flicker amplitudes and peak to peak times were collected for 15, 20, and 25Hz, however no difference was observed in any of the peak to peak times and neither the 15Hz nor 25Hz differed greatly from the 20Hz measurement.

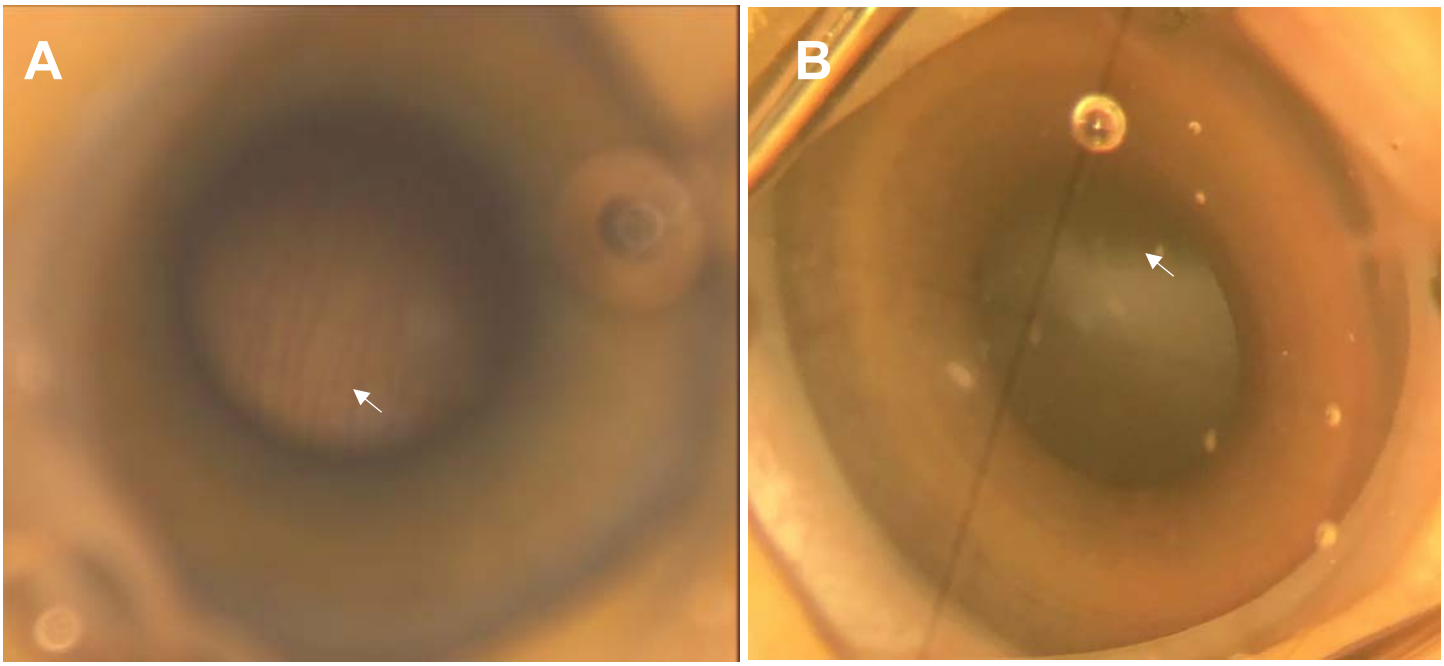


Figure 4. Chick retina as viewed under operating microscope.
Undamaged chick retina visualized through dilated pupil (A). White arrow indicates choroidal markings. Post NMDA Injection chick retina visualized through dilated pupil (B). Note retinal whitening and loss of choroidal markings. White arrow indicates pecten.

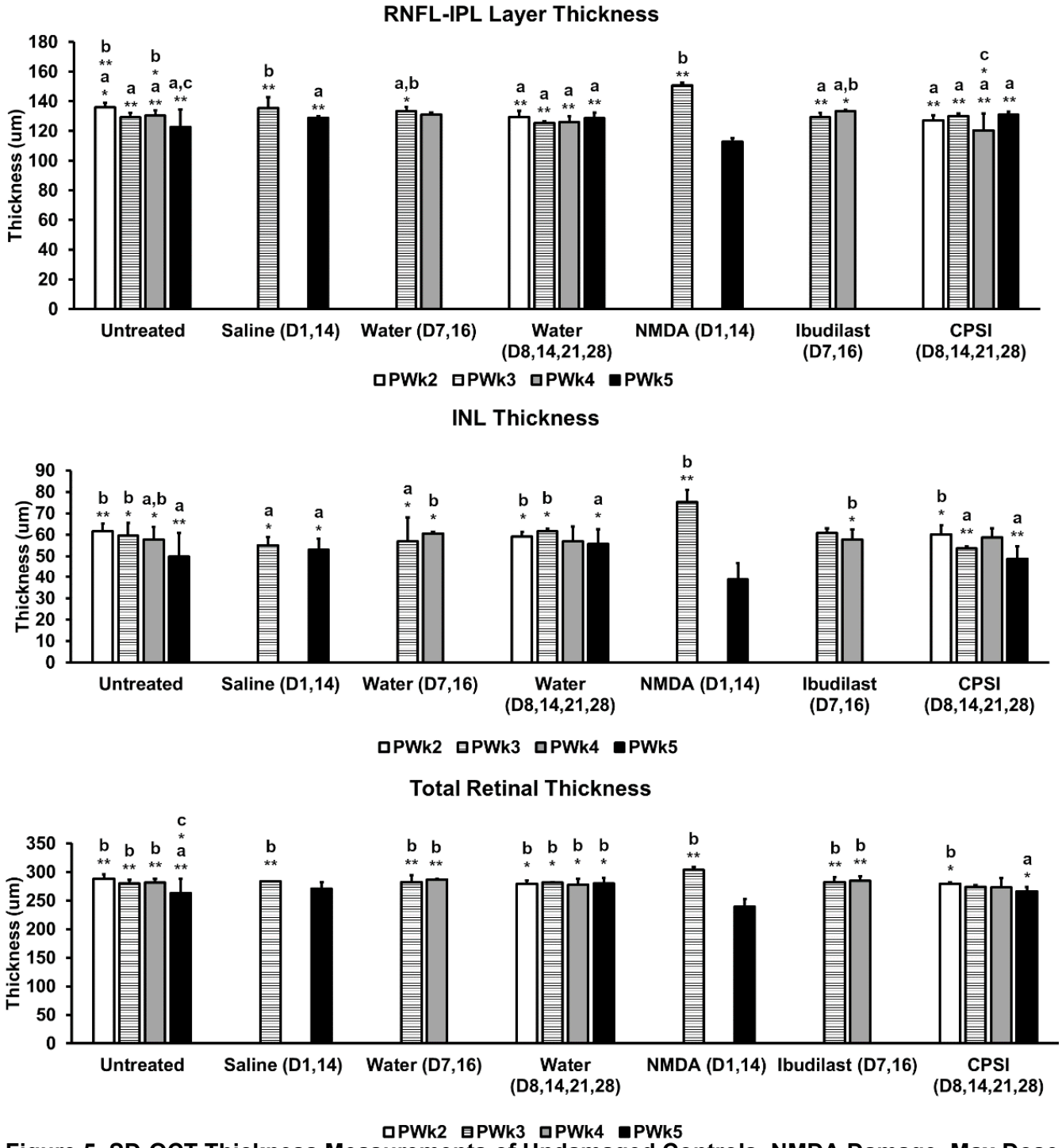


Figure 5. SD-OCT Thickness Measurements of Undamaged Controls, NMDA Damage, Max Dose Ibudilast, and Max Dose CPSI-1306. Measurements of the combined thickness of the retinal nerve fiber layer (RNFL) and the inner plexiform layer (IPL) (top), inner nuclear layer (INL, middle), and total retinal thickness (bottom). Post hatch week age of the chick is shown in the legend (PWk2-5). From left to right, untreated normative database chick eyes, sterile saline treated vehicle eyes (D1 and D14 post-injection), sterile water treated vehicle eyes (D7 and D16 post-injection), sterile water treated vehicle eyes (D8, D14, D21, and D28 post-injection), NMDA treated eyes (D1 and D14 post-injection), max dose ibudilast treated eyes (D7 and D16 post-injection), and max dose CPSI-1306 treated eyes (D8, D14, D21, and D28 post-injection). Max dose ibudilast and max dose CPSI-1306 were not significantly different from vehicle treated and untreated controls. NMDA treatment significantly increased the D1 inner retinal thickness (retinal swelling) followed by significantly decreased retinal thickness at D14. ‘a’ indicates significant difference from NMDA D1 values, ‘b’ indicates significant difference from NMDA D14, ‘c’ indicates significant difference from Untreated PWk2, ‘*’ indicates

significance p<0.05, and '**' indicates significance p<0.01. Statistics were calculated using two-way ANOVA with Tukey post-hoc testing in JMP.

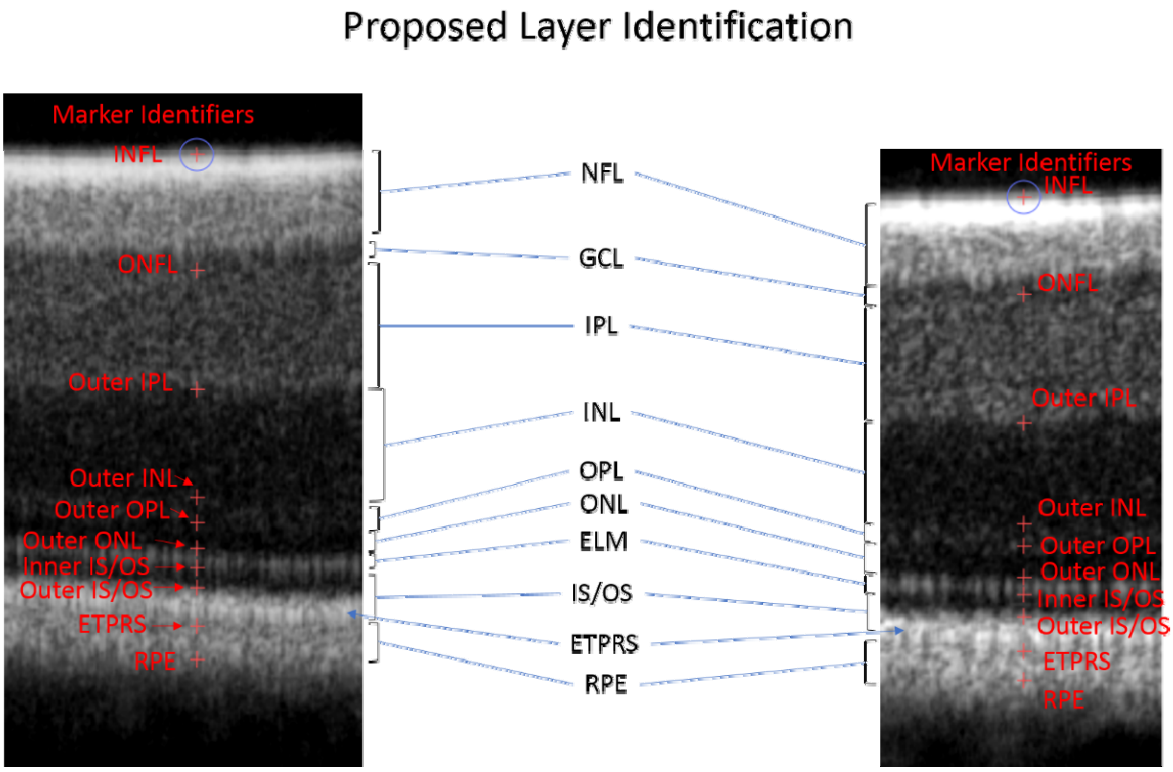


Figure 6. OCT Layering Guide. Guide showing the layers of the eye in OCT capture of two different undamaged chick retinas. The marker identifiers in red on the left and right of the image show what the Diver program allows us to select when performing the measurements. NFL – Nerve Fiber Layer, INFL – Inner Nerve Fiber Layer, ONFL – Outer Nerve Fiber layer, GCL – Ganglion Cell Layer, IPL – Inner Plexiform Layer, INL – Inner Nuclear Layer, OPL – Outer Plexiform Layer, ONL – Outer Nuclear Layer, ELM – External Limiting Membrane, IS/OS – Inner Segments/Outer Segments, ETPRS – End Tip Photoreceptor Segments, RPE – Retinal Pigment Epithelium.

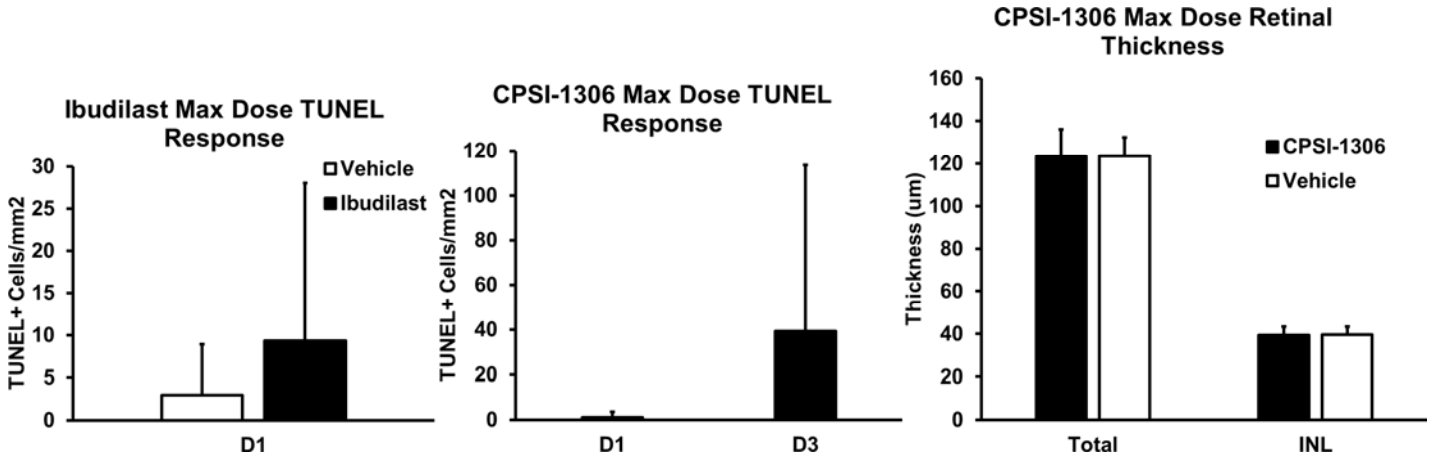


Figure 7. Max Dose Toxicity TUNEL & Histology Results. Ibudilast at its max dose of 4.5mg/ml displayed no significant difference in the number of TUNEL positive cells per mm^2 of retina between the ibudilast and vehicle (sterile water) treated eyes at D1 post-injection (left). CPSI-1306 at its max dose of 1.25mg/ml displayed no difference in the number of TUNEL positive cells per mm^2 of retina between the CPSI-1306 and vehicle (sterile water) treated eyes at D1 and D3 post-injection (center). Vehicle eyes for the CPSI-1306 treatments possessed no TUNEL positive cells and therefore do not appear on the graph. Additionally CPSI-1306 treated eyes were measured at D28 post-injection for any changes in INL or total retinal thickness (right) on DAPI nuclear counterstained sections. No significant difference was detected.

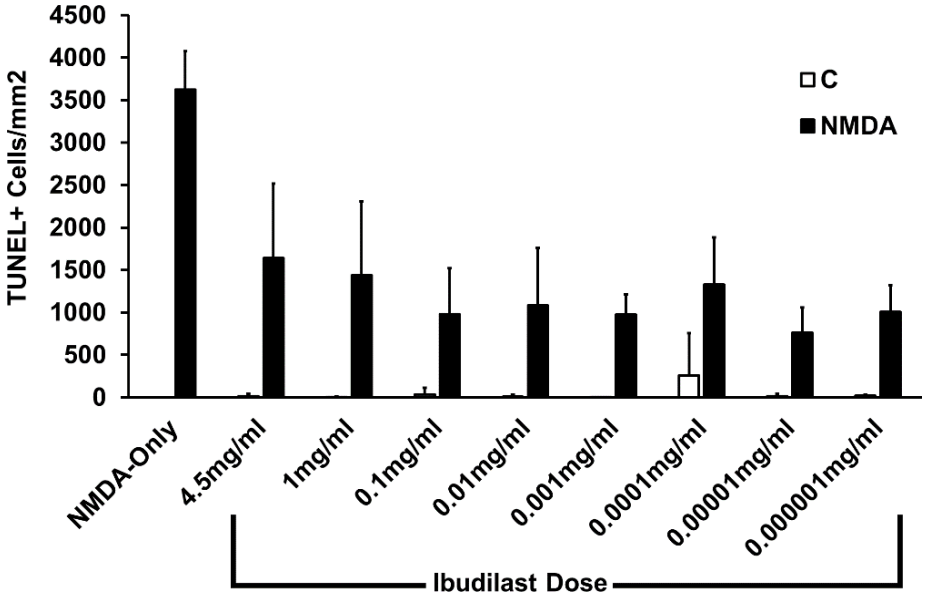


Figure 8. Ibudilast Dose Ranging Results. All ibudilast treatment doses displayed significantly less TUNEL positive cells than the NMDA-only eyes ($p \leq 0.0001$) with Tukey HSD post-hoc testing in JMP. The 10^{-5}mg/ml dose had the highest degree of difference from the NMDA-only eyes, however, none of the different ibudilast doses were found to be significantly different from each other.

Results for Submitted Sample			ATCC Reference Database Profile	
Loci	Query Profile		Database Profile	
Amelogenin	X	Y	X	Y
CSF1PO	11		11	
D13S317	11	12	11	12
D16S539	9	11	9	11
D21S11	28	29	28	29
D5S818	13		13	
D7S820	9	11	9	11
TH01	6	9.3	6	9.3
TPOX	9	11	9	11
vWA	16	19	16	19

Table 1. STR profile results from submitted DNA of ARPE-19 matches the published STR profile.

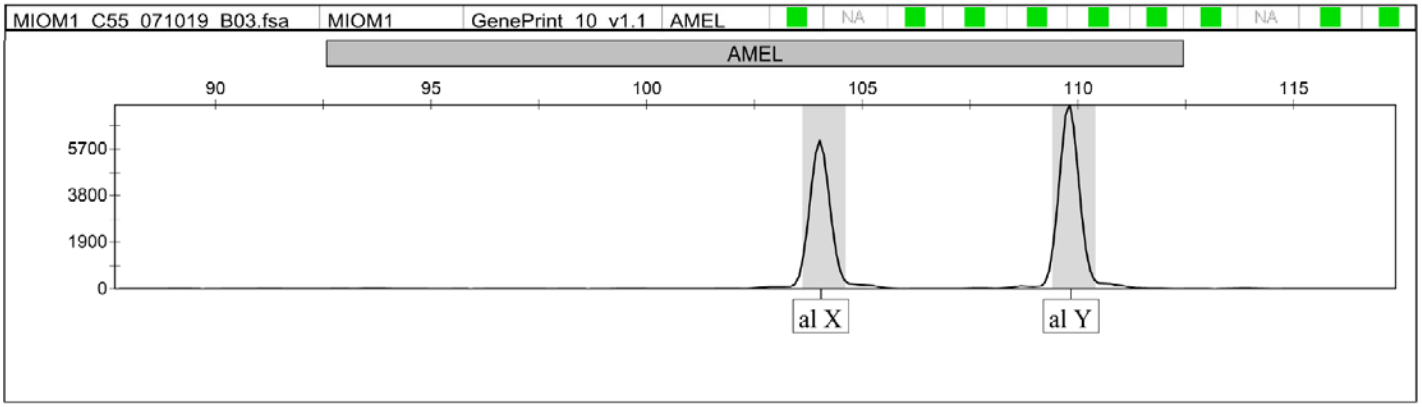


Figure 9. STR profiling of MIO-M1 shows X and Y chromosome-specific PCR products. This suggests the origin of the cell line is from a male donor.

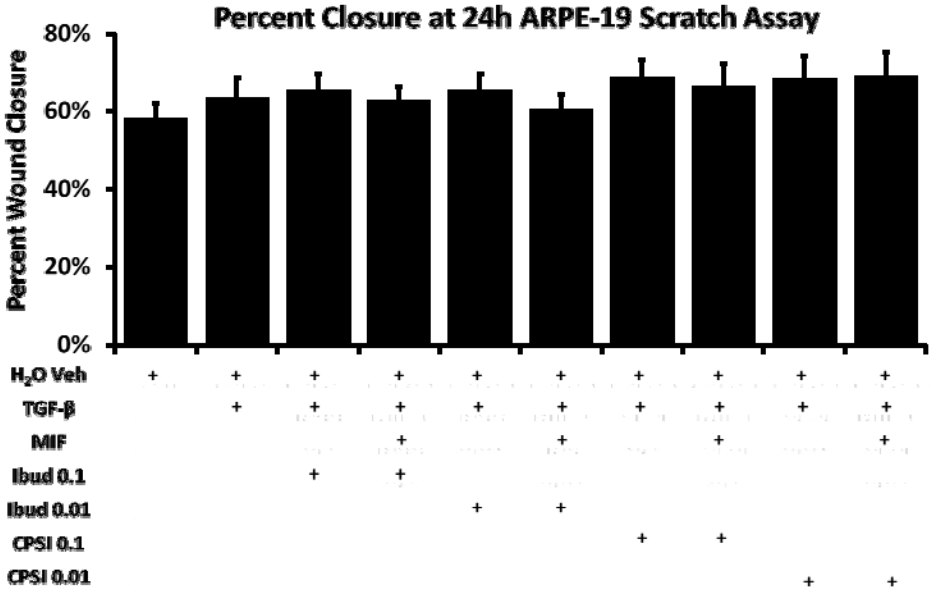


Figure 10. Scratch Assay Results. ARPE-19 scratch assay data shows no significant differences between experimental conditions ($p>0.05$). The addition of ibudilast (0.1 mg/ml and 0.01mg/mL) and CPSI-1306 (0.1 mg/ml and 0.01mg/mL) in the presence of MIF does not significantly increase the migration rate.

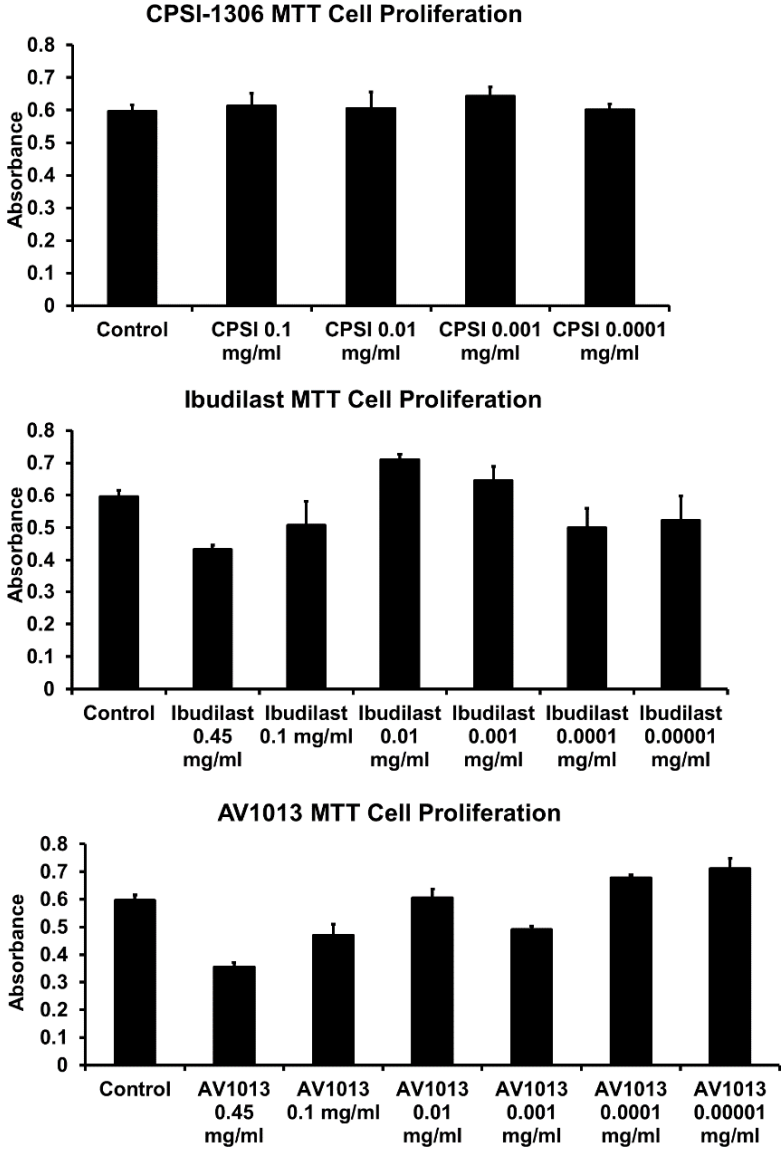


Figure 11. MTT Cell Proliferation Results. MTT absorbance results on CPSI-1306 (top), Ibudilast (middle), and AV1013 (bottom). CPSI-1306 showed no significant differences between the different doses. Ibudilast and AV1013 both showed doses dependent results with the 0.45mg/ml dose inhibiting the most cell proliferation.

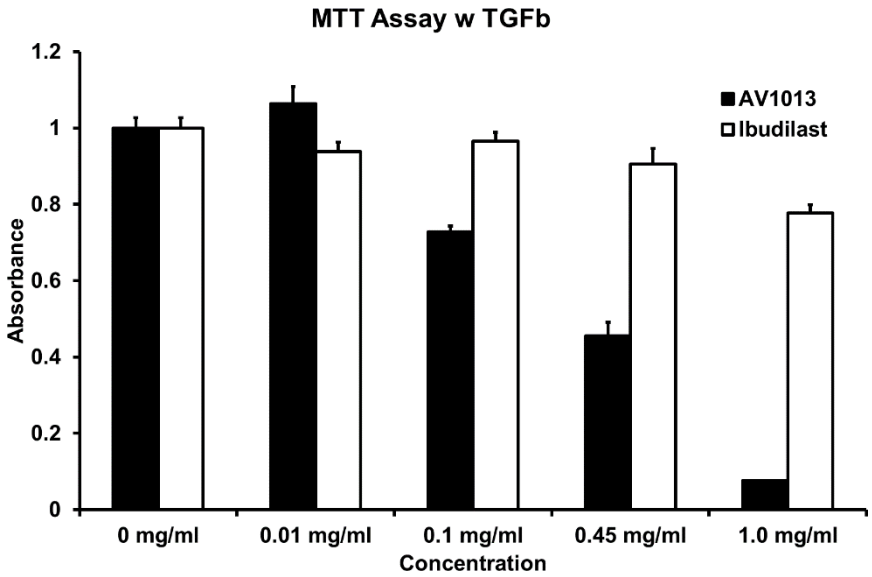


Figure 12. MTT Cell Proliferation with TGFβ. MTT assay comparing the effects of ibudilast and AV1013 on ARPE-19 cells treated with TGFβ to simulate PVR-like conditions.

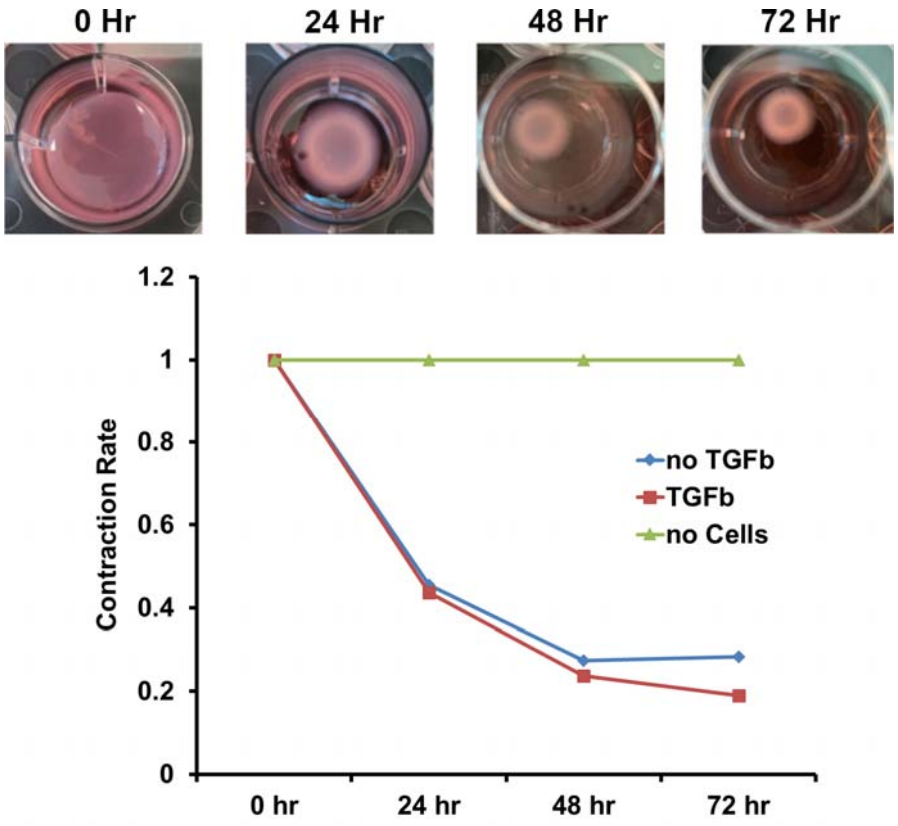


Figure 13. Gel Contraction Preliminary Results. A gel contraction assay was performed on ARPE-19 cells with and without TGFβ. The test was successful displaying contraction in both cell groups.

What opportunities for training and professional development has the project provided?

This project has provided ample opportunity for undergraduates to learn new lab skills under the supervision of senior lab members. Research assistant Elizabeth Urbanski and graduate student Tyler Heisler-Taylor are primarily responsible for training and supervising the undergraduates. Undergraduates gained skills in experimental design, animal work, immunohistochemistry and other wet lab techniques, and analysis and data interpretation. The project introduced them to the rigors of pre-clinical and translational research. Tyler also mentors a Master's Optometry student, Richard Wan, on data analysis, manuscript writing, and experimental design for his Master's thesis.

Dr. Julie Racine has trained Tyler, Elizabeth, and post-doc Mohd Hussain Shah on ERG operation and data analysis and interpretation. Dr. Racine most recently oversaw installation of the new Celeris (Diagnosys LLC) ERG machine which can record both eyes simultaneously and taught lab members how to use and troubleshoot the new system. Lab members have the opportunity to collaborate with an electrophysiologist and to learn ERG, which would not have been possible without the project. Availability and knowledge of ERG has also led to several new collaborations for the lab.

Professional Development Activities:
Abstracts are in preparation for the Association for Research in Vision and Ophthalmology (ARVO).
A manuscript is in preparation.

How were the results disseminated to communities of interest?

Press releases were created to notify the public (see Section 6).

What do you plan to do during the next reporting period to accomplish the goals?

We will finish the analysis of the max dose ibudilast histology studies. We will begin dose finding studies for CPSI-1306 to determine the optimal therapeutic dose. Based on the results of the ibudilast dose finding studies, we will begin dose finding ranges for CPSI-1306 at 100-fold lower intervals starting from the max dose (1mg/mL). We will create chick RDs in preparation for drug studies and start RD drug studies. We will re-create the normative database with the new Celeris ERG system and the Icare tonopen and expand our NMDA normative ERG and SD-OCT database to the 28 day timepoint.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The chick is an excellent model for vision research and the studies performed herein will expand the utility of the model for other vision scientists. The normative database studies on retinal structure and function (particularly with spectral domain optical coherence tomography (SD-OCT) imaging and electrophysiology studies) in normal and damage responses will expand knowledge of the chick eye as it relates to ophthalmic research. By understanding long-term effects of retinal detachment and excitotoxic damage on the chick eye, researchers will be able to extrapolate data from chicks used in research to gain further insight into what happens in the human eye under the same conditions. Chick and human eyes have two important similarities – a cone-driven retina, and the presence of a central high-density photoreceptor region (area centralis and macula, respectively), the point in the retina where visual acuity is highest. Having these features makes the chick eye an especially good model for the human eye, and a favorable alternative to mouse models of eye diseases. Mice dominate animal research for their well-understood genetics, and the ease and diversity of genetic manipulation available. However, mice also lack a macula and have a rod-driven retina as well as very low visual acuity. These differences from the human eye make mice a less-than-ideal model for studying ocular diseases and retinal pathologies. Continued study of normative measurements will allow for further understanding of the chick model of retinal damage. Further development and understanding of the chick retinal damage model may lead to its increased use in ophthalmic research as a fitting model for the human eye.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Due to the high variability of our AVIA Vet tonopen readings, we will continue IOP studies with a new tonopen which has a finer tip and should have less variability for chick eyes (Icare tonometer).
The ERG data showed decreased amplitudes with increased anesthesia time so that the second eye tested had lower amplitudes. Therefore we have started training on a new ERG machine which can record from both eyes simultaneously. This process will eliminate the eye to eye variability of the current system and reduce anesthesia time. It also has improved analysis capabilities.
We will use larger fold-dose changes when performing the dose-finding experiments for CPSI-1306 since even small doses of ibudilast were effective at inhibiting cell death.

Actual or anticipated problems or delays and actions or plans to resolve them

As mentioned above, highly variable readings from our AVIA Vet tonopen have called into question the validity of our IOP data to date. Moving forward, we will continue taking IOP measurements with a new tonometer.

Due to the time-sensitive and time-consuming nature of the animal experiments, the delay due to ACURO review the animal studies are taking longer than we originally anticipated. We will consider taking a no-cost extension to complete the work if needed. We will also try to separate our DoD and non-DoD animal protocols to try to minimize future amendments that would require ACURO re-review.

Changes that had a significant impact on expenditures

We were able to end Year 1 with approximately $27,000 in excess laboratory supply funds. By using our own cryostat and sectioning tissue samples ourselves, we were able to save $6,370 originally allocated for the histology core resource sectioning fee. In other areas, we were able to maximize efficiency of our lab supplies to come in under budget. We are hiring students to assist with various tasks. We have not yet been able to hire a graduate student as originally planned, and research assistant Elizabeth Urbanski was not hired until Quarter 3.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to Report.

  Books or other non-periodical, one-time publications.

  Nothing to Report.

  Other publications, conference papers and presentations.

  Nothing to Report.

- **Website(s) or other Internet site(s)**

  https://wexnermedical.osu.edu/departments/ophthalmology/research/dr-cebulla-mif
  This press release was featured on OSU OneSource and the Research section of the Department of Ophthalmology's website. It provides a brief layperson summary of the impact of the project on the development of therapies for preventing photoreceptor loss and retinal gliosis.

  https://medicine.osu.edu/recent-highlights/ohio-state-awarded-19-million-grant-for-retinal-detachment-recovery-research
  This press release was featured on the OSU College of Medicine's website and was distributed in *MedTips*, a newsletter sent to physicians and departments within Wexner Medical Center.

- **Technologies or techniques**

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**

  Nothing to Report.

- **Other Products**

  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Dr. Colleen M Cebulla
Project Role: Principal Investigator

Researcher Identifier (e.g. ORCID ID): 0000-0002-5636-4968
Nearest person month worked: 4
Contribution to Project: Supervised chick studies, equipment installation, protocol and form generation, data analysis, troubleshooting, and all personnel involved in the study. Edited the report.
Name: Dr. Andy J Fischer
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID):
Nearest Person Month Worked: 1
Contribution to Project: Provided advice about chick TUNEL staining and histology.

Name: Dr. Abhay Satoskar
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0001-5989-1520
Nearest person month worked: 1
Contribution to Project: Advised regarding experimental design for routes of delivery for CPSI-1306 and provided troubleshooting for drug dilution.

Name: Julie Racine
Project Role: Electrophysiologist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Reviewed ERG data and data analysis, oversaw installation of new ERG machine, trained lab members on use of new ERG machine and software.

Name: Tyler Heisler-Taylor
Project Role: Graduate Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0002-3514-0711
Nearest person month worked: 12
Contribution to Project: Ordered equipment, worked on ULAR and IACUC protocols, communicated with ULAR personnel, used slit lamp, used fundus camera, used tonopen, administered isoflurane anesthesia, administered intravitreal injection, collected ocular tissue for histology, provided lab organization, performed laboratory regulatory and safety management, performed clinical exams, sectioned frozen tissue samples, stained, imaged, processed, and analyzed tissue sample images, performed, processed, and analyzed ERG data, operated, processed, and analyzed SD-OCT data, wrote up findings, generated figures, and supervised personnel.
Funding Support: Ohio Lions Eye Research Foundation Norbert Peiker Eye Research Fellowship

Name: Mohd Hussain Shah
Project Role: Post-Doc
Researcher Identifier (e.g. ORCID ID): 0000-0002-3669-1478
Nearest person month worked: 12
Contribution to Project: Used fundus camera, used tonopen, used SD-OCT, administered isoflurane anesthesia, administered intravitreal injection, collected ocular tissue for histology, stained, imaged, processed, and analyzed tissue sample images, used operating microscope, performed clinical exams, performed and analyzed ERG, edited the report, and supervised personnel.

Name: Elizabeth Urbanski
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): 0000-0003-3576-1275
Nearest person month worked: 6
Contribution to Project: Ordered equipment, used slit lamp, used fundus camera, used tonopen, communicated with ULAR personnel, administered isoflurane anesthesia, administered intravitreal injection, used operating microscope, collected ocular tissue for histology, provided lab organization, performed clinical exams, performed laboratory regulatory and safety management, sectioned frozen tissue samples, stained and imaged tissue samples, analyzed TUNEL data, analyzed INL thickness data, performed ERG, performed SD-OCT, wrote up findings, generated figures, trained new undergraduate volunteer, and supervised personnel.

Name: Sumaya Hamadmad
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Stained and imaged tissue samples, performed cell culture experiments, generated figures, wrote up findings.
Funding: Dr. Cebulla's Personal Investigator Fund

Name: Richard Wan
Project Role: Optometry Student Volunteer
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Wrote up findings, generated figures, analyzed IOP and weight data.
Funding: N/A, Volunteer

Name: Alana Y Reese
Project Role: Undergraduate Researcher
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Provided lab organization, performed cell culture experiments, sectioned and stained tissue samples, helped train new undergraduate volunteers, helped with image analysis.
Funding: Dr. Cebulla's Personal Investigator Fund

Name: Hailey Wilson
Project Role: Undergraduate Research Volunteer
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Data entry for ERG, helped with sectioning, immunostaining, and imaging of frozen tissue samples.
Funding: N/A, Volunteer

Name: Bayan Shalash
Project Role: Undergraduate Research Volunteer
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Stained and imaged tissue samples, helped analyze INL thickness data, and helped train new undergraduate volunteer.
Funding: N/A, Volunteer

Name: Krupa Patel
Project Role: Undergraduate Research Volunteer
Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: <1
Contribution to Project: Sectioned frozen tissue samples, stained ocular tissue for histology, and helped analyze INL thickness data.
Funding: N/A, Volunteer

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Colleen M Cebulla

Status: Previously Pending, Now Active
Title: Torsional Indirect Traumatic Neuropathy (TITON): Animal model for diagnostics, drug delivery, and therapeutics for central nervous system injury
Grant Number: W81XWH-15-1-0074 P00001
Role: Co-Investigator
Sponsoring Organization: USAMRAA
Dates Active: 03/29/2019-03/28/2021

Status: Previously Pending, Now Active
Title: Evaluation of an OCA2 enhancer variant as a modifier of the phenotype of BAP1-tumor predisposition syndrome
Grant Number: R21CA219884
Role: Co-Investigator
Sponsoring Organization: NCI/NIH
Dates Active: 06/18/2018-05/31/2020

Andrew J Fischer

Status: Previously Active, Now Closed
Title: Comparative transcriptomic and epigenomic analysis of Müller glia reprogramming
Grant Number: U01 EY027267
Role: Co-Investigator
Sponsoring Organization: NIH/NEI
Dates Active: 9/01/2016 – 08/31/2019

Abhay Satoskar

Status: Previously Active, Now Closed
Title: Prevention and treatment of breast cancer and its metastasis by targeting macrophage inhibitory factor (MIF)
Grant Number: WX81XWH-16-1-0036
Role: Principal Investigator
Sponsoring Organization: USAMRAA
Dates Active: 02/01/2016-01/31/2019

Status: Previously Active, Now Closed
Title: A Systems Biology Approach for Targeted Drug Discovery for Leishmaniasis
Grant Number: R21AI127582
Role: Principal Investigator
Sponsoring Organization: NIAID/NIH
Dates Active: 12/14/2016-11-30/2018

Status: Previously Active, Now Closed
Title: Role of Ibrutinib in modulation of dendritic cell function and innate immune responses
Grant Number: PO# 00013031

Role: Principal Investigator
Sponsoring Organization: Pharmacyclics, Inc.
Dates Active: 02/05/2016-02/05/2019

Status: Previously Active, Now Closed
Title: Multifaceted activity of listeriolysin O during host cell invasion by Listeria
Grant Number: R01AI107250
Role: Co-Investigator
Sponsoring Organization: NIAID/NIH
Dates Active: 02/01/2014-01/31/2019

Status: Previously Active, Now Closed
Title: Trypanosoma cruzi cyclophilin 19 induced host inflammation
Grant Number: R21AI131227
Role: Co-Investigator
Sponsoring Organization: NIAID/NIH
Dates Active: 02/06/2017-01/31/2019

What other organizations were involved as partners?

Nationwide Children's Hospital
Columbus, OH
Donated animal ERG system for data capture.

Robinson Imaging Center (OSU Department of Ophthalmology)
Columbus, OH
The Robinson Imaging Center is in the process of purchasing the rabbit/chick adaptor for the new Celeris ERG unit and other supplies for the equipment (e.g., PERG, probes, and stage). Dr. Matthew Reilly donated use of the new Celeris ERG.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS
See attached.

9. APPENDICES

Nothing to Report.

Macrophage migration inhibitor (MIF) therapeutics for neuroprotection and prevention of scar in traumatic retinal detachment

Log Number: VR170167. W81XWH1810805, Vision Research Program, Technology/Therapeutic Development Award

PI: Colleen M. Cebulla, MD, PhD **Org:** Department of Ophthalmology & Visual Science, The Ohio State University **Award Amount: $1,900,895**

Study/Product Aim(s)

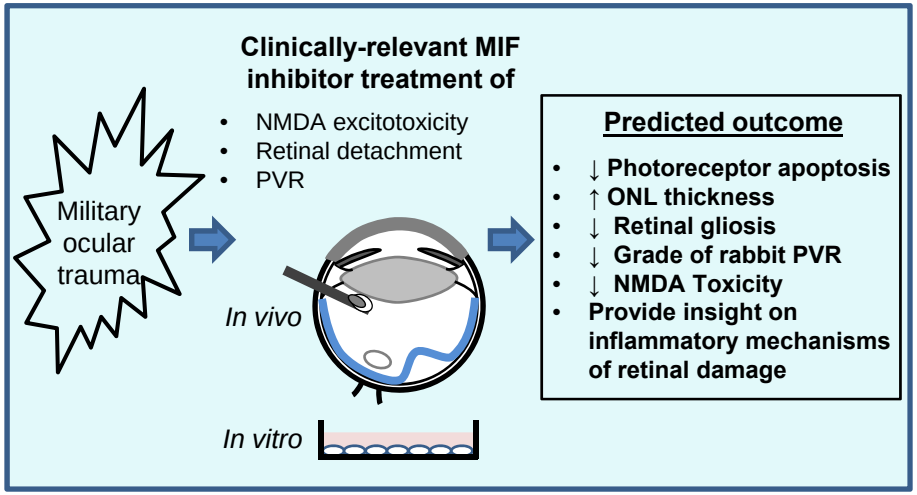
Aim 1: Test the hypothesis that clinically-relevant MIF inhibitors block neuronal apoptosis in our in vivo RD and NMDA damage models. We will evaluate neuroprotection using electrophysiology, spectral domain OCT (SD-OCT), fundus imaging, and histology. Any potential toxicity will be evaluated.

Aim 2: Test the hypothesis that clinically-relevant MIF inhibitors block gliosis and pathologic wound healing in traumatic RD. Studies with cell lines (retinal pigment epithelium and Müller glia) and our in vivo RD, RD-PVR, and NMDA models will be performed. Retinal fibrosis will be evaluated with SD-OCT and histology.

Aim 3: Evaluate the effects on intraocular pressure (IOP) and the ocular pharmacokinetics of clinically relevant MIF inhibitors. Rabbits will be used to determine the effects of the drugs on IOP and the pharmacokinetics of ocular delivery of MIF inhibitors.

Approach

Clinically-relevant MIF inhibitors will be tested on cell lines and administered with single intravitreal injection to eyes with and without retinal damage. Retinas will be evaluated by clinical examination, ERG, SD-OCT and fundus imaging and immunohistochemistry. IOP and pharmacokinetic studies will be performed in rabbit.



Accomplishment: CPSI-1306 has been verified as non-toxic in retinal conditions. Ibudilast extended dosage testing has been completed without observing a loss of effectiveness in neuroprotection. Migration and MTT assays have been completed.

Timeline and Cost

Activities — Fiscal Year (FY)	FY1	FY2	FY3
Task 1a. Dose escalation study of MIF inhibitors *in vivo* on untreated animals	93.9%		
Task 1b. Dose range finding study of MIF inhibitors *in vivo* on retinal damage models	25.7%		
Task 1c. Drug timing study of MIF inhibitors *in vivo* on retinal damage models	5.3%		
Task 2a. Evaluation of anti-gliotic/PVR effects of MIF inhibitors *in vitro*	53.8%		
Task 2b. Evaluation of anti-gliotic/PVR effects of MIF inhibitors *in vivo*		2.0%	
Task 3a. Pharmacokinetic pilot study of MIF inhibitors *in vivo* (chick)	24.1%		
Task 3b. Extended pilot pharmacokinetic study of MIF inhibitors *in vivo* (chick)	14.9%		
Task 3c. Determination of the pharmacokinetics of MIF inhibitors *in vivo (*rabbit)			5.6%
Estimated Budget ($K)	**$583K**	**$660K**	**$658K**

Updated: 10/30/2019

Goals/Milestones

FY1 Goal – Evaluate the clinically relevant MIF inhibitors (CPSI-1306, ibudilast/ AV1013) in our *in vivo* retinal damage models (RD and NMDA) for neuroprotection of photoreceptors.

FY2 Goal – Evaluate the ability of clinically relevant MIF inhibitors to block gliosis and pathological wound healing and to protect against PVR.

FY3 Goal – Determine the ocular pharmacokinetics of clinically relevant MIF inhibitors and potential side effects including on intraocular pressure (IOP) in rabbits.

Comments/Challenges/Issues/Concerns

- AviaVet Tonopen has proven to be too variable in the IOP measurements captured. Seeking to acquire a new method of IOP measurement (Icare).
- ERG amplitudes may be changing due to length of time on anesthesia due to monocular measurement. The new Celeris ERG machine will be able to take binocular data reducing our experimental measurement time significantly.

Budget Expenditure to Date

Projected Expenditure: $576,778.00
Actual Expenditure: $392,651.20