160 Anti-inflammatory; antibiotics; antivirals
Sunday, May 07, 2017 3:15 PM–5:00 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 1079–1095/B0367–B0383
Organizing Section: Physiology/Pharmacology

Program Number: 1079 **Poster Board Number:** B0367
Presentation Time: 3:15 PM–5:00 PM
T1565, a new efficient and safe preservative free hydrocortisone
Celine Olmiere[1], Anne-Laure Raveu[2, 3], Christophe Baudouin[4]. [1]R & D, Laboratoires Thea, Clermont-ferrand, France; [2]UPMC Univ Paris 06, UMR_S 968, Paris, France; [3]Institut de la Vision, Paris, France; [4]Ophthalmology, Quinze-Vingts Hospital, Paris, France.
Purpose: There is a controversy on the use of long term dexamethasone in ophthalmology due to side effects, mainly intraocular pressure rise (IOP). The aim of this preclinical work was to evaluate the efficacy/risk balance of preservative free hydrocortisone. Modifications of IOP were examined after twice daily instillations of T1565 (preservative free hydrocortisone 0.335%), for 5 weeks in a rat model of glucocorticoid-induced ocular hypertension, in comparison to dexamethasone.
Methods: Twenty four albino rats (Sprague Dawley females) were divided into 2 treatment groups (n=12 per group) and twice daily instilled from Day 1 to Day 36 with T1565, or dexamethasone (dex). Everyday a general clinical examination was performed with weighing and IOP was measured each week. In parallel, an *in vitro* comparison of inflammatory marker mRNA was performed in two different in vitro models of dry eye, namely desiccative and hyperosmolar stresses, comparing dexamethasone 0.1% vs hydrocortisone 0.335%.
Results: Animals treated with T1565 had a usual body weight gain variation during the course of the study whereas for dex-treated animals the body weight gain was repressed after 7 days, as expected. All animals were in good health and no particular signs were observed except an agitation of all the animals during IOP measurement. Animals treated with T1565 had a non-significant increase of the mean IOP until Day 15, and then a stabilization. Dex-treated animals showed as expected, an increase of the mean IOP until Day 39 with no sign of stabilization. Moreover, mean IOP at D36 was 9.5±1.6 mmHg vs 8.3±0.7 mmHg at baseline for animals treated with T1565 and 11.6±1.2 mmHg vs 7.7±0.5 mmHg at baseline for animals treated with dexamethasone.
In vitro, with hydrocortisone a comparable decrease with dexamethasone was observed in hyperosmolar stress : decrease of CCL-2, and desiccative stress : decrease of IL-6, IL-8 and CCL-2.
Conclusions: This study shows the safety and efficacy of this new preservative-free hydrocortisone T1565. Twice daily instillations of a preservative-free hydrocortisone T1565 induced a slight IOP elevation after a 2-week dosing period followed by stabilization whilst dexamethasone treatment led to significant continuing IOP increase and drastically reduced body weight gain, as expected with this treatment. Furthermore, ocular inflammatory marker mRNA were reduced as efficiently as with dexamethasone.
Commercial Relationships: Celine Olmiere, Laboratoires THEA (E); **Anne-Laure Raveu**, Laboratoires THEA (F); **Christophe Baudouin**, Laboratoires THEA (F)
Support: Grant from Laboratoires THEA

Program Number: 1080 **Poster Board Number:** B0368
Presentation Time: 3:15 PM–5:00 PM
***Ex-vivo* corneal permeation of nepafenac 0.1% ophthalmic suspension in different species (porcine, canine, equine and feline)**
Roxanne M. Rodriguez Galarza[2], Haley Porter[1], Jayachandra Ramapuram[1], Sue Duran[2], Eva Abarca[3, 2]. [1]Harrison School of Pharmacy, Auburn University, Auburn, AL; [2]Clinical Sciences, Auburn University, Auburn, AL; [3]Vetsuisse-Fakultät, University of Bern, Bern, Switzerland.
Purpose: Nepafenac, the only prodrug NSAID, has not been evaluated for use in dogs, cats or horses. The purpose of this investigation was to evaluate the *ex-vivo* transcorneal permeation of nepafenac 0.1% and compare its permeability profile across the porcine, canine, equine and feline corneas.
Methods: Fresh corneas were obtained from porcine, equine, canine and feline eyes free from corneal disease that were euthanized for reasons unrelated to this study. Corneal buttons (8 mm) were dissected using standard eye bank technique within 2 hours of enucleation. Corneas were mounted horizontally between the donor and the receiving compartments of an all-glass modified Franz diffusion cell (0.20cm^2), which were maintained at 37°C. The donor compartment was filled with 0.1 mL of nepafenac 0.1% formulation (Nevanac, Alcon Laboratories, Inc Fort Worth, Texas, USA) n=4 per species studied. Samples (1ml phosphate buffered saline pH 7.4) were removed from the receiving compartment at set times: 0, 1, 2, 4, 6, 8, 12, 24 hours. High-performance liquid chromatography was used for nepafenac analysis concentration. The cornea and residual solutions were collected at the end of the experiment. Permeability parameters were determined and compared with ANOVA statistical analysis ($P<0.05$).
Results: Mean permeation rates (µg/cm^2/hr ± SEM) were 0.752 ± 0.116, 1.281 ± 0.247, 0.944 ± 0.098 and 2.494 ±0.171 for the porcine, canine, equine and feline corneas, respectively. Permeation rate of nepafenac 0.1% of feline corneas was significantly greater than other species ($p<0.05$).
Conclusions: The results showed that 0.1% Nepafenac is able to permeate the cornea in normal porcine, canine, feline and equine eyes in an *ex-vivo* model. The data obtained demonstrated an interspecies difference with the feline cornea showing a significant increase in the permeation rate which suggests a potential alternative to treat intraocular inflammation in this species.
Commercial Relationships: Roxanne M. Rodriguez Galarza, None; **Haley Porter**, None; **Jayachandra Ramapuram**, None; **Sue Duran**, None; **Eva Abarca**, None

Program Number: 1081 **Poster Board Number:** B0369
Presentation Time: 3:15 PM–5:00 PM
Efficacy and safety of an Iontophoresis platform to control post cataract inflammation and pain
Barbara M. Wirostko[1], Carol M. Assang[1], Brenda Mann[1], Stephen From[1], Michael Raizman[2]. [1]EyeGate Pharmaceutical, Inc, Salt Lake City, UT; [2]Ophthalmic Consultants of Boston, Boston, MA.
Purpose: To determine safety and efficacy of EGP-437 (dexamethasone phosphate solution) delivered via a novel and proprietary iontophoresis platform, the EyeGate II Delivery System, for managing post-surgical inflammation and pain.
Methods: This clinical study was conducted as an open label design with 7 active cohorts (N= 69 patients), varying the iontophoretic dose and dosing regimen as described in Table 1, receiving a 40 mg/ml solution of dexamethasone phosphate. An additional cohort (N=10 patients) received sodium citrate buffer as a placebo #8. Aqueous samples were collected from half of the patients (N=5) from cohort #7 at time of surgery to assess ocular bioavailability of the

dexamethasone. The primary endpoint for all cohorts was Anterior Chamber Cell count (ACC) at day 14, with secondary endpoints of pain and intra-ocular pressure. Patients were followed for 28 days post-surgery.
Results: The iontophoretic dose and treatment regimens for cohorts 2 and 6 resulted in a larger percentage of patients with an ACC of zero at day 14 (30% and 40%, respectively) compared to the placebo group (20%). At day 28, 80% of patients in cohort 2 and 70% in cohort 6 had an ACC = 0. In the placebo group, 80% of the patients had to be rescued prior to day 14. The aqueous samples from cohort 7 had 20.03 ± 8.53 ug/mL of total dexamethasone present. A majority of patients in cohorts 1-7 had a pain score of zero on day 1. Further, no elevation of IOP was noted in these cohorts and the delivery was well tolerated.
Conclusions: Iontophoretic delivery of EGP-437 was demonstrated to be a safe method to deliver adequate amounts of steroid into the ocular tissue to successfully manage post cataract inflammation and pain. This technology has the potential to eliminate the daily need for corticosteroid eye drops, thus leading to improved outcomes for this large patient population.

Cohort #	# Patients	Dose	Treatment regimen
1	9	4.0 mA-min at 1.5 mA	Day 0, 7
2	10	4.5 mA-min at 3.0 mA	Day 0, 1, 4
3	10	9.0 mA- min at 3.0 mA	Day 0, 1, 2*
4	10	9.0 mA- min at 3.0 mA	Day 0, 1, 4*
5	10	14.0 mA-min at 3.5 mA	Day 0, 1, 2*
6	10	14.0 mA -min at 3.5 mA	Day 0, 1, 4*
7	10	14.0 mA-min at 3.5 mA	Day -0**, 1, 4
8	10	Placebo (14mA-min at 3.5 mA)	Day 0, 1, 4

*Potential for an additional treatment at Day 7 based on investigator's judgement.
**Day -0 indicates treatment 30-60 minutes pre-surgery.

Table 1: Cohort Dosing Regimen for EGP-437
Commercial Relationships: Barbara M. Wirostko, EyeGate (F), EyeGate (E), EyeGate (S), EyeGate (I); **Carol M. Assang**, EyeGate (E), EyeGate (I); **Brenda Mann**, EyeGate (F), EyeGate (E), EyeGate (S), EyeGate (I); **Stephen From**, EyeGate (F), EyeGate (E), EyeGate (S), EyeGate (I); **Michael Raizman**, EyeGate (R), EyeGate (F), EyeGate (S), EyeGate (C), EyeGate (I)
Clinical Trial: NCT02571556

Program Number: 1082 **Poster Board Number:** B0370
Presentation Time: 3:15 PM–5:00 PM
Assessment of ophthalmic steroid class adverse event reports for loteprednol etabonate
Megan E. Cavet, Christine M. Sanfilippo, Heleen H. DeCory.
Medical Affairs, Bausch + Lomb, Rochester, NY.
Purpose: Loteprednol etabonate (LE) is a topical ophthalmic steroid which, after binding to the glucocorticoid receptor, is rapidly metabolized to inactive metabolites in the ocular tissue, thus reducing the potential for side effects. The prescribing information for topical ophthalmic steroids includes warnings and precautions related to known steroid class effects such as intraocular pressure (IOP) increase, cataracts, delayed healing, as well as bacterial, viral, and fungal infections. We report on adverse events (AEs) related to these class effects for currently available LE ophthalmic formulations.
Methods: The Bausch + Lomb AE database was queried for all AEs (spontaneous, study, literature) reported to date for LE 0.5% ophthalmic suspension, gel, and ointment, for LE 0.2% ophthalmic suspension, and for a combination LE 0.5% and tobramycin 0.3% suspension. These AEs had been entered into a pharmacovigilance and clinical safety case management database (ARISg™) using preferred MedDRA terms.
Results: From the late 1990s, when the first of the LE formulations was studied and launched, through October, 2016, there were a total of 12 reports of cataract, 136 reports of IOP increase, 17 reports of glaucoma or ocular hypertension, 6 reports of impaired healing, and 42 reports of ocular infections for all LE formulations. With the exception of several glaucoma AEs, most other labeled AEs were categorized as non-serious. During this reporting period, more than 85 million units of LE product were shipped worldwide.
Conclusions: Based on this analysis of AEs reported in the Bausch + Lomb AE database, known steroid class AEs have been infrequently reported for LE ophthalmic formulations, suggesting a very low incidence rate for these AEs with LE. However, due to the voluntary nature of AE reporting, the true incidence rate of these AEs is likely higher. Finally, AE reports do not establish causality.
Commercial Relationships: Megan E. Cavet, Bausch + Lomb (E); **Christine M. Sanfilippo**, Bausch + Lomb (E); **Heleen H. DeCory**, Bausch + Lomb (E)

Program Number: 1083 **Poster Board Number:** B0371
Presentation Time: 3:15 PM–5:00 PM
Antibiotic Resistance in Ocular Pathogens – An Update from the 2016 ARMOR Surveillance Program
Christine M. Sanfilippo[1], Heleen H. DeCory[1], Daniel F. Sahm[2], Penny A. Asbell[3]. [1]Medical Affairs, Bausch + Lomb, Rochester, NY; [2]IHMA, Inc., Shaumburg, IL; [3]Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY.
Purpose: Antibiotic resistance is a serious concern in the treatment of bacterial eye infections. The ongoing Antibiotic Resistance Monitoring in Ocular micRoorganisms (ARMOR) study is the only nationwide antibiotic resistance surveillance program specific to ocular pathogens. Here we report preliminary results for ocular isolates collected in 2016.
Methods: *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* isolates were collected and subjected to antibiotic susceptibility testing. Minimum inhibitory concentrations were determined by broth microdilution for up to 16 antibiotics according to the Clinical and Laboratory Standards Institute guidelines. Isolates were categorized as susceptible or non-susceptible (intermediate and resistant) based on systemic breakpoints, where available.
Results: A total of 359 isolates were collected from 11 participating US sites. *H. influenzae* isolates collected to date from 2016 were susceptible to all antibiotics tested. Although resistance among *P. aeruginosa* isolates continued to be low, preliminary 2016 data indicate that non-susceptibility to fluoroquinolones (7%) more than doubled from 2015. Isolates of *S. pneumoniae* exhibited non-susceptibility to azithromycin (31%) and penicillin (38%) while remaining susceptible to fluoroquinolones and chloramphenicol. Resistance rates for *S. aureus* and CoNS generally remained steady when compared to 2015 data. Among all staphylococci, resistance was most notable for azithromycin (47-63%), oxacillin/methicillin (27-43%), and ciprofloxacin (25-30%), with CoNS isolates also exhibiting high levels of non-susceptibility to tobramycin (20%) and trimethoprim (36%). Non-susceptibility to three or more drug classes was observed in 24% of *S. aureus* and 36% of CoNS isolates collected in 2016, with multidrug resistance remaining prevalent among methicillin-resistant (MR) *S. aureus* (70%) and MRCoNS (77%).
Conclusions: Preliminary 2016 ARMOR surveillance data show continued high levels of antibiotic resistance among staphylococci, especially among MR strains, with many isolates demonstrating multidrug resistance. Continued surveillance of ocular pathogens is

warranted, and susceptibility data obtained from ARMOR should be considered before initiating empiric treatment of common eye infections.
Commercial Relationships: Christine M. Sanfilippo, Bausch & Lomb, Inc. (E); **Heleen H. DeCory**, Bausch & Lomb, Inc. (E); **Daniel F. Sahm**, IHMA, Inc. (E); **Penny A. Asbell**, Bausch & Lomb, Inc. (C)

Program Number: 1084 **Poster Board Number:** B0372
Presentation Time: 3:15 PM–5:00 PM
OcuSurf™-Mediated Antimicrobial Therapies to Treat Multi-Drug Resistant Ocular Infections
Kevin L. Ward[1], Shikha P. Barman[2], Koushik Barman[1], Anne-Marie Cromwick[1], Kathryn S. Crawford[3]. [1]Formulations, Integral BioSystems, Bedford, MA; [2]Executive, Integral Biosystems LLC, Bedford, MA; [3]PharmOcu, Andover, MA.
Purpose: The past two decades have seen an increase in antibiotic resistance among bacterial pathogens in the eye. Ocular staphylococci have developed resistance to methicillin and other antibiotic classes. We have previously presented OcuSurf™: a delivery system that has been designed for rapid and efficient absorption into tissues. Eye-drop formulations utilize insoluble active pharmaceutical ingredients; thus, they exist as drug suspensions, which need to dissolve before they can be absorbed. In the ocular space, rapid fluid turnover and loss *via* the naso-lacrimal duct results in just 5% of the drug being absorbed. Thus, clinical need exists for a drug delivery system that can rapidly permeate and adhere to tissues for enhanced bioavailability and enhanced duration of therapeutic levels of drug. We have developed stable OcuSurf-mediated formulations of Besifloxacin and Vancomycin. Comparative *in-vitro* susceptibility (MIC) and time-kill experiments of these formulations against multi-drug resistant clinical bacterial isolates of s. aureus and p. aeroginosa were performed, Stability, *in-vitro* release, corneal permeability and in-vitro irritation studies were performed to select the best drug product candidate.
Methods: Permeability studies were performed through freshly excised corneal membranes, using Franz-type diffusion cells and permeated drug was quantitated by HPLC. Irritation assessment of the formulations was performed in a standard Epiocular model using corneal cell constructs, using 120 minute incubation of the formulations. Physicochemical characterization was performed, including stability assessments by HPLC.
Results: Of the drugs tested, OcuSurf-formulated Vancomycin and Besifloxacin showed low MIC (<1 microgram/mL) against drug resistant strains of MRSA and MSSA. High corneal permeability of both drugs were observed (10-15% of drug permeated through 22 hours) and fast in-vitro release (30-40%) demonstrating high potential for rapidly absorbing formulations tested *in-vivo*. The formulations were of mean size range 0.250-0.300 nm and demonstrated a liquid crystalline internal microstructure. The formulations were stable at
3 months and were non-irritating by Epiocular testing.
Conclusions: Antimicrobial formulations to treat drug-resistant ocular infections are feasible.
Commercial Relationships: Kevin L. Ward, Integral Biosystems LLC (P), Integral Biosystems LLC (E); **Shikha P. Barman**, Integral Biosystems LLC (P), Integral Biosystems LLC (I); **Koushik Barman**, Integral BioSystems (P), Integral Biosystems LLC (E); **Anne-Marie Cromwick**, Integral BioSystems (P), Integral BioSystems (E); **Kathryn S. Crawford**, Integral BioSystems (C)

Program Number: 1085 **Poster Board Number:** B0373
Presentation Time: 3:15 PM–5:00 PM
In vitro antimicrobial evaluation of ozonized balanced salt solution used for cataract surgery
Alberto Sumitomo, Richard Y. Hida, Ivan C. Teixeira, Alessandra Navarini, Rodrigo A. Silva. Santa Casa de S.Paulo, Sao Paulo, Brazil.
Purpose: To analyze *in vitro* antimicrobial activity of ozonized balanced salt solution for cataract surgery.
Methods: A solution of 200 mL of Balanced Salt Solution was mixed with ozone gas in concentrations between 30 mg/L and 80 mg/L. A wood aerator (Red Sea Berlin Air Lift, USA) was used to produce microbubbles. Temperature was controlled by digital thermometer immersed in the solution. Mueller-Hinton plates seeded with *Staphylococcus aureus* MRSA and ATCC controlled strains were used to analyze antimicrobial effect of this solution. After 24 hours of incubation, readings were compared with controls (non-ozonized balanced salt solution).
Results: There were no inhibition halos against the strains studied in both groups (study group and controls).
Conclusions: Ozonized balanced salt solution in the concentration of 30 mg/L and 80 mg/L has no antimicrobial activity observed *in vitro*.
Commercial Relationships: Alberto Sumitomo, None; **Richard Y. Hida**, None; **Ivan C. Teixeira**, None; **Alessandra Navarini**, None; **Rodrigo A. Silva**, None

Program Number: 1086 **Poster Board Number:** B0374
Presentation Time: 3:15 PM–5:00 PM
Mode-of-action evaluation of the antimycotic effect of a tetracycline-chloramphenicol-colistimethate sodium fixed-dose combination
Anna Rita Blanco[2], Manuela D'Arrigo[1], Maria Grazia Mazzone[2], Andreana Marino[1]. [1]Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Università Messina, Messina, Italy; [2]SIFI SpA, Aci S. Antonio, Italy.
Purpose: Previous our studies showed that the commercially available ophthalmic product, as fixed antibiotic combination (Colbiocin®), containing tetracycline, chloramphenicol and colistimethate sodium, had a good efficacy against *Candida* spp., including resistant strains (Marino et al., ARVO 2016). The aim of this work was to study the mode-of-action of this antibacterial product against yeasts.
Methods: *C. albicans* ATCC and clinical isolated treated with sub-MIC concentrations of Colbiocin eye drop or with antibiotics of the formulation, alone and in combination each other, were examined by propidium iodide staning and MitoTracker red staining to determine cell permeability and mitochondrial function, respectively. Observations under the fluorescence microscope were performed.
Results: Microscopic examination using propidium staining demonstrated that the *C. albicans* cells treated with colistimethate sodium were stained red because they lost in cell membrane integrity. Propidium iodide, a red-fluorescent nuclear stain, is a membrane impermeant dye that is generally excluded from viable cells. Microscopic examination using MitoTracker demonstrated that untreated yeast cells showed intense and definite staining of mitochondria, whereas a diffused staining was observed in the yeast cells treated with tetracycline or chloramphenicol, as result of an altered mitochondrial membrane potential. MitoTracker, fluorescent dye, labels mitochondria within live cells utilizing the mitochondrial membrane potential. The yeast cells treated with the fixed combination Colbiocin® demonstrated both effects, the membrane permeabilization and the change in mithocondrial structure.

Conclusions: The additive mechanism of the fixed combination against *Candida* spp., previously shown, can be explained as follows: the colistimethate sodium binds with anionic lipids disrupting membrane integrity thus allowing to tetracycline and chloramphenicol to penetrate through the lipid bilayer and promote mitochondrial dysfunction. These new results on the mode-of-action, support previously published data and the use of the fixed combination Colbiocin® in the treatment of yeast infections.
Commercial Relationships: Anna Rita Blanco, SIFI SpA (E); **Manuela D'Arrigo**, None; **Maria Grazia Mazzone**, SIFI SpA (E); **Andreana Marino**, None

Program Number: 1087 **Poster Board Number:** B0375
Presentation Time: 3:15 PM–5:00 PM
New Water Based Drug Delivery System for Azithromycin Eye drops (MDV1226). PK Study in Rats vs Oily Azyter
Barbara Melilli[1], Maria G. Saita[1], Danilo Aleo[1], Sandro Dattilo[2], Sergio Mangiafico[1], Melina Cro[1], Sebastiano Mangiafico[1]. [1]R&D, Medivis, Catania, Italy; [2]Istituto per i Polimeri, Compositi e Biomateriali, National Research Council, Catania, Italy.
Purpose: The aim of the study was comparing azithromycin concentration in the cornea and in the bulbar conjunctiva after instillation of 1.5% macrolide antibiotic hyaluronic acid/cyclodextrin based (MDV1226) or oil based (Azyter) formulations in rats.
Methods: Sprague Dawley rats were randomly divided in two groups of 28 animals. One group was instilled with MDV1226 a new eye drops formulation containing sulfobutylether cyclodextrins, hyaluronic acid, EDTA and phosphate buffer (pH=6.8-7.2), while the remaining rats received Azyter. Each group was further divided into 7 subgroups of 4 rats, to be sacrificed at the different time points after last instillation. Before treatment, rats were anesthetized by exposure to sevorane for a few seconds. 30 µl of each drug formulation was dosed by instilling 3 times 10 µl, at 1 min interval in both eyes by means a plastic disposable pipette tip. Rats were sacrificed at 5, 15, 30 min; 1, 4, 8 and 24 hr after the last 10 µl eye drops instillation. Cornea and bulbar conjunctiva from both eyes were collected, weighed and stored at −20°C until analysis. Drug levels in tissue samples were measured by using LC-MS method.
Results: After topical instillation, MDV1226 and Azyter reached the highest concentration in the cornea and in the bulbar conjunctiva at same Tmax (5 min,1st sampling time). Azithromycin levels were significantly greater in the conjunctiva then in the cornea. Antibiotic Cmax after MDV1226 in the cornea was 2.2 fold higher than after Azyter (80.03 vs 36.35 µg/g, respectively), while in the bulbar conjunctiva Cmax were comparable, 111.47 and 111.63 µg/g for MDV1226 and Azyter, respectively.
AUC(0.083-24h) [(µg/g) x h] values in the cornea were 69.07 and 82.09, while in the bulbar conjunctiva were 168.83 and 207.11 for MDV1226 and Azyter® respectively.
Conclusions: This exploratory study on rat model, showed that the extent of bioavailability (AUC) of MDV1226 formulation is comparable with Azyter formulation in the bulbar conjunctiva as well as in the cornea.
Commercial Relationships: Barbara Melilli, Medivis (E); **Maria G. Saita**, Medivis (E); **Danilo Aleo**, Medivis (E); **Sandro Dattilo**, Medivis (E); **Sergio Mangiafico**, Medivis (E); **Melina Cro**, Medivis (E); **Sebastiano Mangiafico**, Medivis (E)

Program Number: 1088 **Poster Board Number:** B0376
Presentation Time: 3:15 PM–5:00 PM
Safety and efficacy of 0.09% Pazufloxacin ophthalmic solution vs gatixifloxacin 0.5% and moxifloxacin 0.5% in subjects with bacterial conjunctivitis: a multicenter randomized controlled trial
Aldo A. Oregon-Miranda, Oscar Olvera, Arieh R. Mercado, Leopoldo Baiza. Clinical research, Laboratorios Sophia S.A. de C.V., Zapopan Lso-841221-6d2, Mexico.
Purpose: To assess the safety and efficacy of the PRO-157 ophthalmic solution in three different dosing regimens, versus Moxifloxacin, versus Gatifloxacin in patients with bacterial conjunctivitis.
Methods: A phase II, double-blind, masked, controlled, multicenter, clinical trial of 300 subjects, randomized to either a three dosing regimens of pazufloxacin 0.09% ophthalmic solution (BID, n = 90; TID, n = 76; QID, n = 68) or moxifloxacin 0.3% (n = 82) or gatifloxacin 0.5% (n = 72). Follow-up was set on days 0, 3, and 7. Assessments of anterior / posterior segment ocular signs were performed. The primary outcome measures included conjunctival culture and clinical signs. Safety variables included adverse events, lisamine green and fluorescein ocular surface stains and clinical signs of tolerability.
Results: The primary efficacy endpoints were similar between groups at baseline. After intervention time bacterial eradication were reported in all groups: pazufloxacin BID 79 %; pazufloxacin TID 84 %; pazufloxacin QID 84 %; Moxifloxacin 80 %; and Gatifloxacin 82 %. Similar results were reported in clinical resolution. However, there were not significantce differences between treatments. sto compare groups. The adverse events were not related to the interventions.
Conclusions: Pazufloxacin in different dose regimens, showed similar bacteriological and clinical efficacy when compared to moxifloxacin and gatifloxacin in patients with bacterial conjunctivitis
Commercial Relationships: Aldo A. Oregon-Miranda, Laboratorios Sophia (F), Laboratorios Sophia (E); **Oscar Olvera**, Laboratorios Sophia (F), Laboratorios Sophia (E); **Arieh R. Mercado**, Laboratorios Sophia (F), Laboratorios Sophia (E); **Leopoldo Baiza**, Laboratorios Sophia (I), Laboratorios Sophia (F)
Clinical Trial: NCT02980523

Program Number: 1089 **Poster Board Number:** B0377
Presentation Time: 3:15 PM–5:00 PM
Solithromycin, a potent next-generation macrolide, for topical ophthalmic use
Kathryn S. Crawford[1], Kevin L. Ward[2], Anne-Marie Cromwick[2], Laura Kaminski[2], Kara Keedy[3], Prabhavathi Fernandes[3], Shikha P. Barman[2]. [1]PharmOcu, Andover, MA; [2]Integral BioSystems, Bedford, MA; [3]Cempra, Chapel Hill, NC.
Purpose: Solithromycin (Soli) is a next-generation macrolide, the first fluoroketolide, which has activity against most macrolide-resistant strains. In addition to its antimicrobial properties, it has been shown to stimulate lysosome and lipid accumulation and overall differentiation of immortilized human meibomian gland epithelial cells, indicating its potential to treat meibomian gland disorder (MGD). However, since the molecule has low aqueous solubility, a suitable ophthalmic formulation was needed to assess the feasibility for topical ocular administration.
Methods: Formulation development evaluated the effects of various solubilizers and excipients on stability of solithromycin in solution. Soli concentration was analyzed by HPLC. Cornea permeability was assessed in a Franz-type diffusion *ex-vivo* bovine cornea permeability model. Tolerability and irritation potential was assessed in rabbits.

Ocular pharmacokinetics in rabbits was calculated by LC/MS/MS bioanalyis of solithromycin concentrations in tears, cornea, aqueous humor, conjunctiva, and eyelids, 0.1-24 hours after administration of various modifications of the Soli ophthalmic formulation.
Results: A stable (6+ months at 5°C), Soli 1% ophthalmic solution was developed which met specifications for an ophthalmic solution and was well-tolerated and non-irritating in rabbits. Soli readily penetrates the cornea and ocular surface tissues, resulting in potentially effective intraocular concentrations (C_{max} 80 ng/mL) as well as sustained levels in ocular surface tissues and tears for up to 12 hours after dosing (C_{max} 20-1,800 μg/g).
Conclusions: Solithromycin can be formulated in a stable solution suitable for ophthalmic use. Soli is 8-16 times more potent than azithromycin *in vitro* and is active against azithromycin-resistant strains. Thus, Soli has the potential to treat a variety of ocular infections, as well as surface diseases such as MGD and blepharitis.
Commercial Relationships: Kathryn S. Crawford, Cempra (C); **Kevin L. Ward**, Integral BioSystems (E); **Anne-Marie Cromwick**, Integral BioSystems (E); **Laura Kaminski**, Integral BioSystems (E); **Kara Keedy**, Cempra (E); **Prabhavathi Fernandes**, Cempra (E); **Shikha P. Barman**, Integral BioSystems (E)

Program Number: 1090 **Poster Board Number:** B0378
Presentation Time: 3:15 PM–5:00 PM
Anti-inflammatory effects of *Hymenaea courbaril* essential oil compounds on pterygium fibroblasts
Magda M. Hata Viveiros[1], Cláudia A. Rainho[2], Márcia G. Silva[3], José G. da Costa[4], Anselmo G. de Oliveira[6], Carlos R. Padovani[5], Silvana A. Schellini[1]. [1]Ophthalmology, Universidade Estadual Paulista "Júlio de Mesquita Filho" - Campus Botucatu, Botucatu, Brazil; [2]Genetics, Biosciences Institute of Universidade Estadual Paulista, Botucatu, Brazil; [3]Pathology, Universidade Estadual Paulista "Júlio de Mesquita Filho" - Campus Botucatu, Botucatu, Brazil; [4]Biological Chemistry, Universidade Regional do Cariri, Crato, Brazil; [5]Biostatistic, Universidade Estadual Paulista "Júlio de Mesquita Filho" - Campus Botucatu, Botucatu, Brazil; [6]Pharmaceuticals and Medicines, Universidade Estadual Paulista "Júlio de Mesquita Filho" - Campus Araraquara, Araraquara, Brazil.
Purpose: The search for new drugs has found in plants an important source of biologically active compounds. The *Hymenaea courbaril* is a tree that grows in South America forests and its essential oil has proved anti-inflammatory effects. Due to the involvement of chronic inflammation in pterygium genesis, we investigated the chemical composition of *H. courbaril* essential oil and its anti-inflammatory effects on pterygium fibroblasts.
Methods: The essential oil was obtained by hydro-distillation using a Clevenger-type apparatus and the identification of the chemical constituents by gas chromatography coupled to mass spectrometry. Primary cultures of pterygium fibroblasts were exposed to the *H. courbaril* essential oil main compounds: trans-caryophyllene and alpha-humulene, separately and together, in triplicates. The cell viability was accessed with MTT assay after 12, 24, 48 and 72 hours of exposure. The IL-1b, IL-6, IL-8, TNF-a and IL-10 levels in the conditioned medium were measured by ELISA, at 12, 24 and 48 hours after exposure. The controls were exposed to the vehicles, in each corresponding concentration. The data were analyzed statistically using Friedman repeated measures analysis of variance on ranks.
Results: The main compounds of *H. courbaril* essential oil are trans-caryophyllene (46,24%), oxide caryophyllene (14,67%) and α-humulene (9,19%). The MTT test showed that α-humulene, trans-caryophyllene and both drugs together had the same cytotoxic effect when used in concentrations of 0.5 to 5μM. The IL-6 levels showed statistically significant reduction (P=0.041) at 48 hours after exposure to trans-caryophyllene at 25 mM. The IL-1b, IL-8, IL-10 and TNF-α levels were not detected.
Conclusions: The trans-caryophyllene compound from *H. courbaril* essential oil in concentration of 25μM presented significant anti-inflammatory effect on IL-6 production of pterygium fibroblasts after 48 hours of exposure when compared to controls. So, *H. courbaril* can be therefore a potential alternative adjuvant agent in the treatment of pterygium.
Commercial Relationships: Magda M. Hata Viveiros, None; **Cláudia A. Rainho**, None; **Márcia G. Silva**, None; **José G. da Costa**, None; **Anselmo G. de Oliveira**, None; **Carlos R. Padovani**, None; **Silvana A. Schellini**, None
Support: São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, grants # 2012/10032-4) and National Program of Post Doctoral (PNPD), grants # 06460/2013 from Coordination for the Improvement of Higher Level Education Personnel – CAPES, Ministry of Education, Brazil

Program Number: 1091 **Poster Board Number:** B0379
Presentation Time: 3:15 PM–5:00 PM
Evaluation of anti-human complement component 5 antibody in humanized C5 mice
Adrianna Latuszek[1], Yashu Liu[2], Randi Foster[3], Irena Lovric[3], Ming Yuan[1], Henry Chen[1], Ying Hu[1], Pamela Krueger[3], Tammy Huang[3], William Poueymirou[4], George Yancopoulos[5], Brian Zambrowicz[4], Jingtai Cao[1], Carl Romano[1], William Olson[3]. [1]Ophthalmology, Regeneron Pharmaceuticals, Tarrytown, NY; [2]Technology & Discovery Centers, Regeneron Pharmaceuticals, Tarrytown, NY; [3]VI Antibody, Regeneron Pharmaceuticals, Tarrytown, NY; [4]Velocigene, Regeneron Pharmaceuticals, Tarrytown, NY; [5]Regeneron Pharmaceuticals, Tarrytown, NY.
Purpose: Complement has been implicated in ocular inflammatory and retinal degenerative diseases. Blocking C5 cleavage by a monoclonal antibody (mAb) is a possible therapeutic strategy for these disorders. The present study was undertaken to evaluate pharmacokinetic/pharmacodynamic (PK/PD) properties of a therapeutic mAb in humanized C5 mice.
Methods: Lead anti-human C5 mAb (REGN3918) was selected by classical (CP) and alternative pathway (AP) hemolysis bioassays in vitro. Humanized C5 mice ($C5^{hu/hu}$, Regeneron) were used to assess PK/PD in vivo/ex vivo. $C5^{hu/hu}$ mice were generated and characterized using VelociGene and VelociMouse technologies. Mice were stratified according to their serum C5 level. Each mouse received a single subcutaneous injection of REGN3918 (15 mg/kg) or isotype control mAb. Mouse serum was collected at different time points: Pre-dose, day 1, terminal bleed time points (days 10-60; n≥4 each). PK was measured by ELISA or Gyros immunoassays. PD was analyzed by ex vivo CP assay. Excess human C3 (80ug/ml) was added ex vivo prior to hemolysis assay. Human C5 and mAb concentrations in serum were evaluated using a LC-MRM-MS method.
Results: REGN3918 blocked CP hemolysis (5% human serum) in a dose-dependent manner, with IC50 of 14.1 nM in a 10 mins lysis assay or 18.3 nM with 60 mins lysis. Maximal inhibition of hemolysis was 91-95%. In this assay REGN3918 also blocked C5a generation in a dose-dependent manner with IC50 of 8.5 nM as measured by C5a ELISA. In AP hemolysis (10% human serum) REGN3918 showed maximal inhibition of 88%, with IC50 of 27.4 nM. The isotype control antibody did not block hemolysis under identical assay conditions. Average serum level of human C5 in $C5^{hu/hu}$ mice was 40.9 μg/ml. There is a difference between male (55.4 ± 1.7 μg/ml, n=47) and female (24.7 ± 0.6 μg/ml, n=49)

mice. Following a single SC dosing of REGN3918, compared with the pre-dose, CP activity induced by the mouse serum ex vivo was significantly reduced at all time points up to and including day 35. This was correlated with the C5/REGN3918 molar ratio measured by LC-MRM-MS method.
Conclusions: Our findings demonstrate that $C5^{hu/hu}$ mice can be used to evaluate PK and PD profiles of anti-human C5 mAb. LC-MRM-MS is a valuable method to confirm the bivalent binding of REGN3918 to C5 and its PD effect in vivo.
Commercial Relationships: Adrianna Latuszek, Regeneron Pharmaceuticals (E); **Yashu Liu**, Regeneron Pharmaceuticals (E); **Randi Foster**, Regeneron Pharmaceuticals (E); **Irena Lovric**, Regeneron Pharmaceuticals (E); **Ming Yuan**, Regeneron Pharmaceuticals (E); **Henry Chen**, Regeneron Pharmaceuticals (E); **Ying Hu**, Regeneron Pharmaceuticals (E); **Pamela Krueger**, Regeneron Pharmaceuticals (E); **Tammy Huang**, Regeneron Pharmaceuticals (E); **William Poueymirou**, Regeneron Pharmaceuticals (E); **George Yancopoulos**, Regeneron Pharmaceuticals (E); **Brian Zambrowicz**, Regeneron Pharmaceuticals (E); **Jingtai Cao**, Regeneron Pharmaceuticals (E); **Carl Romano**, Regeneron Pharmaceuticals (E); **William Olson**, Regeneron Pharmaceuticals (E)

Program Number: 1092 **Poster Board Number:** B0380
Presentation Time: 3:15 PM–5:00 PM
A SOCS1 peptide alleviates inflammation and associated damage to barrier properties in ARPE-19 cells
Chulbul M. Ahmed, Alfred S. Lewin. Molecular Genetics and Microbiology, University of Florida, Gainesville, FL.
Purpose: Parainflammation or overt inflammation are associated with the onset and propagation of several ocular disorders. The purpose of this study was to test a peptide derived from suppressor of cytokine signaling 1 (SOCS1) for protection against inflammation and changes in tight junction properties in ARPE-19 cells.
Methods: The kinase inhibitory region (KIR) of SOCS1 spanning from residues 53 to 68 was conjugated to polyarginine (R9) for cell penetration was tested for its anti-inflammatory properties in ARPE-19 cells treated with TNFα or IL-17A, the major culprits in several ocular pathologies. Induction of inflammatory cytokines and chemokine was evaluated by RT-qPCR. Concomitant secretion of inflammatory cytokine in the supernatant was measured by ELISA. Since these inflammatory cytokines act through the transcription factor, NF-kB, a luciferase reporter linked to NF-kB promoter was used to assess the effect on its activity. Integrity of the cell monolayer was assessed by immunostaining with zona occludin 1 (ZO-1) antibody and by transepithelial electrical resistance (TEER) measurement in cells treated with TNFα or IL-17A in the presence or absence of SOCS1 peptide.
Results: RT-qPCR assays revealed significant induction of IL-1β, IL-6 and CCL-2 in TNFα treated cells, which was attenuated by SOCS1 peptide. Increased secretion of IL-1β in supernatants by TNFα treatment was suppressed when SOCS1 peptide was simultaneously present. Induction of NF-kB activity by TNFα was suppressed in the presence of SOCS1 peptide. Treatment with TNFα or IL-17A led to a decrease in TEER that was prevented in the presence of SOCS1 peptide. Protection of the integrity of the tight junctions was also documented by ZO-1 staining in cells treated with TNFα or IL-17 in the presence of SOCS1.
Conclusions: Since the retinal pigment epithelium cells serve the crucial role of maintaining blood-retinal barrier, our demonstration that SOCS1 peptide exhibits anti-inflammatory properties and protects against the damage to barrier properties suggests that it will have therapeutic potential in treatment of ocular diseases such as autoimmune uveitis and age-related macular degeneration.
Commercial Relationships: Chulbul M. Ahmed, University of Florida (P); **Alfred S. Lewin**
Support: NEI core grant to the University of Florida (P30 EY02172). Funding was also provided by the Shaler Richardson Professorship endowment.

Program Number: 1093 **Poster Board Number:** B0381
Presentation Time: 3:15 PM–5:00 PM
Estimating Total Dose Received by an Ophthalmic Irrigation Solution Used during Cataract Surgery
L David Waterbury[1], Vince Florio[2]. [1]Raven Biosolutions LLC, San Carlos, CA; [2]Omeros Corporation, Seattle, WA.
Purpose: Published methods for determining the total dose received with an ophthalmic irrigation solution are not available. We report a method for estimating the total dose received by canines in a pharmacokinetic study. The total dose received is important to compare accurately with topical dosing and with systemic exposure.
Methods: A formulation of ketorolac (0.3%) and phenylephrine (1%) was administered in irrigation solution in 20 canines during lens replacement surgery. Ketorolac concentrations were determined by LC/MS in aqueous, cornea, conjunctiva, iris-ciliary body, vitreous, retina, choroid, retinal pigment epithelium, sclera, lens capsule and plasma from 4 dogs per time point (0, 2, 6, 8 and 10 hours post-surgery). The tissue weights were obtained upon careful dissection of the tissues, except for aqueous and vitreous samples which were obtained prior to freezing of tissues. The concentrations determined were multiplied by the tissue weights and, for plasma concentrations, by the calculated plasma volume based on the age and weight of the dogs. These calculated values for each animal were summed to determine the estimated total dose of ketorolac received.
Results: The total dose received via intracameral administration in canines was determined to be 12.8 µg, which is less than 10% of the amount received by a 50-µL dose of topical ketorolac. Thus, the amount of systemic exposure of ketorolac is less by the intracameral irrigation route than by topical administration as reflected by 2- to 5-fold lower plasma concentrations. Nonetheless, compared to topical dosing, intracameral delivery provided 2- to 10-fold higher levels of ketorolac in ocular tissues, which is sufficient to inhibit both COX-1 and COX-2 in aqueous and vitreous ten hours after dosing. Also unlike topical dosing, administration via ophthalmic irrigation provides therapeutic concentrations in the vitreous.
Conclusions: A method for estimating the total dose of an ophthalmic drug in an irrigation solution is relevant for determining total systemic exposure to ketorolac. This method is useful as it allows the comparison with topical dosing. Dosing by intracameral irrigation during the surgical procedure provides superior therapeutic concentrations in target ocular tissues at a 90% lower rate of systemic exposure compared to topical dosing.
Commercial Relationships: L David Waterbury, Omeros (F), Omeros (C); **Vince Florio**, Omeros (E)
Support: Unrestricted Grant from Omeros Corporation

Program Number: 1094 **Poster Board Number:** B0382
Presentation Time: 3:15 PM–5:00 PM
The granulocyte colony-stimulating factor (G-CSF) induced stabilization of blood-optic nerve barrier (BOB) in the optic nerve (ON) crush model via PI3K/AKT activation
Yao-Tseng Wen, Rong-Kung Tsai. Buddhist Tzu Chi General Hospital, Hualien, Taiwan.
Purpose: The purpose of this study is to investigate why G-CSF can stabilize BOB in the ON crush model.

Methods: The ONs of adult male Wistar rats (150-180 g) were crushed by a standardized method. The control eyes received a sham operation. G-CSF, G-CSF plus AKT inhibitor, or phosphate-buffered saline (PBS control) was immediately administered after ON crush for 5 days by subcutaneous injection. Rats were euthanized at 1 or 2 weeks after the crush injury. RGC density was counted by retrograde labeling with FluoroGold application to the superior colliculus. TUNEL assay was used to measure the apoptotic RGCs in the RGC layer. BOB stabilization was evaluated by measuring Evans blue extravasation, IHC staining, and Western blot analysis of Claudin-3, ZO-1.
Results: Two weeks after the insult, the RGC densities in the central and mid-peripheral retinas in ON-crushed, G-CSF-treated rats were significantly higher than that of the G-CSF plus AKT inhibitor- or PBS-treated rats. TUNEL assays showed fewer apoptotic cells in the retinal sections in the PBS-treated group and G-CSF plus AKT-treated group. Macrophage infiltration analysis showed more ED-1 positive cells in the G-CSF treated group than the G-CSF plus AKT- or PBS-treated groups. Evans blue extravasation was demonstrated that AKT inhibitor can disrupt G-CSF induced BOB stabilization. Additionally, G-CSF stabilizes the BOB by up-regulating Claudins 3 and ZO-1 in the insulted ON.
Conclusions: We demonstrate G-CSF plays a pivotal role in attenuating neuroinflammation and BBB disruption via activation of the PI3K/Akt pathway in the ON crush model.
Commercial Relationships: Yao-Tseng Wen, None; **Rong-Kung Tsai**, None

Program Number: 1095 **Poster Board Number:** B0383
Presentation Time: 3:15 PM–5:00 PM
Management of chronic anterior uveitis relapses: efficacy of oral vitamin D treatment
Giulia Malaguarnera[2, 1], Davide Scollo[1, 2], Agata Messina[2], Rosario Foti[3, 2], Elisa Visalli[3, 2], Roberta Amato[1, 2], Angela Cantavenera[1, 2], Mario Toro[1], Miriam Gallo Afflitto[1, 2], Caterina Gagliano[1, 2]. [1]Department of Ophtalmology, University of Catania, Catania, Italy; [2]Rare Diseases Center (Ra.Di.Ce.), Santa Marta Hospital, Catania, Italy; [3]Rheumatologic Unit, Catania University, Catania, Italy.
Purpose: The role of vitamin D in immunoregulation has led to the concept of a dual function as both as an important secosteroid hormone for the regulation of body calcium homeostasis and as an essential organic compound that has been shown to have a crucial effect on the immune responses. Altered levels of vitamin D3 have been associated, by recent observational studies, with a higher susceptibility of immune-mediated disorders and inflammatory diseases such as uveitis. This study investigated the effect of vitamin D supplementation in patients with chronic anterior uveitis relapses. The primary end point of our work was the evaluation of relapse frequency in all treated patients, before and after Vitamin D treatment.
Methods: A total of 35 patients (21 men and 14 woman) aged 31–65 years and with relapses uveitis were enrolled in the study. They were treated with oral vitamin D (50.000 I.U. every week). Serum 25-hydroxyvitamin D (25(OH)D) levels were measured. All patients completed a 12-month follow-up therapeutic period.
Results: After 12 months of therapy, no eyes showed relapses uveitis. There was also an improvement in symptoms and signs associated with relapses after Vitamin D treatment, including ocular pain, blurring of vision, pericorneal hyperemia and aqueous or vitreous cells, and flare in 42% of patients. No patients had further reduction in visual acuity, 35% showed stabilization, and 65% showed statistically significant improvement ($P = 0.0001$ by Student's *t*-test and $P = 0.0005$ by Wilcoxon signed rank test).
Conclusions: Our study demonstrate the potential therapeutic role of Vitamin D and its efficacy in eye relapsing diseases.
Commercial Relationships: Giulia Malaguarnera; **Davide Scollo**, None; **Agata Messina**, None; **Rosario Foti**, None; **Elisa Visalli**, None; **Roberta Amato**, None; **Angela Cantavenera**, None; **Mario Toro**, None; **Miriam Gallo Afflitto**, None; **Caterina Gagliano**, None