Appendix 2 Protocol and Protocol Amendments

The protocol V1.0 was not circulated to regulatory authorities or ethics committee, and thus is not included here.

Protocol Version 3.0 dated February 7, 2017

Protocol Version 2.0 dated April 22, 2016

Clinical Protocol P-321-201

Project Number	**P-1003-I101**
Compound Number/ Name	**P-321 Ophthalmic Solution**
Protocol Number	**P-321-201**
Protocol Title	**Randomized, Crossover Study of the Pharmacodynamic Activity of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Characterized by Low Tear Volume**
Sponsor	**Parion Sciences, Inc.** **2800 Meridian Parkway** **Suite 195** **Durham, NC 27713**
Medical Monitor	**Gary N. Foulks, M.D.** **3103 Joy Place** **Wilmington, NC 28409**
Authors	**José L. Boyer, Ph.D.** **Anita Woodring, MS, RAC**
Issue Date	**Version 3.0: 07 February 2017** **Version 2.0: 22 April 2016** **Original Version 1.0: 05 February 2016**

Sponsor Signature and Date José L. Boyer.

PARION SCIENCES, INC.
Clinical Protocol P-321-201
Investigator Signature Page

Project Number	**P-1003-I101**
Compound Number/ Name	**P-321 Ophthalmic Solution**
Protocol Number	**P-321-201**
Protocol Title	**Randomized, Crossover Study of the Pharmacodynamic Activity of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Characterized by Low Tear Volume**
Sponsor	**Parion Sciences, Inc.** **2800 Meridian Parkway** **Suite 195** **Durham, NC 27713**
Issue Date	**Version 3.0: 07 February 2017** **Version 2.0: 22 April 2016** **Original Version 1.0: 05 February 2016**

I have reviewed and understand this protocol and all amendments associated with it. I will administer the protocol in accordance with ICH, FDA, and local regulations and guidelines. I will keep the information provided to me within this protocol and by Parion Sciences, Inc. staff, their representatives, and designees confidential.

Investigator Name (printed or typed):

Victor L Perez

Investigator Signature:

V L Perez

2/9/17

Date

Summary of Changes to the Protocol

The previous version of this protocol (Version 2.0, 22 April 2016) was amended to create the current version (Version 3.0, 07 February 2017).

Key changes in the current version of the protocol are summarized below.

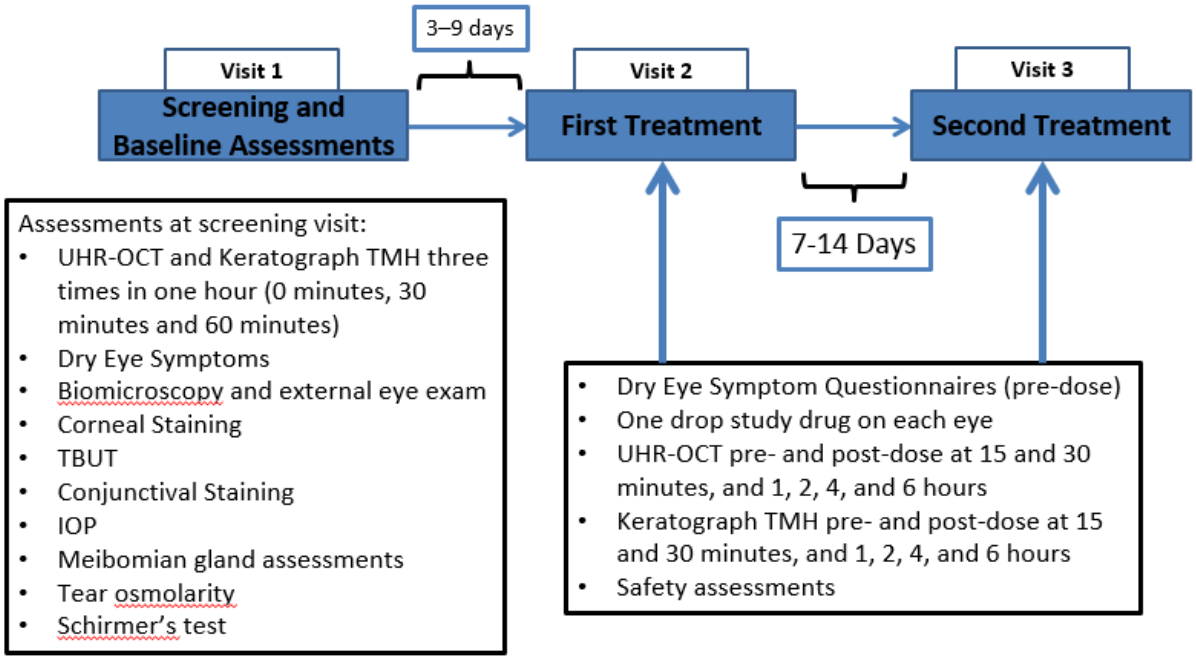
Change	Affected Sections
The following changes were made to the study inclusion criteria; • Added clarification that a history of dry eye disease must be in **both eyes**. • Changed the requirement for average tear meniscus height, as assessed by UHR-OCT, from less than 175 µm to 210 µm or less. This requirement is now in one eye instead of both eyes. • Removed Schirmer's test > 1 mm and < 10 mm as an inclusion criteria. • Added Xiidra® to the list of prohibited medications.	Section 1.0, 5.1 and 7.4.2
The following changes were made to the study exclusion criteria: • Added clarification that external blepharoplasty, not resulting in exposure or abnormal blinking, will be allowed. • Added that scleral lenses are not allowed.	Section 1.0. 5.2 and 7.4.2
Updated study design schematic.	Section 1.0 and 4.1
The requirement to meet the Schirmer's test range at Visit 2 and Visit 3 was removed.	Sections 5.3, 6.2, and 6.3
Definition of "study eye" was added.	Section 5.4
Clarified that informed consent must be obtained before initiating study procedures.	Section 6.1, Appendix I/Table 1
	Section 8.0
Removed phosphorus from the required serum chemistry tests.	Appendix XIII

Typographical and administrative changes were also made to improve the clarity of the document.

1 PROTOCOL SYNOPSIS

Name of Sponsor: Parion Sciences, Inc.		**Study Medication:** 0.017% P-321 Ophthalmic Solution and based on data review potentially 0.01%, 0.017% or 0.05% Ophthalmic Solution
Protocol Number: P-321-201	**Phase:** 2a	**Indication:** Treatment of dry eye disease
Title of the Study: Randomized, Crossover Study of the Pharmacodynamic Activity of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Characterized by Low Tear Volume		
Investigators: Principal Investigator: Victor Perez, MD; Sub-Investigator: Jianhua Wang, MD, PhD		
Study Center: Ocular Surface Center, Bascom Palmer Eye Institute, University of Miami Health System		
Study period (FPFV – LPLV): Approximately 6 months		
Objectives: **Primary objective** • The primary objective of this trial is to assess changes in tear volume by the non-invasive techniques Ultra High Resolution Optical Coherence Tomography (UHR-OCT) following the administration of P-321 Ophthalmic Solution or Placebo in subjects with tear deficient dry eye disease. **Secondary objective** • assess changes in lower tear meniscus height by Keratograph 5M • compare lower tear meniscus height measurements between UHR-OCT and Keratograph 5M • assess the duration of action of P-321 Ophthalmic Solution • compare the pharmacodynamic activity of different strengths of P-321 in subjects with tear-deficient dry eye • monitor safety and tolerability of P-321 Ophthalmic Solution		

Study Design:



This is a single-center, randomized, cross-over placebo controlled study to evaluate the changes in tear volume by P-321 Ophthalmic Solution or Placebo. The study will enroll subjects with tear deficient dry eye disease to receive sequentially two treatments: P-321 Ophthalmic Solution (Treatment A) and Placebo (Treatment B). Subjects will be randomly assigned to receive treatment sequence AB or BA. The study will consist of two Phases: In each Phase, subjects will be treated with a different strength of P-321 Ophthalmic Solution under the same study design.Phase 1 will evaluate 0.017% P-321 Ophthalmic Solution and Phase 2 will evaluate either 0.05%, additional subjects at 0.017% or 0.01% P-321 Ophthalmic Solution as determined by the Data Review Group.

Approximately twenty-four eligible subjects will complete the study with approximately 8 subjects participating in Phase 1 and the remainder of the 24 subjects participating in the phase 2. Decisions on the dose to be used to phase 2 will be made following unmasked data review from subjects in Phase 1. The Principal Investigator, Medical Monitor and Parion Sciences representatives not involved in the day to day conduct of the study will review unmasked data as part of the Data Review Group, which will decide the dose to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur during the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above. The study will consist of three study visits: a Screening Visit (Visit 1), and two treatment visits (Visit 2 and Visit 3). The duration of the study may be up to 23 days. The pharmacodynamic measures of tear volume will be assessed at each visit by two non-invasive methods, UHR-OCT and Keratograph 5M. Dry eye symptom questionnaires will be applied at each visit prior to other assessments. At Visit 1, tear volume will be assessed at three timepoints over 1 hour with measurements taken approximately 30 minutes apart. Tear volume measurements will be followed by other tests that involve contact with the ocular surface (tear osmolarity, tear break up time (TBUT), meibography and meibomian gland assessments, corneal and conjunctival staining, and Schirmer's test). At Visits 2 and 3, tear volume will be assessed serially over 6 hours at pre-dose, and post dose at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours. Safety assessments include adverse events (AEs), biomicroscopy and external eye examination and an assessment of comfort after taking the medication in-clinic.

Subject Population:

This study will complete approximately 24 subjects with dry eye disease with documented low tear volume as measured by non-invasive UHR-OCT. Approximately 8 will participate in Phase 1 and the remainder of the 24 subjects participate in the Phase 2.

Male and female subjects 18-80 years of age who provide written informed consent and have a history of dry eye signs and symptoms of mild to moderate severity in both eyes supported by a previous clinical diagnosis may enter the study. Subjects must have decreased lower tear meniscus height (LTMH) in both eyes assessed by UHR-OCT of not more than 210 µm, normal lid anatomy, have been stable on their current medication regimen for at least 28 days and remain on this regimen for the duration of the study. Additionally, while in the clinic on visit days and for at least 6 hours after dosing with study medication, subjects must be able to withhold ocular topical medications (including artificial tears, topical steroids, Restasis® and Xiidra®), and if contact lens wearers, must be able to withhold wearing contact lenses. To qualify for the study, subjects must not have an identifiable or suspected dry eye caused by pharmacologic, post-traumatic, or post-surgical condition; have undergone refractive eye surgery (e.g., LASIK) in either eye during the past 12 months; uncomplicated cataract surgery in either eye during the past 3 months; previous eyelid surgery in either eye (e.g., blepharoplasty, ptosis repair. External blepharoplasty not resulting in exposure or abnormal blinking will be allowed) and or botulinum toxin (Botox™ or equivalent) injection in the periocular area within 3 months prior to enrollment. The subject must not have lid irregularities or deformities, or severe corneal surface irregularities, a history of glaucoma or intraocular pressure > 25 mmHg at the Screening Visit (Visit 1) or a history of elevated IOP within the past year, a systemic, multi-organ disease requiring active medical or surgical treatment. Additionally, subjects with any significant illness that, in the opinion of the Principal Investigator (PI), could interfere with the study parameters will also be excluded. Other exclusions include: subjects who have punctal plugs, punctal occlusion, scleral lenses, or history of nasolacrimal duct obstruction, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, or other ocular cicatrizing disorders, past or present exposure keratopathy, neurotrophic keratopathy, lagophthalmos, or trichiasis. Use of oral and nasal antihistamines, oral or topical secretagogues such as pilocarpine and Evoxac, or Topical anti-glaucoma medications during the study and for 14 days prior to Visit 1 is prohibited.

Number of Subjects:	**Number of Centers:**
Approximately 24 subjects with tear deficient dry eye disease	1
Test Product and Doses:	**Route of Administration:**
P-321 Ophthalmic Solution and Placebo with dosage strengths of P-321 Opthalmic Solution of 0.017% in Phase 1 and 0.05%, 0.01% or 0.017% in Phase 2	Ocular instillation, one drop per eye

Duration of Treatment:

Subjects will receive a single dose of P-321 Ophthalmic Solution or Placebo in both eyes in random treatment order, one treatment on Visit 2 and the other treatment on Visit 3.

Criteria for Evaluation:

Background characteristics will be evaluated to better characterize subjects at entry. These parameters include: demographics, visual acuity, intraocular pressure, dry eye symptoms upon entry, medical history, concomitant medication use, tear osmolarity, tear break up time, meibography and meibomian gland assessment, corneal and conjunctival staining, and Schirmer's test.

Pharmacodynamics

Detailed measurements of tear volume before and after dose administration will be obtained using non-invasive methods, UHR-OCT (tear volume and tear meniscus height) and Keratograph 5M (tear meniscus height) imaging of the ocular surface.

Safety

Ocular safety measures include: Adverse events (AEs), biomicroscopy and external eye examination and an assessment of comfort after taking the medication.

Statistical Analysis:

Sample Size

A sample size of approximately 24 subjects is proposed for this study. For a two-period, two-treatment, two-sequence crossover design (2x2x2), this study size when all subjects treated with P-321 are analyzed, will provide 98% power to detect a LTMH difference vs placebo of 60 μm at 1 hour or a power of 99% if the average increase in LTMH over the six hours is 60 μm (assuming within-subject SD of 71). In this study it is expected that the study medication will reach its peak effect at 1 hour post dose and that the effect diminishes at 4 and 6 hours post dose (to values still above pre-dose values).

Disposition, Demographic, and Background Characteristics

Subject disposition, demographic, and background characteristics will be summarized using descriptive statistics. Baseline homogeneity with respect to demographic and background characteristics will be assessed via an overall F-test from analysis of variance (ANOVA). For categorical variables, treatment differences will be assessed using the chi-square test or Cochran Mantel Haenszel (CMH) test with modified ridit scores for ordered categorical variables.

Pharmacodynamic Analysis

Treatment group differences for tear volume assessments will be conducted via comparisons of tear meniscus height, and if applicable, tear meniscus volume (TMV). These parameters will be compared between each dose of P-321 Ophthalmic Solution and Placebo using a t-test for study eyes, and for all eligible eyes. Analysis of covariance (ANCOVA) models will be used to adjust for the baseline TMH and TMV for study eyes. For all eligible eyes, a generalized estimation equations (GEE) will also be used to account for the correlation between eyes. For the GEE analysis, an exchangeable correlation matrix will be assumed.

Safety

All safety analyses will be included for all subjects who were randomized and received at least one dose of study medication.

The incidence of AEs will be tabulated by treatment group, by treatment sequence, by severity, and by relationship to study medication.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
ANCOVA	Analysis of covariance
AUC	Area under the curve analysis
BID	Two times daily
C	Celsius
CMH	Cochran Mantel Haenszel (test)
CRF	Case report form
ECG	Electrocardiogram
ENaC	Epithelial sodium channel
F	Fahrenheit
FDA	Food and Drug Administration
GEE	Generalized estimation equations
GLP	Good Laboratory Practices
ICH	International Conference on Harmonization
IOP	Intraocular pressure
IRB	Institutional Review Board
I_{sc}	Short-circuit current
IV	Intravenous
LTMH	Lower tear meniscus height
LTMV	Lower tear meniscus volume
NOAEL	No-observed-adverse-effect level
OSDI	Ocular symptom disease index
PD	Potential difference
PI	Principal investigator
QID	Four times daily
SAE	Serious adverse event
SANDE	Symptom Assessment in Dry Eye questionnaire
SPEED	Standard Patient Evaluation of Eye Dryness questionnaire
SS	Sjögren's syndrome
TBUT	Tear break-up time
TMH	Tear meniscus height
TTMV	Total tear meniscus volume calculated as UTMV + LTMV

UHR-OCT	Ultra high resolution optical coherence tomography
UTMH	Upper tear meniscus height
UTMV	Upper tear meniscus volume

2 BACKGROUND INFORMATION

2.1 Description of Study Medication

Parion Sciences, Inc. is developing P-321 Ophthalmic Solution for the treatment of subjects with dry eye disease. P-321 is a novel potent inhibitor of the epithelial sodium channel (ENaC) and a structural analog of amiloride with unique pharmacokinetic (PK) and pharmacodynamic characteristics designed for ocular topical administration, metabolic stability and limited systemic exposure. The formulation being studied is a sterile non-preserved aqueous solution of P-321.

ENaC is a transmembrane sodium channel important in the regulation of epithelial sodium absorption that is present in the eye and other tissues such as lung, kidney, colon, and sweat glands. ENaC has been extensively characterized in the lung where it plays a major role in the regulation of the composition of the airway surface liquid and is tightly linked to the maintenance of airway surface hydration and mucocilliary clearance (Barker, 1998). The absorption of sodium from the epithelial surface liquid via ENaC osmotically entrains water into the epithelium, decreasing the level of hydration of the mucosal surface.

The dynamics of the ocular tear film are maintained through an integrated system known as the lacrimal functional unit that consists of the ocular surface epithelium (cornea and conjunctiva), the lacrimal glands, the meibomian glands, and their neural and immunological components (Stern, 2004).

ENaC is a key ion channel in this process, mediating the absorption of sodium (and hence water) from the tear fluid through the ocular surface epithelium (cornea and conjunctiva) where ENaC is expressed (Levin, 2006; Thelin, 2012; Krueger. 2012; Yu, 2012a; Yu, 2012b). Therefore, ENaC provides an absorptive pathway for tear fluid loss, which regulates the composition and volume of tears under non-stimulated or “basal” conditions. The inhibition of ENaC with highly potent, small molecule channel blockers represents a unique ocular hydration strategy, working by preventing the absorption of tear fluid and thereby maintaining the protective and lubricating actions of tears on the ocular surface.

2.2 Non-Clinical Studies for P-321 Ophthalmic Solution

2.2.1 Pharmacology

***In vitro* studies:**

The inhibitory effect of P-321 on ENaC was studied in human and canine primary cultures of bronchial epithelial cells. Apical administration of P-321 produced a concentration-dependent inhibition of ENaC-mediated short-circuit currents (Isc) with a potency of 3.247 ± 1.231 nM (n=12) and 1.901 ± 0.7543 nM (n=3) for canine and human airway epithelial cells, respectively.

Primary cultures of human conjunctival epithelia grown on a permeable support at an air-liquid interface were used to assess the effect of P-321 on the absorption of fluid by the conjunctiva. Addition of fluid to the apical surface of these cells resulted in rapid absorption of fluid into the tissue. The presence of P-321 on the apical surface inhibited the "tear" fluid absorption of the conjunctiva, suggesting that ENaC-mediated sodium absorption plays an important role on fluid absorption and tear dynamics.

Among the unique characteristics built in the design of P-321 are the low systemic exposure following administration on the ocular surface, and the retention of the drug on the ocular surface to increase the duration of action. The penetration of P-321 into the corneal epithelium was measured by confocal microscopy imaging, taking advantage of the intrinsic fluorescence of P-321. *Ex vivo* imaging of mouse corneas treated with P-321 or amiloride indicated that in corneas treated with P-321, the drug remained associated with the mucosal surface of the cornea, whereas under identical conditions, amiloride had fully penetrated the epithelium, the stroma, and the endothelium, suggesting that P-321 is selectively retained by the epithelium of the ocular surface.

***In vivo* Studies**

The effect of P-321 on the activity of ENaC in the ocular surface was studied in C57BL/6 mice by *in vivo* measurements of the transepithelial potential difference (PD) driven by sodium movement through ENaC**.** Topical ocular instillation of P-321 produced a concentration-dependent inhibition of the ocular PD measurements with a potency of 57.2 nM, (95% Confidence Interval: 35.2 to 93.0 nM). These studies are consistent with *in vitro* results obtained in airway cells and suggest that P-321 is a potent inhibitor of the ocular ENaC-mediated epithelial sodium transport *in vivo*.

The ability of P-321 to increase tear volume was studied in the ExLac dry eye animal model in rats in which the main lacrimal glands have been surgically removed (Fujihara, 2001). ExLac rats exhibit approximately 50 to 60% reduction in the basal tear volume compared to normal animals. A single ocular instillation of P-321 produced a concentration-dependent increase of tear volume that reached tear volumes similar to those observed in normal animals and at maximally effective concentrations. The increase in tear levels was maintained for several hours following a single administration of P-321.

The effect of repeat dosing of P-321 on tear volume was also studied in the ExLac rat animal model.

Animals were treated with repeated administrations of 0.001% P-321 or vehicle control for five consecutive days at two dosing frequencies, twice daily (BID) and four times daily (QID). Animals treated with vehicle control showed no significant changes in tear volume throughout the five days of dosing. In contrast, in the group of animals treated with P-321 administered BID or QID, a gradual increase in tear volume was observed on each day of treatment reaching by the fifth day of treatment a steady state level similar to the tear volume of normal rats. On the first day of treatment, the animals receiving P-321 QID had a larger increase in tear volume than the animals treated BID, however, both groups had similar levels of tear volume by the end of treatment on Day 5. These results show that a maximum effect on tear volume can be achieved with low concentrations of P-321 (0.001%) when administered either BID or QID over five consecutive days.

In vivo pharmacology studies have shown that epithelial sodium channel inhibitors, including P-321 have a stimulatory effect on tear volume in an animal model of dry eye disease (Thelin, 2012).

These *in vivo* and *in vitro* pharmacological actions together with the PK properties of P-321 provide a strong scientific rationale for the use of P-321 Ophthalmic Solution for the treatment of diseases of impaired ocular hydration such as dry eye.

2.2.2 Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution, metabolism, and excretion profile of P-321 was characterized in *in vitro* and *in vivo* studies. P-321 was not metabolized in plasma from different species (rat, rabbit, dog and human) or by incubation with rat and dog hepatocytes.

Oral administration of P-321 to rats did not produce measurable plasma drug concentrations, suggesting that P-321 is not orally available.

The PK and systemic clearance of P-321 was characterized in rats following intravenous (IV) administration. P-321 displayed biphasic elimination from plasma with a long terminal half-life and a high volume of distribution. The PK of P-321 in plasma showed no difference among sex, and no accumulation was observed after multiple days of IV administration.

The renal elimination of P-321 and its potential for inhibition of ENaC in the kidney was studied in rats treated with P-321 by topical ocular administration or by IV infusion. The amount of P-321 recovered in urine over 24 hours following IV and ocular administration accounted for only a small fraction of the total dose administered corresponding to 0.57-1.3% and 0.19%, respectively. Ocular administration of P-321 Ophthalmic Solution in dogs given four times per day over multiple days also indicated that very low amounts of P-321 were excreted in the urine and these concentrations of drug were not associated with changes in urine electrolyte excretion, the most sensitive measurement of the effect of ENaC blockers in the kidney.

The ocular distribution and PK following topical ocular administration of P-321 was assessed in Dutch Belted rabbits following a single dose of 0.1% P-321 Ophthalmic Solution or during QID administration for 14 days. The ocular distribution of P-321 was limited only to the external surface of the eye. P-321 had a long terminal half-life in tears of approximately 24 hours. Sustained drug levels were also observed in the palpebral and bulbar conjunctiva and eyelids for up to 48 hours following a single administration. After multiple days of QID dosing, P-321

concentrations in these tissues, increased relative to the levels observed following a single administration, and reached a steady-state level after approximately 5 days of dosing. P-321 had minimal or no penetration into internal regions of the eye such as the retina and aqueous humor, or the main lacrimal glands. Furthermore, the systemic exposure of P-321 following ocular instillation was low, as evidenced by low or non-detectable drug levels in the plasma following a single dose or during 14 days of QID administration.

The toxicokinetics of P-321 were estimated in Good Laboratory Practices (GLP) studies conducted in rabbits and dogs following ocular administration four times per day for 28 days. The systemic exposure of P-321 in rabbits was low with only the highest dose tested (0.5% P-321, QID) exhibiting quantifiable plasma drug levels. P-321 was rapidly absorbed following ocular administration and rapidly cleared from plasma, with mean $t_{1/2}$ values ranging from 0.183 to 0.254 hours on Days 1 and 28, respectively. No accumulation was observed over 28 days of dosing based on plasma drug concentrations. Note, the nominal ocular dose that produced systemic exposure in rabbits is 120-fold larger than the initial dose to be given to the subjects in this study, and 40-fold larger than the high dose planned for this study (0.05%). The systemic exposure in dogs was also low, and only observed with the highest dose tested (0.05% QID) on Day 28. P-321 was rapidly absorbed following ocular administration and rapidly cleared from plasma, with mean $t_{1/2}$ values ranging from 2.28 to 2.68 hours. Note, the nominal ocular dose that produced systemic exposure in dogs is 12-fold larger than the initial dose to be given to the subjects in this study, and 4-fold larger than the high dose planned for this study (0.05%).

In summary, P-321 is metabolically stable. Following IV administration P-321 is rapidly cleared primarily via non-renal mechanisms, and is not orally available. The ocular tissue distribution of P-321 following ocular instillation is essentially limited to its site of action on the ocular surface (tears, palpebral conjunctiva, bulbar conjunctiva, and cornea) with no penetration to internal structures of the eye (aqueous humor, retina) or the main lacrimal glands. Topical ocular administration of P-321 Ophthalmic Solution at pharmacologically active concentrations results in minimal to no systemic exposure and also failed to produce any renal effect. Furthermore, in subjects with dry eye disease treated with 0.01% P-321 BID for 28 days, we were not able to detect any drug present in plasma or urine from these subjects.

2.2.3 Toxicology

Multiple toxicology studies have been conducted using P-321Ophthalmic Solution. Systemic administration of P-321 via IV, oral and ocular routes of administration, in both acute and repeat-dose nonclinical studies, demonstrated that P-321 Ophthalmic Solution at pharmacologically active concentrations is well tolerated. Extensive ocular toxicology studies for up to 28 days of administration at higher frequencies than those used in this study have been conducted in rabbits and dogs, with the dog being the most sensitive species. The primary finding associated with ocular administration of P-321 was a dose-dependent increase of ocular irritation, described as minimal or slight, that reversed upon discontinuation of treatment. No significant effects of P-321 Ophthalmic Solution were observed at any dose level for both species on electroretinograms, corneal thickness, and corneal endothelial cell density. For the dose limiting factor of ocular irritation, the no-observed-adverse-effect levels (NOAEL) of 0.8 mg/eye/day in rabbits and 0.024 mg/eye/day in dogs exceeds the human doses planned for of this study. The NOAEL of 0.05 mg/kg/day in rats administered P-321 IV exceeds the potential exposure from

the proposed initial ocular dose of $2.2661x10^{-4}$ mg/kg/day (assuming bilateral dosing of 0.017% P-321, QD with a 40 µl drop size in a 60 kg person) by 225-fold on a Human Equivalent Dose basis. For the high dose planned in this study, the dose of $6.666x10^{-4}$ mg/Kg/day (assuming bilateral dose of 0.05% P-321 QD in a 60 Kg person, the systemic safety margin is 75-fold on a Human Equivalent Dose basis.

No systemic effects at any dose level in dogs or rabbits were observed following ocular administration of P-321 Ophthalmic Solution.

In a battery of genotoxicity studies, P-321 did not cause mutations in the Ames or mouse lymphoma assay in vitro with or without S9 metabolic activation. Additionally, the compound was negative in a rat micronucleus study. Thus, P-321 can be considered to be non-mutagenic and non-clastogenic, with no evidence of disruption of the mitotic apparatus.

2.3 Clinical Experience with P-321 Ophthalmic Solution

There has been one study completed with P-321 Ophthalmic Solution in which 40 subjects with mild to moderate dry eye disease were treated with P-321 Ophthalmic Solution. In this study, P-321 Ophthalmic Solution was well-tolerated at concentrations of 0.0005%, 0.0015%, 0.005% instilled twice daily for up to 15 days and 0.01% instilled twice daily for up to 28 days. There were no clinically relevant adverse drug-related, dose-related or time of treatment-related effects on any safety measure. No serious adverse events (SAEs) were reported. No evidence of systemic exposure of P-321 was observed.

Although this study was not powered for effects on efficacy measurements, improvements in the frequency and severity of symptoms of dry eye disease that approached statistical significance compared with placebo were observed. In addition, consistent with the proposed mechanism of action of P-321, the treatment difference relative to placebo favored P-321 for the measurements of tear meniscus height (TMH) observed in this study.

2.4 Justification for Route of Administration and Dose Selection

P-321 Ophthalmic Solution is expected to exert its biological activity through direct interaction with ENaC on the corneal and conjunctival surface of the eye. Since the intended route of administration for P-321 is topical ocular, this route of administration will be used in this study. The dose planned for this study was selected based upon results of the first clinical study and preclinical safety studies.

This study is a single day crossover comparing P-321 Ophthalmic Solution and Placebo on the pharmacodynamic measure, tear volume. During Phase 1, a single dose of 0.017% will be administered and during Phase 2 and based on the recommendation of the Data Review Group, a single dose of either 0.05%, 0.017% or 0.01% will be administered. These doses on a single instillation frequency and are covered by the safety profile obtained in the most sensitive species (dog) with safety margins that range from 6-fold to 1.2-fold.

2.5 GCP Compliance

This clinical trial will be conducted in compliance with the protocol, International Conference on Harmonization (ICH) Good Clinical Practice guidelines, and other applicable regulatory requirements.

2.6 Population to be Studied

Dry eye disease is a multifactorial debilitating disease of the ocular surface characterized by ocular signs of corneal and conjunctival impairment and damage of the protective epithelial surface, as well as, decreased tear volume, decreased tear break-up time, and symptoms of discomfort that can range from mild to severe such as, burning, pain, stinging, itching, swelling, foreign body sensation, photophobia, and ocular fatigue. Frequent instillation of artificial tears is currently the most commonly used treatment for mild to moderate dry eye signs and symptoms.

According to recent prevalence estimates, dry eye disease can affect up to 20 million people in the US, with a disproportionate number of women and elderly being afflicted. However, with the spread use of digital technologies in recent years, increased incidence of dry eye in younger adults is being observed. There is a heterogeneous collection of diseases with many shared characteristics that can precipitate dry eye disease. Dry eye disease is one of the hallmarks of the Sjögren's syndrome (SS); however, they represent only a fraction of subjects who experience dry eye symptoms. Dry eye is also observed in approximately 40 to 60% of subjects who have undergone hematopoietic stem cell transplantation and develop graft versus host disease (Saboo, 2015). One core abnormality of the disease is the decrease in tear volume that can be originated by a deficiency of tear secretion or composition which in turn triggers the loss of volume and compromises the protective barrier of the ocular film. Additionally, dry eye symptoms can be the side effect of many commonly used medications such as anticholinergics, antidepressants, and environmental factors such as air conditioning and focusing on video displays for prolonged periods of time. These symptoms of dry eye can vary from mild to severe and in the most severe cases, can result in significant vision impairment and permanent damage of the ocular surface. In this study, subjects with tear deficient dry eye disease will be recruited for participation.

Approximately 24 subjects with tear deficient dry eye disease documented by low tear volume as measured by UHR-OCT will be completed in this single-center, randomized, cross-over, placebo controlled study to evaluate the changes in tear volume by P-321 Ophthalmic Solution or Placebo. The study consists of two Phases. Approximately 8 subjects participating in Phase 1 will receive 0.017% P-321 Ophthalmic Solution or Placebo, and the remainder of the 24 subjects participating in Phase 2 will receive either 0.05%, 0.017% or 0.01%. The decision on the dose administered in Phase 2 will be made following unmasked data review from subjects in Phase 1. In each Phase, subjects will receive sequentially two treatments: P-321 Ophthalmic Solution (Treatment A) and Placebo (Treatment B). Subjects will be randomly assigned to receive treatment sequence AB or BA, with each subject in Phase 1 and Phase 2 being treated with P-321 Ophthalmic Solution and placebo The Principal Investigator, Medical Monitor and Parion Sciences representatives not involved in the day to day conduct of the study will review unmasked data as part of the Data Review Group, which will decide the dose to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur during the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above.

3 TRIAL OBJECTIVES AND PURPOSE

The primary objective of this trial is to assess changes in tear volume by the non-invasive techniques Ultra High Resolution Optical Coherence Tomography (UHR-OCT) following the administration of P-321 Ophthalmic Solution or Placebo in subjects with tear deficient dry eye disease.

The secondary objectives are to:

- assess changes in lower tear meniscus height by Keratograph 5M
- compare lower tear meniscus height measurements between UHR-OCT and Keratograph 5M
- assess the duration of action of P-321 Ophthalmic Solution
- compare the pharmacodynamic activity of different strengths of P-321 in subjects with tear-deficient dry eye
- monitor safety and tolerability of P-321 Ophthalmic Solution

4 INVESTIGATIONAL PLAN

4.1 Overall Study Design

This is a single-center, randomized, cross-over, placebo-controlled, Phase 2a trial designed to evaluate the pharmacodynamic effects of tear volume of P-321 Ophthalmic Solution in tear-deficient dry eye subjects. Approximately 24 eligible subjects will complete the study which consists of two Phases: Approximately 8 subjects participating in Phase 1 will receive 0.017% P-321 ophthalmic Solution and Placebo, and the remainder of the 24 subjects participating in Phase 2 will receive either 0.05%, 0.017% or 0.01% P-321 and placebo. The decision on the dose administered in Phase 2 will be made following unmasked data review from subjects in Phase 1. In each Phase, subjects will receive sequentially two treatments: P-321 Ophthalmic Solution (Treatment A) and Placebo (Treatment B). Subjects will be randomly assigned to receive treatment sequence AB or BA, in a 1:1 ratio.

The pharmacodynamic measures of tear volume will be assessed by two methods: UHR-OCT and Keratograph 5M. Optical coherence tomography (OCT) has been used to image the anterior segment of the eye including tears on the ocular surface. The method is non-invasive and non-contact which is suitable for imaging the tear meniscus around both upper and lower lids. The system at Bascom Palmer Eye Institute is a custom made OCT system (UHR-OCT) with an ultra-high resolution (3 μm). Many previous studies have been done using UHR-OCT and its clinical application has been well documented. With the wide scan of the UHR-OCT, cross-sectional images of the tear menisci can be imaged and tear meniscus volume can be calculated. The Keratograph 5M is a corneal topographer with a built in camera optimized for external imaging. The Keratograph 5M has a feature that enables the non-invasive assessment of the tear meniscus height of the inferior eyelid from high resolution images obtained under infrared light. With the use of a built-in measurement tool the height of the tear meniscus is obtained.

The study will consist of three study visits: a Screening Visit (Visit 1), and two treatment visits (Visit 2 and Visit 3). The duration of the study may be up to 23 days.

At Visit 1, three tear volume measurements will be conducted over 1 hour with measurements taken approximately 30 minutes apart (initial timepoint, and at 30 and 60 minutes later) and prior to any invasive ocular assessments. The average of these three measurements will be obtained and used as baseline tear volume and meniscus height and to determine the eligibility of the subject to participate in the study.

At Visits 2 and 3, tear volume will be assessed serially over 6 hours: Before administration of assigned study medication (time = 0, pretreatment), and at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after study medication administration. Subjects will be asked to arrive to the clinic for Visit 2 and Visit 3 between 8:00AM and 11:00AM and at approximately the same time as Visit 1.

Safety assessments including adverse events (AEs), biomicroscopy and external eye examination and an assessment of comfort immediately after taking the medication will be conducted following each in-clinic dose. Changes in medical conditions and medication, abbreviated physical exam changes and vital signs will be recorded periodically throughout the study. A post

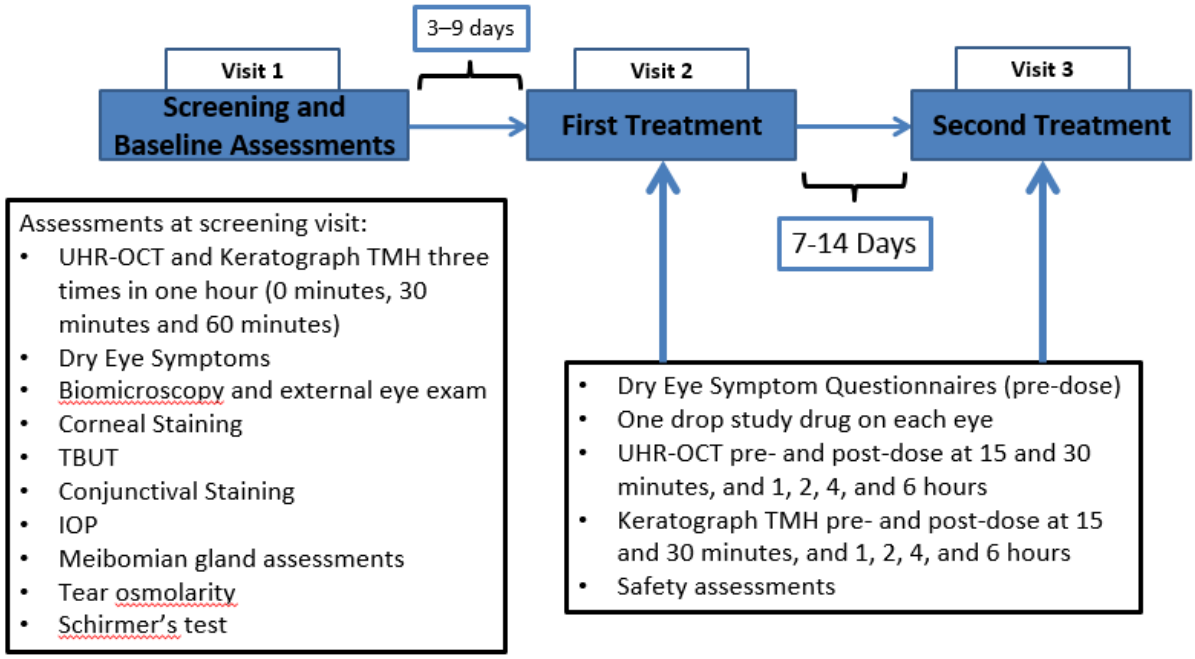
instillation dry eye symptom assessment will be conducted at Visits 2 and 3 immediately prior to the 2 hour post dose time point.

Subjects may be asked to stop or withhold certain medications, and on visit days and for at least 6 hours after dosing with study medication, withhold certain medications and not wear contact lenses on visit days. After the assessments of Visit 3 are completed, the subject will be discharged from the study.

A detailed schedule of the time and events for the study is provided in Appendix I.

A schematic of the study design is shown in Figure 1.

Figure 1: P-321-201 Study Schematic



4.2 Endpoints

4.2.1 Primary Endpoint

The primary endpoint for this study is the change in measurements of tear volume over time as measured by the UHR-OCT following the administration of P-321 Ophthalmic Solution and Placebo in subjects with tear deficient dry eye disease.

4.2.2 Secondary Endpoints

The secondary endpoints are to:

- assess changes in lower tear meniscus height by Keratograph 5M
- compare lower tear meniscus height measurements between UHR-OCT and Keratograph 5M

- assess the duration of action of P-321 Ophthalmic Solution
- compare the pharmacodynamic activity of different strengths of P-321 in subjects with tear-deficient dry eye
- monitor safety and tolerability of P-321 Ophthalmic Solution

4.2.3 Safety and Tolerability Endpoints

Safety endpoints include: AEs, biomicroscopy and external eye examination and comfort assessment (after each in-clinic dose).

4.3 Duration of Participation

The study will consist of three study visits: A Screening Visit (Visit 1), and two Treatment Visits (Visit 2 and Visit 3). The duration of the study may be up to 23 days. The start of each visit will take place between 8:00AM and 11:00 AM and at approximately the same time as Visit 1.

Visit 1 is expected to last approximately 2 hours and Visits 2 and 3 are expected to last approximately 7 hours.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Subject Inclusion Criteria

Subjects must meet the following criteria at Screening to be enrolled in the study:

1. Provide written informed consent
2. Male or female subjects aged 18 to 80 years
3. Are willing and able to follow instructions and can be present for the required study visits for the duration of the study
4. Have a history of predominantly tear-deficient dry eye of mild to moderate severity, in both eyes, supported by a previous clinical diagnosis
5. Subjects must have in at least one eye an average tear meniscus height (TMH) of the lower eye lid of 210 μm or less as assessed by UHR-OCT
6. Have normal lid anatomy
7. Female subjects of child bearing potential must have a negative serum pregnancy test at Screening and agree to use a medically acceptable form of birth control (e.g., intrauterine device, birth control pill, patch or subcutaneous implant, condoms, diaphragm, or abstinence) throughout the duration of the study. Females who are breast feeding an infant are not eligible to participate in the study.
8. Subjects must:
    a. Remain on current medications for the duration of the study
    b. Be on the current medication regimen at least during the past 28 days
    c. Be able to withhold ocular topical medications, including artificial tears, topical steroids, Restasis®, Xiidra®, autologous serum, and lid wipes and scrubs on visit days while in the clinic, and for at least 6 hours after dosing with study medication
    d. If they are contact lens wearers, be able to withhold wearing contact lenses on visit days, while in the clinic, and for at least 6 hours after dosing

5.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria at Screening or during the study will be excluded from the study.

1. Have an identifiable or suspected dry eye caused by pharmacologic, post-traumatic, or post-surgical condition
2. Have undergone refractive eye surgery (e.g., LASIK) in either eye during the past 12 months
3. Have undergone uncomplicated cataract surgery in either eye during the past 3 months
4. Have undergone previous eyelid surgery in either eye (e.g., blepharoplasty, ptosis repair). External blepharoplasty, not resuting in exposure or abnormal blinking, will be allowed.
5. Have lid irregularities or deformities
6. Have severe corneal surface irregularities

7. Have undergone botulinum toxin (Botox™ or equivalent) injection in the periocular area within 3 months prior to Visit 1
8. Have a history of glaucoma or IOP > 25 mmHg at the Screening Visit (Visit 1) or a history of elevated IOP within the past year
9. Subjects that have a systemic, multi-organ disease (with the exception of subjects with SS or GVHD) requiring active medical or surgical treatment are excluded, **except** if the following conditions are met:
    a. the condition is chronic (> 1 years' duration), stable, of mild severity and adequately controlled
    b. the condition does not or is unlikely to have ocular manifestations
    c. the condition is one of the following:
        i. essential hypertension
        ii. coronary artery disease
        iii. Type II diabetes mellitus without diabetic retinopathy
        iv. thyroid dysfunction without thyroid eye disease
        v. non-morbid obesity
        vi. remote history of cancer (> 5 years from diagnosis)
10. Have punctal plugs, punctal occlusion, or history of nasolacrimal duct obstruction or wear scleral lenses,
11. Stevens-Johnson syndrome, ocular cicatricial pemphigoid, or other ocular cicatrizing disorders
12. Past or present exposure keratopathy, neurotrophic keratopathy, lagophthalmos, or trichiasis
13. Subjects taking the following medications within 14 days of Visit 1 or during the study are excluded
    a. Oral, nasal and ocular antihistamines
    b. Oral or topical secretagogues such as pilocarpine and Evoxac
    c. Topical anti-glaucoma medications
14. History of allergies to any of the components of the study medication
15. Are pregnant or breast feeding an infant.
16. Have any significant illness that, in the opinion of the Principal Investigator (PI), could interfere with the study parameters
17. Use of any investigational product or device within 28 days prior to the Screening Visit or during the study
18. Are unable in the opinion of the PI to comply fully with the study requirements or to complete the study

5.3 Randomization Criteria

At Visit 2 (First Treatment Visit) and Visit 3 (Second Treatment Visit), an eligible subject must:

1. Continue to meet all clinical inclusion/exclusion criteria as defined in Section 5.1 and Section 5.2, with the exception of the inclusion criteria #5 (UHR-OCT measurement) .

A log will be maintained at the sites for those subjects that do not meet eligibility criteria. Minimally this log will include the subject's age, sex and race and the reason that they were not eligible. Screen failure data will not be collected in the clinical database.

5.4 Study Eye

The Study eye will be the eye that meets entry criteria as defined in Section 5.1, Section 5.2, and Section 5.3. If both eyes meet entry criteria, the study eye will be the eye with the lower average TMH by OCT. If both eyes have the same average TMH, then the qualifying sStudy eye will be the **right eye**.

5.5 Subject Withdrawal Criteria and Stopping Rules

Treatment may be discontinued and the subject withdrawn at any time during the study at the discretion of the investigator, Medical Monitor or Parion Sciences for any reason including but not limited to occurrence of an AE which precludes subsequent participation, withdrawal of Informed Consent, or requirement of an ocular surgery or intervention. Also in the event that a woman becomes pregnant while participating on the study, she will be withdrawn from the study. In the event that discontinuation of treatment is necessary, the investigator will make every attempt to complete all subsequent safety assessments and the Early Termination Visit. The reason for premature discontinuation should be entered onto the paper case report form (CRF). Subjects who withdraw from the study will be replaced.

Additionally, the trial or parts of the trial may be discontinued by Parion Sciences, Inc. or at the recommendation of the Investigator or Medical Monitor after consultation with Parion. This may be based on a significant number of AEs of a similar nature that warrant such action or at the request of Parion Sciences, Inc.

6 PROCEDURES

The procedures performed for this study are listed below. The times indicated in the following sections and Appendices are based on the visit starting in the AM with each visit starting at a consistent timeframe relative to Visit 1.

6.1 Visit 1: Screening (3 to 9 days prior to Visit 2 – Randomization)

The subject should initiate screening procedures within 14 days of signing the ICF. Prior to conducting study procedures, informed consent will be obtained.

The following procedures will be completed at Visit 1 which will start between 8:00AM and 11:00AM:

- Review eligbility criteria
- Review of the medical history
- Review concomitant medication history in the past 28 days
- Administer ocular symptom questionnaires (SPEED, OSDI, and SANDE Part 1 only) before other procedures
- Biomicroscopy and external eye examination
- Visual acuity
- Abbreviated physical exam (including respiratory, cardiovascular, musculoskeletal, gastrointestinal, dermatological)
- Vital signs (pulse, blood pressure, temperature and respiration rate) and height and weight
- Conduct pretreatment measurements in both eyes by UHR-OCT and Keratograph 5M three times in one hour (at 0 minutes, 30 ± 10 minutes and 60 ± 20 minutes). NOTE: throughout the study, UHR-OCT will be conducted first, immediately followed by Keratograph 5M.
- Tear osmolarity
- TBUT
- Assessment of meibomian glands
- Corneal staining
- Conjunctival staining
- Schirmer's test
- IOP
- Collect blood for hematology, chemistry and pregnancy tests

Monitoring of adverse events will begin from the time the Informed consent is signed

Eligible subjects will be instructed to return to the clinic between 3 and 9 days for the first treatment (Visit 2, First Treatment Visit) with the visit occurring in the AM, at approximately the same time of day as Visit 1. Subjects will be reminded not to use ocular medications or wear contact lenses on the day of Visit 2.

6.2 Visit 2: First Treatment Visit (3 -9 days after Visit 1)

The following procedures will be completed at Visit 2 which will take place between 8:00AM and 11:00 AM and at approximately the same time as Visit 1:

- Subjects will be asked to remain on site through this visit and limit use of devices with video display screens.
- Confirm continued eligibility through review of inclusion and exclusion criteria as outlined in Sections 5.1 and 5.2 of the protocol with the exception of the inclusion criteria #5 (UHR-OCT dry eye).
- Review of concomitant medications and medical changes since last visit
- Administer symptom questionnaires (SPEED, OSDI, and SANDE Part 1 and Part 2) before other procedures and before dosing
- Assess adverse events since the last visit and during this visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate) pre-treatment
- Conduct pretreatment measurements in both eyes by UHR-OCT and Keratograph 5M (at approximately the same time as conducted at Visit 1) immediately prior the administration of study medication. UHR-OCT will be conducted first, immediately followed by Keratograph 5M.
- Subjects will be assigned a treatment number at this visit.
- Administer one drop of assigned study medication in each eye immediately after the pretreatment assessment (the drug administration will be conducted by the PI or clinical site personnel). NOTE: Remove study medication from refrigerator at least 15 minutes prior to administration.
- At approximately 5 minutes after drug administration, administer drop instillation comfort assessment
- Repeat assessments by UHR-OCT and Keratograph 5M post dose at 15 ± 5 minutes, 30 ± 10 minutes minutes, 1 hour ± 20 minutes, 2 hours ± 30 minutes, 4 hours ± 30 minutes, and 6 hours ± 30 minutes.
- Immediately prior to the 2 hour time point for tear volume measurement, a post instillation dry eye symptom assessment will be completed
- After the 6-hour tear volume assessment, conduct a biomicroscopy and external examination
- At the end of the visit, the subject will be instructed to return to the clinic between 7 and 14 days for the second treatment (Visit 3, Second Treatment Visit) with the visit occurring in the AM, at approximately the same time of day as Visit 1 and Visit 2
- The subjects will be instructed to maintain the same medications and study restrictions between visits to continue being eligible to remain in the study. Subjects will be reminded not to use ocular medications or wear contact lenses on the day of Visit 3

6.3 Visit 3: Second Treatment Visit (7-14 days after Visit 2)

- Subjects will be asked to remain on site through this visit and limit use of devices with video display screens.

- Confirm continued eligibility through review of inclusion and exclusion criteria as outlined in Sections 5.1 and 5.2 of the protocol with the exception of the inclusion criteria #5 (UHR-OCT dry eye).
- Review of concomitant medications and medical changes since last visit
- Administer symptom questionnaires (SPEED, OSDI, and SANDE Part 1 and Part 2) before other procedures and before dosing
- Assess adverse events since the last visit and during this visit
- Collect vital signs pre-treatment (pulse, blood pressure, temperature and respiration rate)
- Conduct pretreatment measurements in both eyes by UHR-OCT and Keratograph 5M in the AM (at approximately the same time as conducted at Visit 1) immediately prior the administration of study medication. NOTE: throughout the study, UHR-OCT will be conducted first, immediately followed by Keratograph 5M.
- Administer one drop of assigned study medication in each eye immediately after the pretreatment assessment (the drug administration will be conducted by the PI or clinical site personnel). NOTE: Remove study medication from refrigerator at least 15 minutes prior to administration.
- At approximately 5 minutes after drug administration, administer drop instillation comfort assessment
- Repeat assessments by UHR-OCT and Keratograph 5M post dose at 15 ± 5 minutes, 30 ± 10 minutes minutes, 1 hour ± 20 minutes, 2 hours ± 30 minutes, 4 hours ± 30 minutes, and 6 hours ± 30 minutes.
- Immediately prior to the 2 hour time point for tear volume measurement, a post instillation dry eye symptoms assessment will be completed
- After the 6-hour tear volume assessment, conduct a biomicroscopy and external eye examination
- At this point, the subject will be discharged from the study.
- Subjects may resume previously used ocular medications when discharged from the study.

6.4 Early Termination Visit

The following assessments will be performed at the Early Termination Visit for randomized subjects who are withdrawn from the study prematurely:

- Assess adverse events since the last visit and during this visit
- Assess concomitant medications and medical changes since last visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate) and height and weight
- Conduct an abbreviated physical examination (including respiratory, cardiovascular, musculoskeletal, gastrointestinal, and dermatological)
- Conduct a biomicroscopy and external eye examination

6.5 Selecting Dosage Strength in Phase 2

The decision of dose strength for P-321 Opthalmic Solution in Phase 2 of the study will be the responsibility of the Data Review Group constituted by the Prinicipal Investigator (or a designee not directly involved in the conduct of the study), the Medical Monitor and representatives of Parion Sciences. Decision on the dose level to be given in Phase 2 will be made based on unmasked review of the study data from the first 8 subjects by the review committee. Data to be considered will include safety and efficacy measures. A detailed criterion for Dose selection for Phase 2 is described in Appendix XIV and outlined below.

The dose level may be increased to 0.05% in Phase 2 in the event that:

1) No cases of serious, suspected related, unexpected events are noted in Phase 1 OR
2) No cases of serious ocular safety events are noted in Phase 1
3) Lack of evidence that P-321 produced an increase in tear volume based on OCT and/or Keratograph 5M, or evidence that additional improvements in tear volume (or the duration of effect) may be obtained with a higher dose.

The dose level may be decreased to 0.01% in Phase 2 in the event that:

1) No cases of serious, suspected related, unexpected events are noted in Phase 1
2) No cases of serious ocular safety events are noted in Phase 1
3) There is evidence of a significant increase in tear volume and the duration of effect based on OCT and/or Keratograph 5M

The dose level may remain at 0.017% (the same as in Phase 1) in the event:

1) No cases of serious, suspected related, unexpected events are noted in Phase 1
2) No cases of serious ocular safety events are noted in Phase 1
3) There is not compelling data to either increase or decrease the strength for Phase 2.

The Data Review Group will make a recommendation to Parion Sciences to increase the dose, decrease the dose or maintain the dose.

Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above.

6.6 Potential Toxicity Management

All preclinical evidence indicates that systemic exposure of P-321 via ocular topical administration is none or negligible at concentrations up to 10-fold higher than the concentration planned in this study (the estimated systemic safety margins for the doses anticipated in this study range from 75- to 375-fold). However, since P-321 is an ENaC inhibitor, a previous potential safety signal of concern following the administration of P-321, was drug-induced hyperkalemia. This possibility was carefully investigated in Study P-321-101. No changes in plasma or serum potassium were noted in this study in subjects administered 0.01% P-321 Ophthalmic Solution BID for up to 28 days.

No concerns for systemic toxicity in this single-dose administration study are based on the lack of safety findings from the P-321-101 study when treatment with P-321 was administered BID for up to 28 days. In addition, in the same study, the pharmacokinetic results demonstrated a lack of systemic availability of 0.01% P-321 Ophthalmic Solution given twice daily for 28 days. In this study, treatment with P-321 Ophthalmic Solution at doses up to 0.05% will be given only **once** to each participating subject. Thus, no post dose monitoring of serum potassium is planned for this trial.

Any additional questions regarding toxicity management should be managed by appropriate medical professional at the clinical site and directed to Parion Sciences Medical Monitor.

6.7 Collection of Data

Source documentation for data collected in the study will be maintained at the investigative site. In cases where no source documents will be used (i.e., data will be recorded directly onto the CRF without first being recorded on another document, such as the subject symptom questionnaires, for which data will be recorded directly on the CRF pages by the subject), the original data will be included in the CRF and this will be noted in the investigator files.

7 TREATMENT OF SUBJECTS

7.1 Treatment Administration

Study medication will be administered by the PI or clinical site personnel at Visits 2 and 3. At Visit 2, eligible subjects will be randomized (1:1 ratio) to receive one of two treatment sequences: P-321 Ophthalmic Solution at Visit 2, and Placebo at Visit 3, or Placebo at Visit 2 and P-321 at Visit 3. The order of the treatment is randomized, neither the subjects nor site personnel will know which treatment the subject receives at which visit. Assessments will be conducted before and at the indicated times after study medication administration in the indicated order in Section 6.

7.2 Trial Treatments

Subjects will receive P-321 Opthalmic Solution or Placebo in random order at Visit 2 and 3. The study treatment order will be determined by the assignment of the randomization code. P-321 Ophthalmic Solution and Placebo.

P-321 Ophthalmic Solution is a sterile, aqueous, non-preserved solution of approximately pH 5, and tonicity of approximately 290 mOsm/kg. Placebo solution has identical composition without the active ingredient. Both solutions are packaged in low density, polyethylene dropper bottles. Each bottle of study medication will contain sufficient volume of ophthalmic solution for the intended dosing (1 drop in each eye). Only one drop should be instilled in each eye.

For Phase 1, one pouch of study medication will be used at each clinical visit, the label on the pouch will indicate the visit number at which the drug will be administered (Visit 2 or Visit 3). The labels on the kit and the pouches will minimally contain the following information: Study ID, kit number, storage temperature, and "Caution: New Drug – Limited by Federal Law to Investigational use". The study medications will be stored at approximately 2-8°C in a secure area with limited access to study personnel.

For Phase 2, the dose selected of P-321 Ophthalmic Solution and Placebo will be provided by a compounding pharmacy in individual vials similar to those of the Phase 1 study, based on a randomization code. No medication kits will be prepared for Phase 2. The pharmacy will be unmasked to distribute according to the randomization scheme. The study medications will be stored at approximately 2-8°C in a secure area with limited access to study personnel and should be labeled and "Caution: New Drug – Limited by Federal Law to Investigational use". Pharmacy records should include Study ID, randomization number, visit number, date, and subject identifiers.

The medication will be administered by the PI or clinical site personnel at each of Visit 2 and Visit 3. There will be no study medication administered at an Early Termination Visit.

7.3 Methods to Minimize Bias

To minimize bias, the treatment regimen will be randomized and masked. The randomization code will be generated by an independent designee of Parion Sciences, Inc. who is not involved in the day-to-day conduct of the clinical study. The randomization code will be provided to the designee of Parion Sciences, Inc. who is responsible for the manufacture, packaging and labeling

of the clinical supplies; further the randomization code will be maintained in a secure location separate from the clinical study site personnel.

The Medical Monitor and Principal Investigator and members of the Parion team not involved in the conduct of the study will be unmasked as part of the Data Review Group for decision making on the strength of P-321 Opthalmic Solution to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined in Section 6.5.

7.4 Concomitant Medications

7.4.1 Medications Permitted

All medications including prescription, over-the-counter, and natural remedies, that the subject has taken within 28 days prior to Visit 1 or during the study will be recorded in the CRF and in the subjects' source documents. The name of the drug, dose, route of administration, duration of treatment (including start and stop dates), frequency, and indication will be recorded for each medication. The use of fluorescein and lissamine green do not need to be recorded on the concomitant medications page.

Subjects should use medications consistently throughout the study and for 28 days prior to Visit 1 with the exceptions outlined in Section 7.4.2.

7.4.2 Medications Not Permitted

Use of the following products on visit days, while in the clinic, and for at least 6 hours after dosing will not be allowed (use of these all other times during the study is permitted):

- Topical ocular medications including artificial tears, topical steroids, Restasis®, Xiidra®, autologous serum, and lid wipes and scrubs.
- Contact lens wear

Use of the following medications during the study will NOT be allowed within 14 days of Visit 1 or during the study:

- Oral, nasal and ocular antihistamines
- Oral or topical secretagogues such as pilocarpine or Evoxac
- Topical anti-glaucoma medications

Use of the following medications during the study or within 28 days prior to Visit 1 will NOT be allowed:

- Investigational product or device

Use of the following medications during the study or within 3 months prior to Visit 1 will NOT be allowed:

- Botulinum toxin (Botox® or equivalent) injection in the periocular region

7.5 Treatment Compliance

Because this is a single day crossover, with study medication administered by the PI or clinical site personnel, treatment compliance will not be calculated. Time of administration of study medication and kit number will be captured in the CRF.

7.6 Drug Accountability

Study medication accountability will occur during monitoring visits by Parion Sciences, Inc, or its designee. Accountability will be ascertained by performing reconciliation between numbers of Kits/bottles of study medication received on site/compounded for distribution, number of bottles dispensed to subjects according to the protocol-specified dosing regimen, and the remaining unused study medication at the time of reconciliation.

Clinical trial materials and bulk supplies will be shipped to the investigational site/pharmacy under sealed conditions. Study medication shipment records will be verified by comparing actual quantity of drug received against the shipment inventory sheet accompanying the drug received at the site. Accurate records of receipt and disposition of the study medications (e.g., dates, quantity, subject number, dose dispensed, returned, etc.) must be maintained by the investigator or his/her designee. Study medication will be stored at the site in an area free of environmental extremes, at controlled temperatures between 2 – 8°C (36 - 46°F). This area should be limited and have controlled access.

At the end of the study, all study materials, including unused study medication bottles and bulk supplies will be returned to Parion Sciences, Inc. (or its designee) for disposal. The study monitor or designee should verify drug accountability at routine monitoring visits.

7.7 Maintenance of Randomization and Procedure for Breaking the Code

The treatment assignments will be masked to the investigator, medical monitor, study site personnel, subjects in the study and those involved in the conduct of the study.

Only in the case of a medical emergency, or occurrence of a suspected related, unexpected, SAE, may the randomization code be unmasked and made available to the investigator, medical monitor, subject, and/or other personnel involved in the monitoring or conduct of this study. In the absence of medical need, the randomization code will not be available to the above personnel until after the study is completed and the database is locked.

The Data Review Group will review unmasked data. The Medical Monitor and Principal Investigator (or designee) and members of the Parion team not involved in the conduct of the study will be unmasked as part of the Data Review Group for decision making on the strength of P-321 Opthalmic Solution to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined in Section 6.5.

For Phase 1, a a randomization list will be generated and the kits will be labeled in a masked fashion. The randomization assignments will be kept in a secure area at Parion Sciences, Inc. or designee. For Phase 2, a randomization list will be provided to the pharmacy and the pharmacy will distribute the assigned drug according to the randomization schedule with subjects remaining masked.

In case of a medical need, the investigator will treat the subject as needed regardless of randomized treatment. Since there is no specific antidote to P-321 Ophthalmic Solution, immediate emergency unmasking is not necessary. If the investigator feels it is necessary to unmask a subject's treatment assignment after an emergency situation, the investigator must call the medical monitor and notify Parion Sciences, Inc. The treatment assignment will be revealed on a subject-by-subject basis, leaving the masking of the remaining subjects intact.

8 STATISTICS

8.1 Statistical Methods

8.1.1 Subject Disposition, Demographic and Background Characteristics

Subject disposition, demographic, and background characteristics will be summarized by using descriptive statistics. Baseline homogeneity with respect to demographic and background characteristics will be assessed via an overall F-test from analysis of variance (ANOVA). For categorical variables, treatment differences will be assessed using the chi-square test or Cochran Mantel Haenszel (CMH) test with modified ridit scores for ordered categorical variables.

Background characteristics include: demographics, tear volume measurements by UHR-OCT and Keratograph 5M, visual acuity, symptoms upon entry, medical history, concomitant medication use, tear osmolarity, tear break up time, meibomian gland assessments, corneal and conjunctival staining, Schirmer and IOP.

8.1.2 Pharmacodynamic parameters

UHR-OCT will assess the following parameters in both eyes: UTMH, LTMH, UTMV, LTMV, and TTMV (UTMV + LTMV). Treatment group differences for tear volume assessments will be conducted via comparisons of tear meniscus height, and if applicable, tear meniscus volume (TMV). These parameters will be compared between each dose of P-321 Ophthalmic Solution and Placebo using a t-test for study eyes, and for all eligible eyes. Analysis of covariance (ANCOVA) models will be used to adjust for the baseline TMH and TMV for study eyes. For all eligible eyes, a generalized estimation equations (GEE) will also be used to account for the correlation between eyes. For the GEE analysis, an exchangeable correlation matrix will be assumedArea under the curve (AUC) analysis will also be performed as an exploratory analysis to compare OCT assessments and Keratograph 5M LTMH for Placebo vs each dose of P-321.

8.1.3 Analysis of Efficacy

This is a pharmacodynamic and tolerability study of a single administration of P-321 Ophthalmic Solution or Placebo, and therefore, no primary or secondary efficacy endpoints will be formally assessed.

8.1.4 Analysis of Safety

All safety data collected on or after the date that study medication was first dispensed up through the date of the final post dose assessment will be summarized.

The objective of the statistical analysis of the safety parameters is to investigate the data for any effects of study medication on clinical tolerability. All such parameters will be summarized by treatment group and time point as indicated below. Because this study, is a cross over design and, at the cross over treatment, the subjects may no longer be treatment naive, analysis will check for sequence/period effects. If sequence/period effects are detected, the analysis of safety will use just the first period results. Provided that no sequence/period effects are seen, analysis will use the most-recent treatment received as the treatment group.

In the absence of any predefined hypotheses, the general strategy of the safety analysis will be to examine the data summaries for any trends amongst the treatment groups. No formal hypothesis testing will be carried out.

The incidence of AEs will be tabulated by treatment group overall, by treatment sequence, by severity, and relationship to study medication.

Change from baseline values (or shifts from baseline, if more appropriate) will be presented for other safety measures for the visits at which they are assessed.

8.1.5 Interim Reviews of Data

A Data Review Group will review unmasked data when approximately 8 subjects have completed Phase 1. The Data Review Group will include the Prinicpal Investigator (or designee), the Medical Monitor and representative of Parion Sciences not involved in the conduct of the study. Other study site personnel, subjects in the study and those involved in the conduct of the study will remain masked. Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined in Section 6.5.

8.2 Sample Size Estimation

An initial sample size of approximately 24 subjects is proposed for this study. The study design includes OCT measurements at time 0 (pretreatment) and post dose at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours. Shen et al. (Shen, 2009) reported that among the measures of tear volume and tear film thickness obtainable through OCT, the most sensitive and specific measure of dry eye is the low tear meniscus height (LTMH). Power estimations for a two-period, two-treatment, two-sequence crossover design (2x2x2),this study size when all subjects treated with P-321are analyzed, will provide 98% power to detect a difference of 60 µm at 1 hour or a power of 99% if the average increase in LTMH over the six hours is 60 µm (assuming within-subject SD of 71). In this study it is expected that the drug will reach its peak effect at 1 hour post dose and that the effect diminishes at hours 4 and 6 (to values still above pre-dose values).

8.3 Level of Significance

Each hypothesis will be tested at a significance level of 0.05.

8.4 Criteria for Termination of an Individual Subject

An individual subject may be terminated from the trial for any reason including, but not limited to the following:

- Subject withdrawing consent to continue in the trial
- Occurrence of an AE which precludes subsequent participation
- Requirement of an ocular surgery or intervention that is exclusionary for the trial
- Physician discretion based on the ability of the subject to continue schedule or treatment regimens according to protocol, or for the safety of the subject
- In the event that a woman becomes pregnant while participating on the study, she will be withdrawn from the study

8.5 Criteria for Termination of the Trial

The trial may be discontinued by Parion Sciences, Inc. or at the recommendation of the principal investigator after consultation with Parion. This may be based on a significant number of AEs of a similar nature that warrant such action, or at the request of Parion Sciences, Inc. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies) and Institutional Review Board (IRB). In terminating the study, Parion Sciences and the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

8.6 Procedure for Accounting for Missing, Unused, or Spurious Data

All analyses will be based on observed data only. No attempts will be made to impute or estimate missing data.

8.7 Procedure for Reporting Deviations from the Statistical Plan

Any deviations from the statistical plan will be described and a justification given in the final clinical report.

8.8 Subjects to be Included in the Analysis

All safety analyses will include all subjects who were randomized and received at least one dose of study medication.

9 ASSESSMENT OF PHARMACODYNAMICS

Detailed measurements of tear volume before and after dose administration will be obtained using UHR-OCT (tear volume and tear meniscus height) and Keratograph 5M (tear meniscus height). Procedures for these measurements are located in Appendix X.

10 ASSESSMENT OF SAFETY

10.1 Safety Parameters

Subject safety will be evaluated by a medical professional during the study. Tolerance of study medication as well as general subject well-being will be assessed. Subjects may be discontinued from the study at any time by the investigator if this action is considered in the subject's best interest.

Post dose safety parameters are measured as described in Appendix I and include:

- Assessment of adverse events (Appendix II)
- Biomicroscopy and external eye examination (Appendix IV)
- Assessment of comfort in taking the medication will be conducted (Appendix XI)

10.2 Procedures for Adverse Events Reporting

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, which does not necessarily have to have a causal relationship with this treatment. AEs will be documented upon signing the informed consent. A treatment emergent AE (TEAE) is an AE that either commenced following initiation of study treatment or was present prior to study treatment but increased in frequency or severity following initiation of study treatment.

A non-serious AE is any AE that does not meet the definitions for SAEs as described in Section 10.3.

AEs that occur prior to subject randomization at Visit 2 will be documented in source documents. TEAEs will be monitored throughout the study and will be recorded on the CRF with the date and time of onset, date and time of resolution, intensity, seriousness, causality (relationship to study medication), treatment required and the outcome.

If an AE occurs, the investigator will institute support and/or treatment as deemed appropriate. If a non-serious AE is unresolved at the time of the last visit, a reasonable effort will be made to follow up until the AE is resolved or stabilized, the subject is lost to follow-up, or there is some other resolution of the event.

To elicit AEs simple questions with minimal connotations should be used as the initial questions at all evaluation points during the trial. For example:

- How have you felt since your last assessment?
- Have you had any health problems since your last assessment?

10.3 Serious Adverse Event Reporting

An SAE is any untoward medical occurrence occurring on this trial that results in any of the following outcomes:

- Death

- A life-threatening adverse drug experience (i.e., the subject is at immediate risk of death at the time of the event)
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

The Investigator or other study personnel must immediately inform Parion Sciences or designee by phone or email of any AE considered serious or otherwise significant, as described above. **In addition, a completed SAE report form must be submitted to Parion within 24 hours of initial awareness of the event.**

SAE Reporting
Dedicated Safety Email Address: safety@parion.com

It is the responsibility of the investigator or their designee to report any event of this nature to Parion Sciences, Inc. within 24 hours of the event being brought to the investigator's or their staffs' attention. It is also the responsibility of the investigator to report all SAEs to their IRB according to their requirements and provide a copy of this notification to Parion Sciences. The investigator should make every attempt to follow all SAEs to resolution.

Information to submit when reporting an SAE to Parion Sciences, Inc. is located in Appendix II.

10.4 Procedures for Reporting Pregnancy

Any subject found to be pregnant at any time during the study will be withdrawn from the study immediately. Any pregnancy outcome that meets the criteria for an SAE will be reported as an SAE. All pregnancies will be reported to Parion Sciences within 24 hours of the event being brought to the investigator's or their staffs' attention. It is also the responsibility of the investigator to report all pregnanciess to their IRB according to their requirements and provide a copy of this notification to Parion Sciences. The investigator should make every attempt to follow all pregnancies to resolution (i.e., birth or voluntary or spontaneous termination of the pregnancy).

11 DIRECT ACCESS TO SOURCE DATA and DOCUMENTS

The principal investigator will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to source data and documents.

12 QUALITY CONTROL AND QUALITY ASSURANCE

The progress of the study will be monitored by on-site, written, e-mail and telephone communications between personnel at the study center and Parion Sciences, Inc. (or designated monitor). The investigator will allow Parion Sciences, Inc. clinical monitors, designees, auditors, and regulatory authorities to inspect all CRFs; subject records (source documents), signed informed consent forms; records of study medication receipt, storage, and dispensation; and regulatory files related to this study.

At the time of database lock, the clinical database will be audited in order to ensure accuracy of all data as well as to provide an estimated error rate for the final, locked database. The results of the audit will be provided in the final study report. The audit will involve a comparison of CRF values with values from data listings generated from the clinical database. Values of all variables will be confirmed for the five randomly selected subjects. If the database fails to meet an accuracy rate of 99.5% (less than 1 error / 200 fields), a second set of 5 subjects will be selected, If a combined sample fails to meet an accuracy rate of 99.5%, the remainder of the database will be rechecked or take other action taken agreed with by Parion.

13 ETHICS

This protocol is written in accordance with the principles established by the 18th World Medical Assembly General Assembly (Helsinki, 1964) and amendments and clarifications adopted by subsequent General Assemblies. The investigator will make sure that the study described in this protocol is conducted in full conformance with those principles, the protocol, current FDA regulations, ICH Good Clinical Practices (GCP) guidelines, Good Laboratory Practices (GLP) guidelines, local ethical and regulatory requirements, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (CFR) (title 21), any Ethics Committee (EC) requirements relative to clinical studies. As required by the US FDA, the study drug may not be shipped to any participating investigator until the requisite study documentation has been submitted to the IND.

13.1 Institutional Review Board

The EC/IRB must be a properly constituted board or committee operating in accordance with 21 CFR Part 56, "Institutional Review Boards." This protocol, any protocol amendments and the associated informed consent forms and translations must be submitted to the EC/IRB for review and approved before the enrollment of any subject into the trial.

All subject recruitment materials and advertising information, including translated versions of these documents, must be submitted to Parion or its designee and to the EC/IRB for review and approval prior to implementation. EC/IRB approval of any protocol amendments must be received before any of the changes outlined in the amendments are put into effect, except when the amendment has been enacted to eliminate a potential hazard to study subjects. In such cases, the chair of the EC/IRB should be notified immediately and the amendment forwarded to the EC/IRB for review and approval.

In addition, the Clinical Investigator Brochure should be submitted to the IRB.

Written IRB approval must adequately identify the materials approved. Copies of all approved materials, all correspondence with the IRB, and written approval from the IRB must be made available to Parion Sciences, Inc. (or designated monitor) *prior* to shipment of study medication and the start of subject enrollment into the study.

SAEs will be reported to the IRB by the principal investigator as required by the IRB.

13.2 Informed Consent Requirements

Written informed consent will be obtained from each subject prior to any study related procedures being performed. A copy of the signed and dated consent document will be given to each subject. The original signed and dated informed consent document must be maintained in the study files at the investigative site and be available for Parion Sciences, Inc. or designee, and regulatory authorities for review.

Each informed consent form will contain contact information with a telephone number the subject should contact if they have medical concerns 24-hours a day.

13.3 Data Handling and Record Keeping

All procedures for the handling and analysis of data will be conducted using good clinical practices meeting ICH and U.S. Food and Drug Administration (FDA) guidelines for the handling and analysis of data for clinical trials.

13.4 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks and manual data reviews will be implemented and applied to the database. Query reports pertaining to data omissions and discrepancies will be forwarded to the clinical investigator and monitor(s) for resolution. The study database will be updated in accordance with the resolved query reports. All changes to the study database will be documented.

CRFs will be provided by Parion Sciences, Inc. All data relating to the trial must be recorded in the CRFs. The Investigator must verify that all data entries in the CRFs are accurate and complete. The CRF must be signed and dated by the Investigator upon subject withdrawal or completion of the study.

During monitoring visits, CRFs will be reviewed by study monitor(s) for completeness, accuracy, and legibility. CRFs will be compared with the source documents to ensure that there are no discrepancies. All CRF entries, corrections, and alterations must be made by an Investigator or his/her designee. Corrections must be made in such a way that the original entry is not obliterated. Correction fluid must NOT be used. The correct data must be inserted, dated and initialed by the Investigator or his/her designee. The study monitor is not allowed to make entries on the CRF pages.

The original CRF page will be retrieved by the monitor and returned to Parion Sciences, Inc. after complete review of the case. A copy must be maintained by the Investigator. If documented corrections to a CRF are needed after removal of the original CRF copy from the center, a Data Correction Form (DCF) will be used. The Investigator or a staff member must verify, sign, and date each DCF.

A log will be maintained at the sites for those subjects that do not meet eligibility criteria. Minimally this log will include the subject's age, sex and race and the reason that they were not eligible. Screen failure data will not be collected in the clinical database.

13.5 Records Retention

The study center will retain all records related to the study in compliance with ICH Good Clinical Practices Guidelines.

13.6 Publication Policy

The institution and investigators participating in this trial shall have no rights to publish, disclose or present the results of this study without prior written consent of Parion Sciences, Inc.

14 REFERENCES

Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the Meibomian glands in a normal population. Ophthalmology. 2008;115:911–915.

Barker, P. M., Nguyen, M. S., Gatzy, J. T., Grubb, B., Norman, H., Hummler, E., Koller, B. (1998). Role of gammaENaC subunit in lung liquid clearance and electrolyte balance in newborn mice. Insights into perinatal adaptation and pseudohypoaldosteronism. The Journal of Clinical Investigation, 102(8), 1634–40. doi:10.1172/JCI3971.

Foulks G, Bron AJ. A clinical description of meibomian gland dysfunction. Ocul Surf. 2003;1:107–126.

Fujihara, T., Murakami, T., Fujita, H., Nakamura, M., Nakata, K. (2001). Improvement of corneal barrier function by the P2Y(2) agonist INS365 in a rat dry eye model. Investigative Ophthalmology & Visual Science, 42(1), 96–100.

Korb D.R., Herman J.P., Greiner J.V., Scaffidi R.C., Finnemore V.M., Exford J.M., Blackie C.A., Douglass T. (2005) Lid Wiper Epitheliopathy and Dry Eye Symptoms. Eye and Contact Lens, 31(1), 2-8.

Krueger, B., Schlötzer-Schrehardt, U., Haerteis, S., Zenkel, M., Chankiewitz, V. E., Amann, K. U., Korbmacher, C. (2012). Four subunits (αβγδ) of the epithelial sodium channel (ENaC) are expressed in the human eye in various locations. Investigative Ophthalmology & Visual Science, 53(2), 596–604. doi:10.1167/iovs.11-8581.

Lemp, M. A. (1995). Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. The CLAO Journal : Official Publication of the Contact Lens Association of Ophthalmologists, Inc, 21(4), 221–32.

Levin, M. H., Kim, J. K., Hu, J., & Verkman, A. S. (2006). Potential difference measurements of ocular surface Na+ absorption analyzed using an electrokinetic model. Investigative Ophthalmology & Visual Science, 47(1), 306–16. doi:10.1167/iovs.05-1082.

Ngo W., Situ P., Keir N., Korb D., Blackie C., Simpson T., (2013). Psychometric Properties and Validation of the Standard Patient Evaluation of Eye Dryness Questionnaire. Cornea, (32):1204–1210.

Saboo, U., Amparo, F., Abud, T., Schaumberg, D., Dana, R., (2015). Vision-Related Quality of Life in Patients with Graft-versus-Host Disease. Ophthalmology, 122 (8), 1669-1674.

Schaumberg, D. A., Gulati, A., Mathers, W. D., Clinch, T., Lemp, M. A., Nelson, J. D., Dana, R. (2007). Development and validation of a short global dry eye symptom index. The Ocular Surface, 5(1), 50–7.

Schiffman R.M., Christianson M.D., Jacobsen G., Hirsch J.D., Reis B.L. (2000). Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol., 118:615-621.

Shen M., Li J., Wang J., Ma H., Cai C., Tao A., Yuan Y., Lu F (2009). Upper and lower tear menisci in the diagnosis of dry eye. Invest. Ophthalmol. Vis. Sci., 50:2722-2726

Stern, M. E., Gao, J., Siemasko, K. F., Beuerman, R. W., & Pflugfelder, S. C. (2004). The role of the lacrimal functional unit in the pathophysiology of dry eye. Experimental Eye Research, 78(3), 409–16.

Thelin, W. R., Johnson, M. R., Hirsh, A. J., Kublin, C. L., & Zoukhri, D. (2012). Effect of topically applied epithelial sodium channel inhibitors on tear production in normal mice and in mice with induced aqueous tear deficiency. Journal of Ocular Pharmacology and Therapeutics : The Official Journal of the Association for Ocular Pharmacology and Therapeutics, 28(4), 433–8. doi:10.1089/jop.2011.0157.

Yu, D., Thelin, W. R., Randell, S. H., & Boucher, R. C. (2012a). Expression profiles of aquaporins in rat conjunctiva, cornea, lacrimal gland and Meibomian gland. Exp Eye Res. (103), 22–32.

Yu, D., Thelin, W. R., Rogers, T. D., Stutts, M. J., Randell, S. H., Grubb, B. R., & Boucher, R. C. (2012b). Regional differences in rat conjunctival ion transport activities. Am J Physiol Cell Physiol., 303(7), C767–780.

APPENDIX I: SCHEDULE OF VISITS AND PROCEDURES.

Table 1 Schedule of Visits and Procedures

Event	Visit 1	Visit 2		Visit 3	ET
	Screening Visit	First Treatment Visit (3 to 9 days after Visit 1)	Wash-out (7 to 14 Days)	Second Treatment Visit (7 to 14 days after Visit 2)	Early Termination Visit
Informed Consent	X[a]				
Eligibility Criteria Reviewed	X	X		X	
Medical History/Changes	X	X		X	X
Concomitant Medication/Changes	X[b]	X		X	X
Abbreviated Physical Exam	X				X
Vital Signs	X	X[c]		X[c]	X
Tear Osmolarity	X				
Assessment of Meibomian Glands[d]	X				
Pregnancy Test	X				
Serum Chemistry	X				
Hematology	X				
Dry Eye Symptoms[e]	X	X		X	
Study Medication Administration	N/A	One drop per eye in AM[f]		One drop per eye in AM[f]	
UHR-OCT Assessments[g]	X[g]	X[i]		X[i]	
Keratograph 5M	X[g]	X[i]		X[i]	
Drop Instillation Comfort Assessment[j]		X		X	

Event	Visit 1	Visit 2		Visit 3	ET
	Screening Visit	First Treatment Visit (3 to 9 days after Visit 1)	Wash-out (7 to 14 Days)	Second Treatment Visit (7 to 14 days after Visit 2)	Early Termination Visit
Post Instillation Dry Eye Symptoms Assessment		X[k]		X[k]	
Visual Acuity	X				
Biomicroscopy and External Eye Examination	X	X[l]		X[l]	X
Corneal Staining	X				
Conjunctival Staining	X				
TBUT	X				
Intraocular Pressure	X				
Schirmer's Test	X				
Adverse Event Monitoring	X	X		X	X

a. Prior to conducting study procedures, informed consent will be obtained.
b. Concomitant medications taken within 28 days of screening will be reviewed
c. To be completed pre-treatment
d. Meibomian gland assessment to include clinical exam and meibography
e. Dry eye symptom questionnaires (OSDI, SPEED, and SANDE with SANDE part 1 at visit 1, 2 and 3 and Part 2 at Visit 2 and Visit 3 only) will be administered pre-dose and before other procedures.
f. Remove study medication from refrigerator at least 15 minutes before administration
g. Measurementes include: UTMH, LTMH, UTMV, LTMV, and TTMV (UTMV + LTMV)
h. To be measured three times in one hour (at 0 minutes, 30 minutes, and 60 minute interval)
i. Timepoints: Pre dose and post dose at 15±5 minutes, 30±10 minutes, 1 hour±20 minutes, 2 hours±30 minutes, 4 hours±30 minutes, and 6 hours±30 minutes
j. To be completed approximately 5 minutes after dosing
k. To be completed immediately prior to the 2 hour post dose tear volume measurements
l. To be completed after 6 hour tear volume assessment

APPENDIX II: ADVERSE EVENT REPORTING

Adverse Event Definitions

Adverse Event (AE): Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product; which does not necessarily have a causal relationship with this treatment.

Treatment Emergent Adverse Event (TEAE): A treatment emergent AE (TEAE) is an AE that either commenced following initiation of study treatment or was present prior to study treatment but increased in frequency or severity following initiation of study treatment.

A **SERIOUS ADVERSE EVENT (SAE)** is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience (i.e., the subject is at immediate risk of death at the time of the event)
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

A **NON-SERIOUS ADVERSE EVENT** is any adverse event that does not meet the definitions for serious adverse events as described above.

Adverse Event Classification

Each **Adverse Event** will be classified as **SERIOUS** or **NONSERIOUS** using the definitions provided above.

The **INTENSITY** of each Adverse Event will be classified as **MILD, MODERATE, or SEVERE.**

The **CAUSALITY** of the Adverse Event will be classified as **NOT RELATED to study medication, POSSIBLY RELATED to study medication, or RELATED to study medication defined as follows:**

Not related: An event that does not follow a reasonable temporal sequence from administration to the suspected drug, is not a known response pattern to the suspect drug or due wholly to the subject's clinical state or other factors.

Possibly related: An event that follows a reasonable temporal sequence form administration of the study medication, follows a known or expected response pattern to the suspected drug, but that could be explained by the subject's clinical state or other factors.

Related: An event that follows a distinct temporal sequence from administration of the study medication, follows a known or expected response pattern to the suspected drug, and cannot be explained by subject's clinical state or other factors

Serious Adverse Event Reporting

The following information should be provided when an SAE is reported to Parion Sciences:

1. Protocol Number
2. Site ID
3. Subject Initials
4. Subject Number
5. Subject Demographic information, including:
    a. Date of Birth
    b. Sex
    c. Race
6. Start date of study medication administration
7. Date of last dose of study product
9. SAE information, including:
    a. SAE Term (diagnosis only; if known or serious signs/symptoms)
    b. Description of SAE/narrative
    c. Date of onset
    d. Outcome
    e. Date of resolution or death
    f. Duration of SAE if less than 24 hours
    g. Relationship to study medication
    h. Action taken with Study medication
    i. If ocular, which eye(s) were affected
10. Criteria for classifying the event as serious, including whether the SAE resulted in any of the following:
    a. Death

b. Life-threatening

c. In-patient hospitalization or prolongation of existing hospitalization

d. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

e. Congenital anomaly/birth defect

f. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

11. Concomitant medications
12. Relevant history
13. Possible causes of SAE other than study medication
14. Copy of the Adverse Event page from the Case Report Form if the subject has been randomized
15. Signed copy of the SAE form

APPENDIX III: VISUAL ACUITY ASSESSMENT

Visual acuity will be measured by the M&S Technologies Smart System II (Park Ridge, IL). This is a computer generated visual acuity test. The Smart System II comprises a computer processor, using a flat LCD screen monitor, and an interactive keypad controller. The LCD screen monitor is wall-mounted and manufactured to M&S Technologies' specifications, with high resolution and a 450:1 nominal contrast ratio. The examiner uses the keypad to access directly each primary acuity test, specific optotypes, randomization options, and to increase or decrease the size of the optotype on demand. The system allows the examiner to present random letter sequences to preclude subjects from memorizing the sequence to pass each line. The Smart System II can be calibrated for lane lengths of 6 to 22 feet.

APPENDIX IV: BIOMICROSCOPY/EXTERNAL EYE EXAMINATION

Slit lamp biomicroscopy will be performed and the observations will be graded as described below:

Lashes

0= Normal
1= Abnormal

Eyelid

Erythema

0 = Normal, without any redness
+1 = A low grade flushed reddish color
+2 = Diffused redness encompassing the entire lid margin
+3 = Deep diffused reddish color of lid margins and superior or inferior eyelid

Edema

0 = Normal, no swelling of the lid tissue
+1 = Slight diffuse swelling above normal
+2 = General swelling
+3 = Swelling sufficient to close the lid

Conjunctiva

Erythema

0 = Normal
+1 = A flush, pink color predominantly confined to the palpebral or bulbar conjunctiva; slight localized injection
+2 = More prominent red color of the palpebral or bulbar conjunctiva
+3 = Marked redness of the palpebral or bulbar conjunctiva

Edema

0 = Normal, no swelling of the conjunctiva
+1 = Slight diffuse or regional swelling of the conjunctiva
+2 = General swelling of the conjunctiva
+3 = Extensive swelling of the conjunctiva

Tear Film Debris

0 = None; absence of debris
+1 = Mild; presence of debris in inferior tear meniscus
+2 = Moderate; presence of debris in inferior tear meniscus and in tear film overlying cornea
+3 = Severe; presence of debris in inferior tear meniscus and in tear film overlying cornea
presence of mucus strands in inferior fornix or on bulbar conjunctiva
+4 = Very Severe; presence of debris in inferior tear meniscus and in tear film overlying cornea;
presence of numerous AND/OR adherent mucus strands in inferior fornix and on bulbar conjunctiva or filamentary keratitis

Cornea

Endothelial Changes

0 = Normal
+1 = Slight pigment, keratoprecipitates, guttata
+2 = Moderate pigment, keratoprecipitates, guttata
+3 = Dense pigment, keratoprecipitates, guttata

Edema

0 = None, transparent and clear
+1 = Mild, dull glassy appearance
+2 = Moderate, dull glassy appearance of epithelium with large number of vacuoles
+3 = Severe, epithelial bullae and/or stromal edema, localized or diffuse, with or without stromal striae.

Anterior Chamber

Cells

0 = No cells seen
+1 = + cells
+2 = ++ cells
+3 = +++ cells
+4 = ++++ cells, Hypopyon formation

Flare

0 = No Tyndall effect
+1 = Tyndall beam in the anterior chamber has a mild intensity
+2 = Tyndall beam in the anterior chamber is of strong intensity
+3 = Tyndall beam is very intense; the aqueous has a white, milky appearance

Lens Pathology

0 = Normal; no opacity in the lens
1 = Abnormal; existing opacity in the lens; aphakic or pseudophakic eyes or other abnormal findings.

APPENDIX V: FLUORESCEIN TEAR BREAK-UP TIME (TBUT)

Non-preserved fluorescein will be used to conduct this assessment. When conducting all assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

TBUT should be conducted as specified in Appendix I: Schedule of Visits and Procedures.

Fluorescein Tear Break-Up Time

- Perform the examination with the slit lamp at 10X magnification and cobalt blue illumination.
- Draw 5 µL of 2% sodium fluorescein into the micropipette, allowing a drop to form.
- Gently touch the drop at the tip of the delivery dropper to the lower palpebral conjunctiva of the right eye.
- In order to thoroughly mix the fluorescein with the tear film, ask the subject to blink several times and move his/her eye around.
- Using a stop watch, measure the time it takes for the appearance of the first dry spot after blinking.
- Ask the subject to blink again and repeat the measurement of the time it takes for the appearance of the first dry spot after blinking. Repeat this procedure once more for a total of three measurements.

NOTE: If this procedure is completed within two minutes, proceed to measure corneal staining in the same eye as described in Appendix VI without applying more fluorescein.

- Repeat these steps for the left eye.
- Using these three assessments calculate the mean time in seconds for each eye and record this time in the subject's chart as well as the case report form.

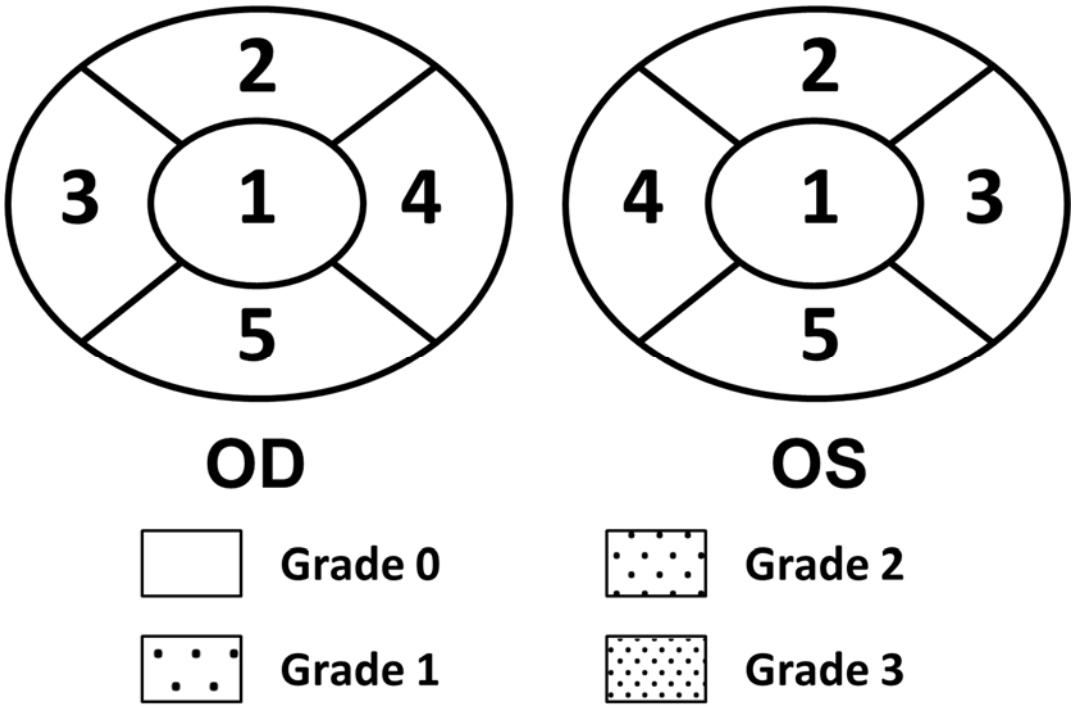
APPENDIX VI: FLUORESCEIN CORNEAL STAINING

The cornea will be stained with non-preserved, 2% fluorescein as described by Lemp et al. (Lemp, 1995). When conducting all assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

Corneal Staining should be conducted as specified in Appendix I: Schedule of Visits and Procedures.

Corneal Staining

- Using a yellow barrier filter (Wratten or Tiffen #11 or #12) and the slit lamp with cobalt blue illumination, with a 3 mm slit width and 10X or 16X magnification.
- Draw a 5 µL drop of fluorescein in the right eye.
- In order to thoroughly mix the fluorescein with the tear film, ask the subject to blink several times and move his/her eye around.
- Wait two minutes.
- Evaluate the cornea for staining referencing the diagram below.
- Punctate staining is recorded using a standard grading system of 0-3 for each of the five areas shown below.
- Repeat this procedure for the left eye.

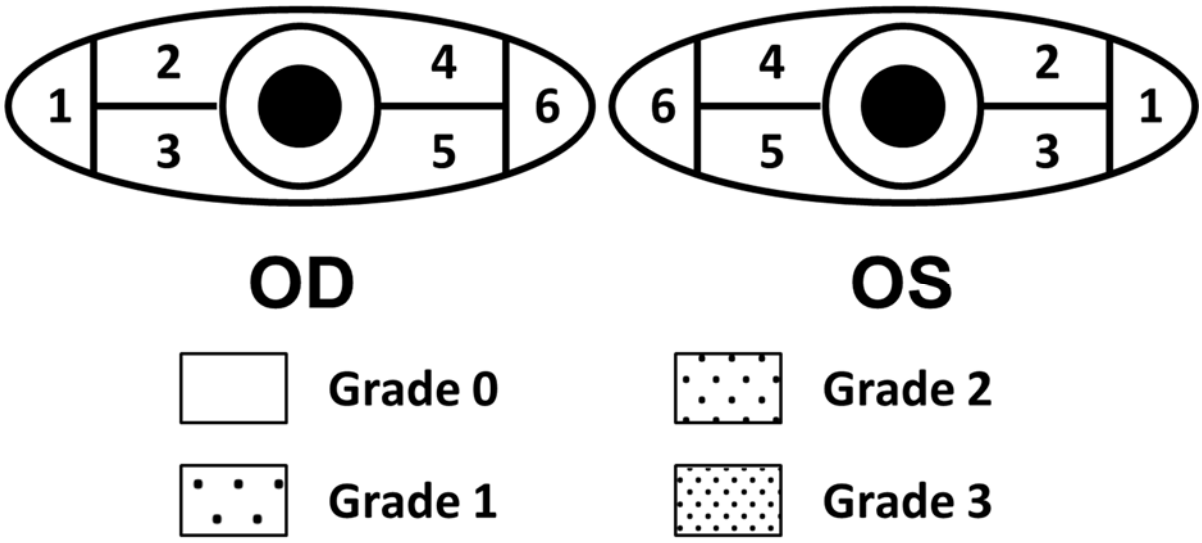


APPENDIX VII: LISSAMINE GREEN CONJUNCTIVAL STAINING

The conjunctiva will be stained with non-preserved, 1% lissamine green as described by Lemp et al. (Lemp, 1995). When conducting all assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

Conjunctival Staining should be conducted as specified in Appendix I: Schedule of Visits and Procedures.

- Without flushing the eye from the previous assessment, instill a 20 µl drop of lissamine green to the right eye using a micropipette, allowing a drop to form.
- Gently touch the drop at the tip of the delivery dropper to the lower palpebral conjunctiva of the right eye.
- In order to thoroughly coat the ocular surface with the lissamine, ask the subject to blink several times and move his/her eye around.
- After 1 minute and before 4 minutes have elapsed, using white light of moderate intensity, grade the areas of the conjunctiva of the right eye with the 0-3 scale shown below.
- Repeat this procedure for the left eye.



APPENDIX VIII: INSTILLATION OF MEDICATION

Study personnel will instill medication at Visit 2 (First Treatment Visit) and Visit 3 (Second Treatment Visit).

Instilling the Medication

- The study personnel will remove the study medication from the refrigerator at least 15 minutes prior to planned administration.
- The study personnel will record the kit number in the subject's records and the Subject ID number on the labels of the kit and vial pouches before proceeding with dosing the subject:
- Confirm that the visit number on the pouch corresponds to the subject's visit
- Open the pouch
- Instruct subject to slightly tilt his/her head back and look upward
- The study personnel should gently squeeze the bottle allowing one drop to coat the ocular surface.
- Repeat procedure for left eye using the same bottle.
- The subject should gently close his or her eyes for a few seconds.
- Use one pouch of assigned study medication for Visit 2 and one pouch for Visit 3.
- The used bottle should be returned to its pouch and the pouch returned to its kit box.
- After dose administration at Visit 2, return the kit containing one used, and one unused bottle to the refrigerator.
- After dosing at Visit 3, return the bottle into the pouch and place the pouch in the kit box. At this time, the kit containing two used bottles can be stored at room temperature before returning to the sponsor for disposal.

APPENDIX IX: DRY EYE SYMPTOM QUESTIONNAIRES

The following subject reported outcomes measurements will be collected as noted in Appendix I:

- SANDE
- SPEED
- OSDI

SYMPTOM ASSESSMENT IN DRY EYE QUESTIONNAIRE (SANDE) (Schaumberg, 2007)

Part 1 (administer at Visits 1, 2, and 3)

PLEASE COMPLETE THE FOLLOWING QUESTIONS REGARDING THE FREQUENCY AND SEVERITY OF YOUR DRY EYE SYMPTOMS

1. Frequency of symptoms:

Please place an '**I**' on the line to indicate how often, on average, your eyes felt **dry and/or irritated** in the past day:

Rarely ——————————————— **All the time**

2. Severity of symptoms:

Please place an '**I**' on the line to indicate how severe, on average, you feel your symptoms of **dryness and /or irritation** were in the past day:

Very Mild ——————————————— **Very Severe**

Part 2 (administer at only Visits 2 and 3)

PLEASE COMPLETE THE FOLLOWING QUESTIONS REGARDING THE FREQUENCY AND SEVERITY OF YOUR DRY EYE SYMPTOMS

1. Frequency of symptoms:

Please place an '**I**' on the line to indicate how often, on average, your eyes feel dry or irritated **now** *compared to at your last visit*

2. Severity of symptoms:

Please place an '**I**' on the line to indicate how severe, on average, you feel your symptoms of dryness and irritation are **now** *compared to at your last visit*

SPEED QUESTIONNAIRE (Ngo, 2013 and Korb, 2005)

1. Report the type of SYMPTOMS you experience and when they occur:

	At this Visit		Within the past 72 hours		Within past 3 months	
Symptoms	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Dryness						
Grittiness or Scratchiness						
Soreness or Irritation						
Burning or Watering						
Fatigue						

2. Report the FREQUENCY of your symptoms using the rating list below:

Symptoms	**0**	**1**	**2**	**3**
Dryness				
Grittiness or Scratchiness				
Soreness or Irritation				
Burning or Watering				
Fatigue				

0 = Never **1** = Sometimes **2** = Often **3** = Constant

3. Report the FREQUENCY of your symptoms using the rating list below:

Symptoms	**0**	**1**	**2**	**3**	**4**
Dryness					
Grittiness or Scratchiness					
Soreness or Irritation					
Burning or Watering					
Fatigue					

0 = No problems
1 =Tolerable; not perfect, but not uncomfortable
2 = Uncomfortable; irritating, but does not interfere with my day
3 = Bothersome; irritating and interferes with my day
4 = Intolerable; unable to perform my daily tasks

OCULAR SYMPTOM DISEASE INDEX (Schiffman, 2000)

Please answer the questions by checking the box that best represents your answer

HAVE YOU EXPERIENCED ANY OF THE FOLLOWING *DURING THE LAST WEEK:*

	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

HAVE PROBLEMS WITH YOUR EYES LIMITED YOU IN PERFORMING ANY OF THE FOLLOWING *DURING THE LAST WEEK:*

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

HAVE YOUR EYES FELT UNCOMFORTABLE IN ANY OF THE FOLLOWING SITUATIONS *DURING THE LAST WEEK:*

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

APPENDIX X: TEAR VOLUME ASSESSMENTS:

Three assessments of tear volume are used in the study: Ultra high resolution optical coherence tomography (UHR-OCT) measuring tear volume and tear meniscus height, lower tear meniscus height measured by Keratograph 5M, and Schirmer's test. The sections below briefly describe procedures for each.

Tear meniscus imaged Ultra High Resolution Optical Coherence Tomography (UHR-OCT)

The custom-built ultra-high resolution (3 microns axial resolution) spectral domain OCT can image a full 15mm width scan at up to 48 images per second. The tear meniscus around the upper and lower eyelids can be imaged and measured with the OCT, a unique capability. The OCT images obtained with this system show the tear menisci around both eyelids (Figure 1).

Figure 1: Tear menisci imaged with UHR-OCT

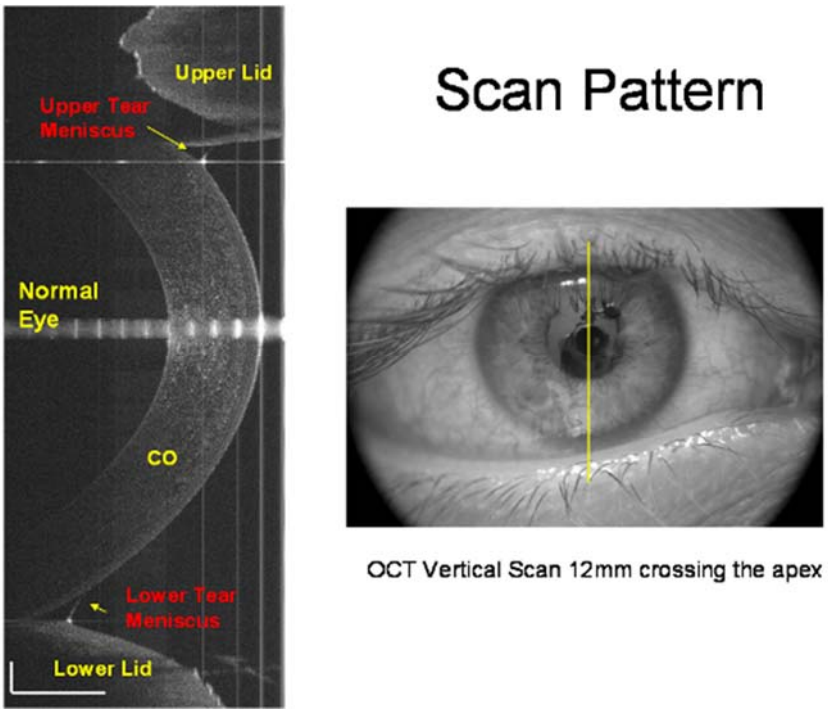


Figure 1: Upper and lower tear menisci imaged with ultra-high resolution optical coherence tomography. A custom built 3 µm resolution ultra-high resolution optical coherence tomography was used to image both upper and lower tear menisci. The boundaries of the tear meniscus were clearly visualized. The bars denote to 500 µm.

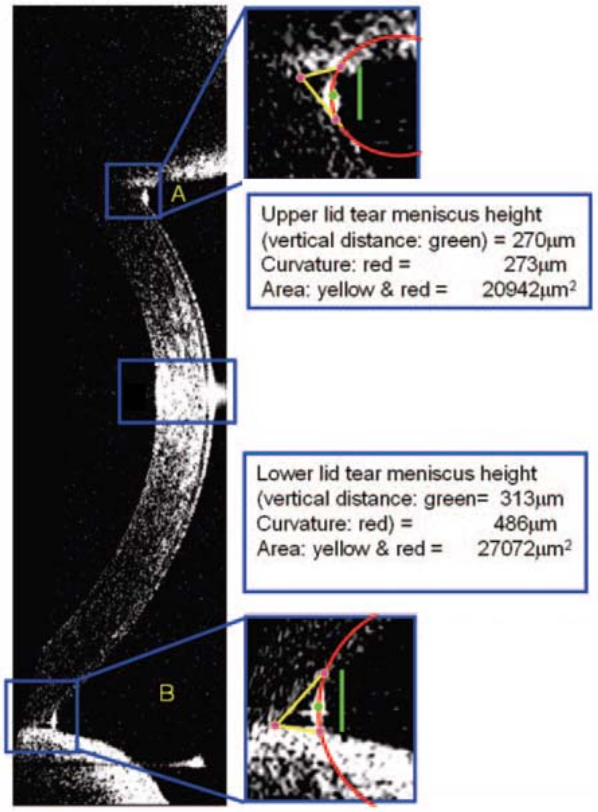
OCT imaging

Each subject will be asked to sit in front of a slit-lamp, on which an OCT probe is mounted. An internal fixation target will be provided to facilitate the subject to keep the eye on a straight forward direction. The central OCT beam as indicated as a green line on the OCT monitor is set to be on the corneal apex (Figure 1), which a specular reflection is normally detected. Immediately after eye opening, corneal images with the OCT will be recorded. A vertical OCT scan with a scan width of up to 13 mm will be used so that the entire cornea and both upper and lower eyelids will be imaged. The measurements will be acquired in one scan with 3 images in the same fashion. The first image will be processed for the measurement. If the first image is not good, the second or third image will be used. The measurements of the image analyzed will be collected in the CRF.

Image process to yield the results.

All meniscus variables of upper (**A**) and lower (**B**) tear menisci are measured with a custom software program with operator inputs (TDanalyer, ver. 1.0) (Figure 2). Touch points (*pink dots*) between two elements (eyelid, cornea, and tear meniscus) and a middle point (*green dot*) of the front edge of the tear meniscus are marked. After that, the software process the image, to yield the results. The three-point method is used to fit a circle to yield tear the meniscus height and area. Tear meniscus height and area of both upper and lower tear menisci will be used. In addition, the eye lid length will be photographied and measured to calculate tear meniscus volumes in both upper and lower tear menisci.

Figure 2: Measuring menisci variables with UHR-OCT



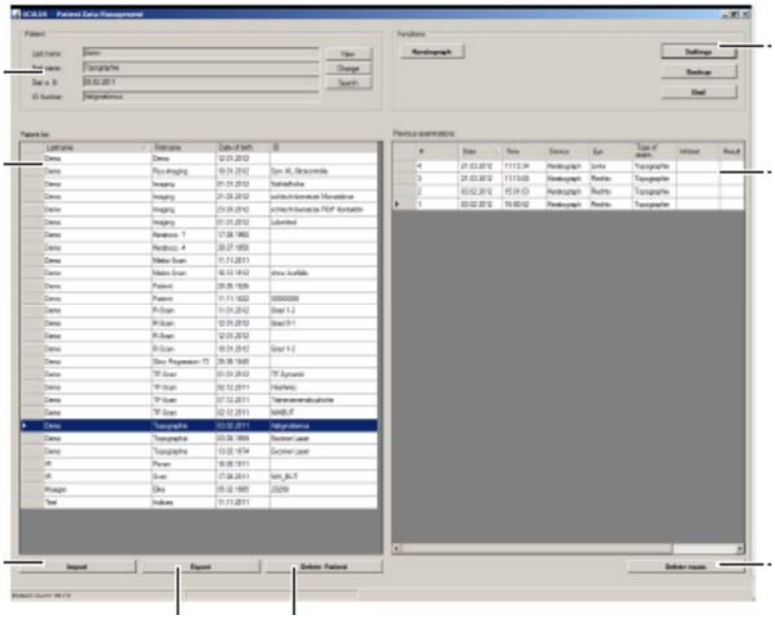
Tear Meniscus Height measured by Keratograph 5M

Evaluation of tear film volume will be assessed via tear meniscus height measurements using the Keratograph 5M. High resolution images of the lower tear meniscus from both eyes are obtained under infrared light as indicated in the Keratograph 5M user manual. Using integrated magnification and measurement tools, the tear meniscus height of the lower lid margin are estimated. Detailed description of the operation of the instrument and software is described in Keratograph 5M user manual provided by Oculus.

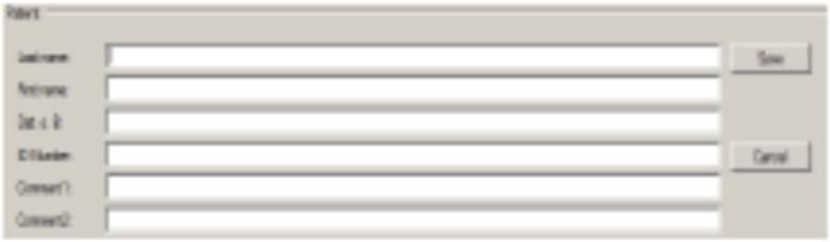
Instructions:

- Turn on the computer. After turning on the computer the operating system loads the Keratograph 5M program. If necessary click on the Keratograph 5M icon  .
- Turn on the Keratograph 5M

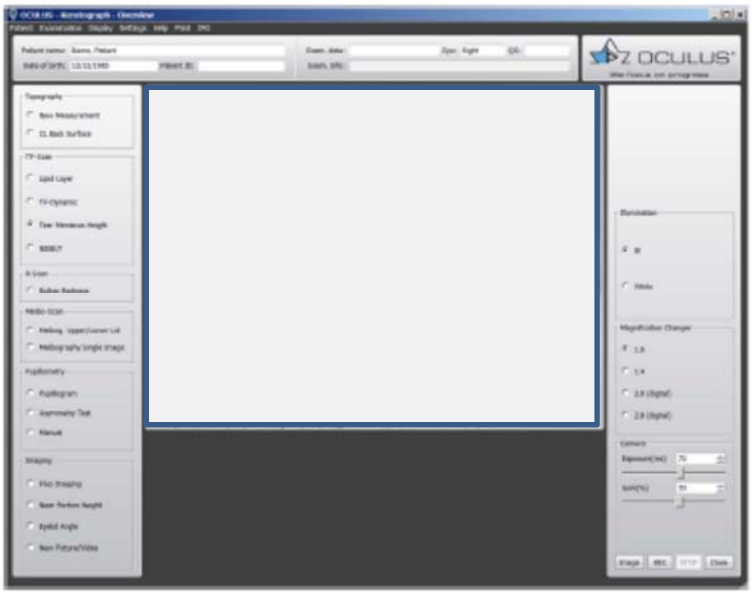
- Enter Subject information in the Patient Data Management Page.



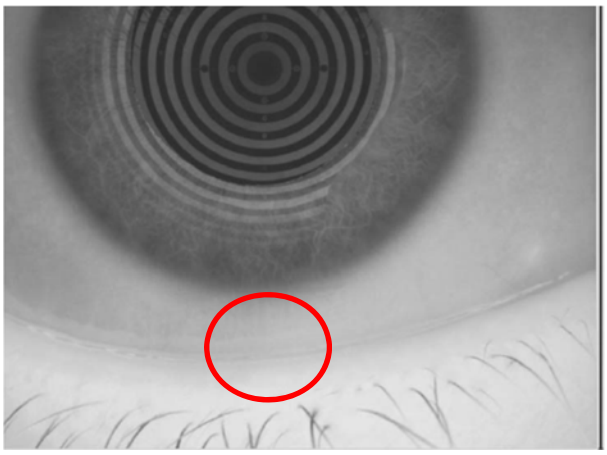
- In the "Patient" box on the top left, select **[New].** The following screen will appear:



- Enter the patient's last name, first name, and date of birth and click **[Save]**.
- Click **[Keratograph]** in the "Functions" box to transition from the Patient Data Management to the Keratograph Overview.
- In the "Examination" menu click **[New]** and the following screen will appear:



- Activate the **[Tear Meniscus Height]** function button on the "TF Scan" Box on the left.
- In the illumination box on the right-hand side of the screen, select **[IR]** lighting.
- If necessary, adjust the camera for optimal focusing on the tear meniscus as seen on the example in the image below (i.e. at approximately the 6:00 position).



- After a blink, focus the camera on the central part of the tear meniscus of the lower eyelid as indicated in the red circle above.
- Click on the **[Image]** button to record the image.
- The magnification changer and camera settings located at the right-hand side of the screen are set to optimal settings. No adjustments should be needed.
- Confirm that the image is in focus at the central area of the eyelid (below the center of the iris), if not, repeat the image.

Note: This procedure is repeated to obtain 3 quality images per eye

Repeat the procedure for the other eye.

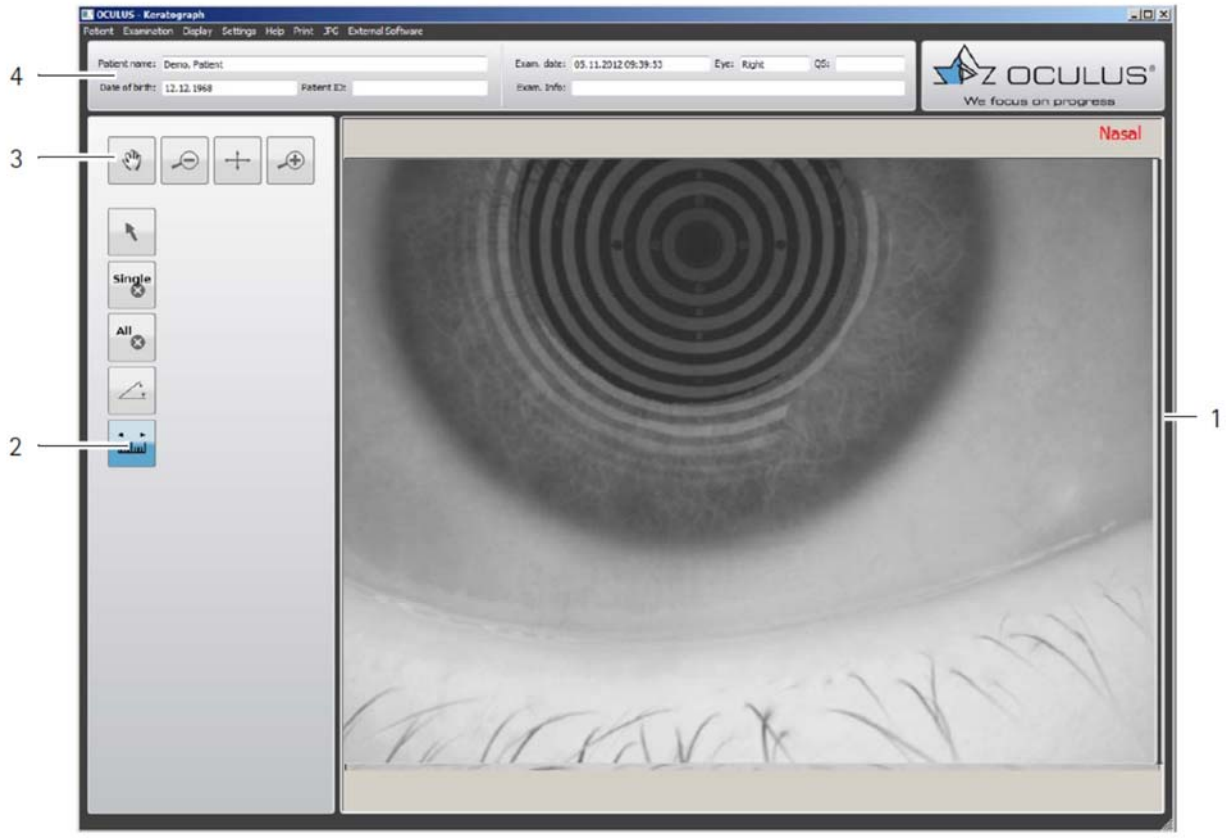
- Select **[Close]** after the three images have been taken.

Measurement of the Tear Meniscus Height (TMH)

- From the three images from each eye obtained above, the tear meniscus height is measured with a software-integrated ruler. The evaluations can be made at different magnification levels. Select a higher magnification if necessary.

If the TMH is not measured at the time the image is captured, select the patient and the assessment from the Patient Data Management Page by double clicking on the assessment on the right of the screen for the selected patient on the left.

The following screen will be displayed:

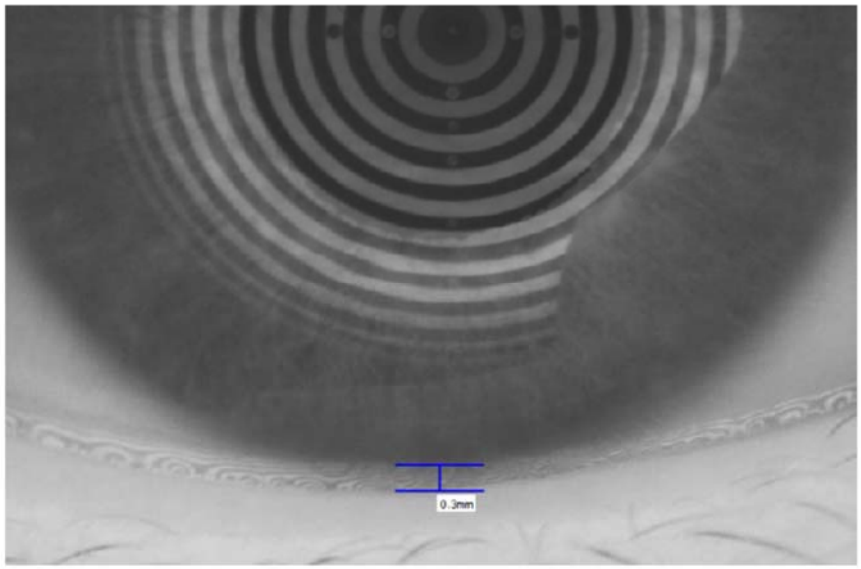


1 *Camera image*
2 *Buttons for tear meniscus measurement*
3 *Buttons to work in the camera image*
4 *Examination and patient data*

- Measure the tear meniscus height with the ruler option.
- Select the following button  from the left of the screen with the left mouse button.

- The button will be highlighted in blue.
- Click once with the left mouse button on the edge of the lower eyelid to position one of the edges of the measurement.
- Move the cursor vertically from this edge to the upper tear meniscus margin and
- click the left mouse button again.

- The measurement length will appear on the image (in millimeters) as indicated below:



- The system will display the real measurement regardless of the magnification used for the measurement.
- If the selection of the tear meniscus height is not accurate, delete the measurement and repeat the procedure.
- Record the distance of the TMH.
- Repeat the procedure for the all three images of each eye.
- Click **[Close]** and the image with the measurement will be saved in the patient file.

Schirmer's Test

Schirmer's strips will be supplied to the clinical site. When conducting assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

Schirmer's Test without Anesthesia

- While still in the plastic sheath, fold the notched end of the Schirmer at the apex of "v". Additionally, fold a partial second fold at the halfway point of the strip so that the strip does not lie directly in the subject's line of sight.
- Remove the right eye strip from the sheath.

- Ask the subject to look up and gently draw the right lower lid in a downward and temporal direction.
- Place the rounded end of the strip toward the temporal one-third of the lower eyelid.
- Repeat this procedure in the left eye.
- Darken slightly the room, and instruct the subject to relax and look at an object in the room while blinking normally or have subjects gently close eyes.
- Strips are removed after five minutes.
- After removing the strips, with a sharp pencil draw a horizontal line across the leading edge of moisture and a second horizontal line across the lowest point of moisture.
- Using a ruler and/or the millimeters recorded on the strips, measure a point halfway between the two lines and record this as the amount of wetting.

APPENDIX XI: POST INSTILLATION QUESTIONNAIRES

DROP INSTILLATION COMFORT MEASUREMENT

Evaluation of the comfort of the eye drop will be conducted approximately 5 minutes following initial dosing at Visits 2 and 3 for subjects.

The subject will respond to the following question upon instillation of the study medication:

"Did you experience any discomfort when the drop was placed in your eyes?"

The response to the question will be graded using the following scale:

1 = None
2 – Mild
3 = Moderate
4 = Severe

Note: The drop instillation comfort response is NOT considered an AE regardless of severity unless it results in discontinuation of the subject from the study.

If the subject experiences discomfort symptoms within 15 minutes after the drop instillation comfort assessment is completed, then the site should record these symptoms as AEs.

POST INSTILLATION DRY EYE SYMPTOMS ASSESSMENT

The subject will respond to the following question immediately prior to the 2 hour post dose tear volume measurements at Visits 2 and 3:

Did you experience any change in your dry eye symptoms after instillation of the eye drop?

-1 = Worsening
0 = No change
+1 = Improvement

APPENDIX XII: MEIBOGRAPHY ASSESSMENT

Meibomian gland evaluation will be conducted in both eyes using the clinical grading scores indicated below for plugging, character of secretions, and ease of expression (modified from Foulks, 2003). In addition, meibomian gland loss as determined by non-contact Meibography as described by Arita (Arita, 2008).

Plugging (nasal third of lower lid)

0 no glands plugged
1 1-2 glands plugged
2 3- 4 glands plugged
3 5 glands plugged

Character of secretion

0 clear
1 turbid
2 turbid with clumps or granular
3 thick or paste-like

Ease of expression

0 easily expressed
1 light pressure
2 moderate pressure
3 heavy pressure

Noncontact meiboscopy (Arita et al[2]): Scored for LL and UL0 no gland loss
1 gland loss 33% of total area
2 gland loss, 33%–67% of total area
3 gland loss >67% of total area

Scores of upper and lower lid summed

Scale range: 0 – 6

APPENDIX XIII: SERUM CHEMISTRY AND HEMATOLOGY ANALYTES

Serum Chemistry Panel includes the following analytes:

- Sodium
- Potassium
- BUN/Urea
- Creatinine
- Glucose
- Calcium
- Total Protein
- Albumin
- AST(SGOT)
- ALT(SGPT)
- Alkaline Phosphatase

Hematology Panel includes:

- WBC
- Haemoglobin
- Haematocrit
- RBC
- MCV
- MCH
- MCHC
- RDW
- Platelet Count
- Differential (% and absolute)

Serum pregnancy test in women of child bearing potential.

APPENDIX XIV: DOSE SELECTION FOR PHASE 2 AND DATA REVIEW DURING STUDY

The decision on dosage strength to be used in Phase 2 will be the responsibility of the Data Review Group constituted by the Prinicipal Investigator (or designee), Medical Monitor and representative(s) of Parion Sciences not involved in the conduct of the study. The Data Review Group may request the participation of an independent statistician to assist in the assessment of the safety of the study medication and the dosage strength selection for Phase 2.

The Data Review Group will review unmasked data in order to make a decision on the dose to be administered in Phase 2. The Data Review Group will convene when approximately 8 subjects have completed the study,or earlier if needed. The trial will continue enrolling during the time the review by the Data Review Group is being prepared and being held. The Data Review Group will review the following to assist in making their recommendation for dose selection in Phase 2:

- Adverse events
- Biomicroscopy and external eye examination
- Drop instillation comfort assessments
- Abnormal alert laboratory results
- Tear volume via OCT
- Tear volume via Keratograph 5M

Recommendations by the Data Review Group may include:

1) The dose level may be increased to 0.05% in Phase 2 in the event that:
    a) No cases of serious, suspected related, unexpected events are noted in Phase 1 AND
    b) No cases of serious ocular safety events are noted in Phase 1
    c) Lack of evidence that P-321 produced an increase in tear volume based on OCT and/or Keratograph 5M, or evidence that additional improvements in tear volume (or the duration of effect) may be obtained with a higher dose
2) The dose level may be decreased to 0.01% in Phase 2 in the event that:
    a) There is evidence of a significant increase in tear volume and duration of effect in Phase 1 based on OCT and/or Keratograph 5M
    b) No cases of serious, suspected related, unexpected events are noted in Phase 1
    c) No cases of serious ocular safety events are noted in Phase 1
3) The dose level may remain at 0.017% (the same as in Phase 1) in the event that:
    a) There is no compelling data to either increase or decrease the strength for Phase 2.
    b) No cases of serious, suspected related, unexpected events are noted in Phase 1
    c) No cases of serious ocular safety events are noted in Phase 1
4) Discontinue the study.
5) Amend the protocol

The Data Review Group will make a recommendation to the Parion Sciences following the meeting and review of data and via a written memo. If additional data is required, the Data Review Group may request additional data for their review. If the Data Review Group requires

additional unmasked data in order to safeguard the safety of the study subjects, this data will be provided.

The Data Review Group will make every effort to arrive to a decision by consensus.
Upon receipt fo theData Review Group recommendation, Parion representatives will make a decision that is consistent with the safeguard of the interests of study subjects.

Additional Data Reviews

Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above.

Unmasking the study

In the event of a medical emergency where breaking the mask of the study is being considered, the Principal Investigator may request unmasking by contacting the Medical Monitor and the Parion Representative. If the Medical Monitor and Parion Representative grant the unmasking, the unmasking will be clearly documented with the date the code is broken indicated, who authorized the unmasking and other details regarding the unmasking. A copy of this unmasking memo will be provided to the sponsor for their records and a copy should remain in the source.

Clinical Protocol P-321-201

Project Number	**P-1003-I101**
Compound Number/ Name	**P-321 Ophthalmic Solution**
Protocol Number	**P-321-201**
Protocol Title	**Randomized, Crossover Study of the Pharmacodynamic Activity of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Characterized by Low Tear Volume**
Sponsor	**Parion Sciences, Inc. 2800 Meridian Parkway Suite 195 Durham, NC 27713**
Medical Monitor	**Gary N. Foulks, M.D. 3103 Joy Place Wilmington, NC 28409**
Authors	**José L. Boyer, Ph.D. Anita Woodring, MS, RAC**
Issue Date	**Version 2.0: 22 April 2016 Original Version 1.0: 05 February 2016**
Sponsor Signature and Date	José L. Boyer April 22, 2016

PARION SCIENCES, INC.
Clinical Protocol P-321-201
Investigator Signature Page

Project Number	**P-1003-I101**
Compound Number/ Name	**P-321 Ophthalmic Solution**
Protocol Number	**P-321-201**
Protocol Title	**Randomized, Crossover Study of the Pharmacodynamic Activity of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Characterized by Low Tear Volume**
Sponsor	**Parion Sciences, Inc.** **2800 Meridian Parkway** **Suite 195** **Durham, NC 27713**
Issue Date	**Version 2.0: 22 April 2016** **Original Version 1.0: 05 February 2016**

I have reviewed and understand this protocol and all amendments associated with it. I will administer the protocol in accordance with ICH, FDA, and local regulations and guidelines. I will keep the information provided to me within this protocol and by Parion Sciences, Inc. staff, their representatives, and designees confidential.

Investigator Name (printed or typed):

Victor L Perez MD

Investigator Signature:

4/29/16
Date

1 PROTOCOL SYNOPSIS

Name of Sponsor: Parion Sciences, Inc.		**Study Medication:** 0.017% P-321 Ophthalmic Solution and based on data review potentially 0.01%, 0.017% or 0.05% Ophthalmic Solution
Protocol Number: P-321-201	**Phase:** 2a	**Indication:** Treatment of dry eye disease
Title of the Study: Randomized, Crossover Study of the Pharmacodynamic Activity of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Characterized by Low Tear Volume		
Investigators: Principal Investigator: Victor Perez, MD; Sub-Investigator: Jianhua Wang, MD, PhD		
Study Center: Ocular Surface Center, Bascom Palmer Eye Institute, University of Miami Health System		
Study period (FPFV – LPLV): Approximately 6 months		

Objectives:

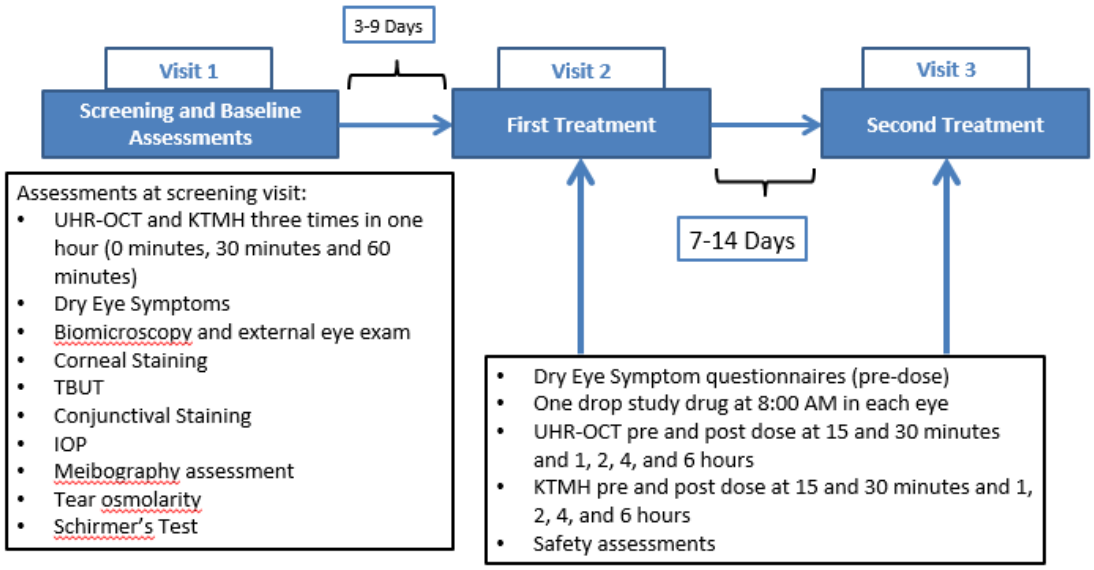
Primary objective

- The primary objective of this trial is to assess changes in tear volume by the non-invasive techniques Ultra High Resolution Optical Coherence Tomography (UHR-OCT) following the administration of P-321 Ophthalmic Solution or Placebo in subjects with tear deficient dry eye disease.

Secondary objective

- assess changes in lower tear meniscus height by Keratograph 5M
- compare lower tear meniscus height measurements between UHR-OCT and Keratograph 5M
- assess the duration of action of P-321 Ophthalmic Solution
- compare the pharmacodynamic activity of different strengths of P-321 in subjects with tear-deficient dry eye
- monitor safety and tolerability of P-321 Ophthalmic Solution

Study Design:



This is a single-center, randomized, cross-over placebo controlled study to evaluate the changes in tear volume by P-321 Ophthalmic Solution or Placebo. The study will enroll subjects with tear deficient dry eye disease to receive sequentially two treatments: P-321 Ophthalmic Solution (Treatment A) and Placebo (Treatment B). Subjects will be randomly assigned to receive treatment sequence AB or BA. The study will consist of two Phases: In each Phase, subjects will be treated with a different strength of P-321 Ophthalmic Solution under the same study design.Phase 1 will evaluate 0.017% P-321 Ophthalmic Solution and Phase 2 will evaluate either 0.05%, additional subjects at 0.017% or 0.01% P-321 Ophthalmic Solution as determined by the Data Review Group.

Approximately twenty-four eligible subjects will complete the study with approximately 8 subjects participating in Phase 1 and the remainder of the 24 subjects participating in the phase 2. Decisions on the dose to be used to phase 2 will be made following unmasked data review from subjects in Phase 1. The Principal Investigator, Medical Monitor and Parion Sciences representatives not involved in the day to day conduct of the study will review unmasked data as part of the Data Review Group, which will decide the dose to be used in Phase2 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur during the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above. The study will consist of three study visits: a Screening Visit (Visit 1), and two treatment visits (Visit 2 and Visit 3). The duration of the study may be up to 23 days. The pharmacodynamic measures of tear volume will be assessed at each visit by two non-invasive methods, UHR-OCT and Keratograph 5M. Dry eye symptom questionnaires will be applied at each visit prior to other assessments. At Visit 1, tear volume will be assessed at three timepoints over 1 hour with measurements taken approximately 30 minutes apart. Tear volume measurements will be followed by other tests that involve contact with the ocular surface (tear osmolarity, tear break up time (TBUT), meibography and meibomian gland assessments, corneal and conjunctival staining, Schirmer's test). At Visits 2 and 3, tear volume will be assessed serially over 6 hours at pre-dose, and post dose at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours. Safety assessments include adverse events

(AEs), biomicroscopy and external eye examination and an assessment of comfort after taking the medication in-clinic.

Subject Population:

This study will complete approximately 24 subjects with dry eye disease with documented low tear volume as measured by non-invasive UHR-OCT. Approximately 8 will participate in Phase 1 and the remainder of the 24 subjects participate in the Phase 2.

Male and female subjects 18-80 years of age who provide written informed consent and have a history of dry eye signs and symptoms of mild to moderate severity supported by a previous clinical diagnosis may enter the study. Subjects must have decreased lower tear meniscus height (LTMH) in both eyes assessed by UHR-OCT of not more than 175µm, Schirmer's test >1mm and <10mm, normal lid anatomy, have been stable on their current medication regimen for at least 28 days and remain on this regimen for the duration of the study. Additionally, while in the clinic on visit days and for at least 6 hours after dosing with study medication, subjects must be able to withhold ocular topical medications (including artificial tears, topical steroids, and Restasis) and if contact lens wearers, must be able to withhold wearing contact lenses. To qualify for the study, subjects must not have an identifiable or suspected dry eye caused by pharmacologic, post-traumatic, or post-surgical condition; have undergone refractive eye surgery (e.g., LASIK) in either eye during the past 12 months; uncomplicated cataract surgery in either eye during the past 3 months; previous eyelid surgery in either eye (e.g., blepharoplasty, ptosis repair) and or botulinum toxin (Botox™ or equivalent) injection in the periocular area within 3 months prior to enrollment. The subject must not have lid irregularities or deformities, or severe corneal surface irregularities, a history of glaucoma or intraocular pressure > 25 mmHg at the Screening Visit (Visit 1) or a history of elevated IOP within the past year, a systemic, multi-organ disease requiring active medical or surgical treatment. Additionally, subjects with any significant illness that, in the opinion of the Principal Investigator (PI), could interfere with the study parameters will also be excluded. Other exclusions include: subjects who have punctal plugs, punctal occlusion, or history of nasolacrimal duct obstruction, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, or other ocular cicatrizing disorders, past or present exposure keratopathy, neurotrophic keratopathy, lagophthalmos, or trichiasis. Use of oral and nasal antihistamines, oral or topical secretagogues such as pilocarpine and Evoxac, or Topical anti-glaucoma medications during the study and for 14 days prior to Visit 1 is prohibited.

Number of Subjects:	**Number of Centers:**
Approximately 24 subjects with tear deficient dry eye disease	1
Test Product and Doses:	**Route of Administration:**
P-321 Ophthalmic Solution and Placebo with dosage strengths of P-321 Opthalmic Solution of 0.017% in Phase 1 and 0.05%, 0.01% or 0.017% in Phase 2	Ocular instillation, one drop per eye

Duration of Treatment:

Subjects will receive a single dose of P-321 Ophthalmic Solution or Placebo in both eyes in random treatment order, one treatment on Visit 2 and the other treatment on Visit 3.

Criteria for Evaluation:

Background characteristics will be evaluated to better characterize subjects at entry. These parameters include: demographics, visual acuity, intraocular pressure, dry eye symptoms upon entry, medical history, concomitant medication use, tear osmolarity, tear break up time, meibography and meibomian gland assessment, corneal and conjunctival staining, and Schirmer.

Pharmacodynamics

Detailed measurements of tear volume before and after dose administration will be obtained using non-invasive methods, UHR-OCT (tear volume and tear meniscus height) and Keratograph 5M (tear meniscus height) imaging of the ocular surface.

Safety

Ocular safety measures include: Adverse events (AEs), biomicroscopy and external eye examination and an assessment of comfort after taking the medication.

Statistical Analysis:

Sample Size

A sample size of approximately 24 subjects is proposed for this study. For a two-period, two-treatment, two-sequence crossover design (2x2x2), this study size when all subjects treated with P-321 are analyzed, will provide 98% power to detect a LTMH difference vs placebo of 60 µm at 1 hour or a power of 99% if the average increase in LTMH over the six hours is 60 µm (assuming within-subject SD of 71). In this study it is expected that the study medication will reach its peak effect at 1 hour post dose and that the effect diminishes at 4 and 6 hours post dose (to values still above pre-dose values).

Disposition, Demographic, and Background Characteristics

Subject disposition, demographic, and background characteristics will be summarized using descriptive statistics. Baseline homogeneity with respect to demographic and background characteristics will be assessed via an overall F-test from analysis of variance (ANOVA). For categorical variables, treatment differences will be assessed using the chi-square test or Cochran Mantel Haenszel (CMH) test with modified ridit scores for ordered categorical variables.

Pharmacodynamic Analysis

Treatment group differences for tear volume assessments will be conducted via comparisons of tear meniscus height, and if applicable, tear meniscus volume (TMV). These parameters will be compared between each dose of P-321 Ophthalmic Solution and Placebo using a t-test for each eye separately and for the within-subject average. Analaysis of covariance (ANCOVA) models will be used to adjust for the baseline TMH and TMV for each eye separately and for the average of the two eyes. Generalized estimation equations (GEE) will also be used to account for the correlation between eyes. For the GEE analysis, an exchangeable correlation matrix will be assumed.

Safety

All safety analyses will be included for all subjects who were randomized and received at least one dose of study medication.

The incidence of AEs will be tabulated by treatment group, by treatment sequence, by severity, and by relationship to study medication.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
ANCOVA	Analysis of covariance
AUC	Area under the curve analysis
BID	Two times daily
C	Celsius
CMH	Cochran Mantel Haenszel (test)
CRF	Case report form
ECG	Electrocardiogram
ENaC	Epithelial sodium channel
F	Fahrenheit
FDA	Food and Drug Administration
GEE	Generalized estimation equations
GLP	Good Laboratory Practices
ICH	International Conference on Harmonization
IOP	Intraocular pressure
IRB	Institutional Review Board
I_{sc}	Short-circuit current
IV	Intravenous
LTMH	Lower tear meniscus height
LTMV	Lower tear meniscus volume
NOAEL	No-observed-adverse-effect level
OSDI	Ocular symptom disease index
PD	Potential difference
PI	Principal investigator
QID	Four times daily
SAE	Serious adverse event
SANDE	Symptom Assessment in Dry Eye questionnaire
SPEED	Standard Patient Evaluation of Eye Dryness questionnaire
SS	Sjögren's syndrome
TBUT	Tear break-up time
TMH	Tear meniscus height
TTMV	Total tear meniscus volume calculated as UTMV + LTMV

UHR-OCT	Ultra high resolution optical coherence tomography
UTMH	Upper tear meniscus height
UTMV	Upper tear meniscus volume

2 BACKGROUND INFORMATION

2.1 Description of Study Medication

Parion Sciences, Inc. is developing P-321 Ophthalmic Solution for the treatment of subjects with dry eye disease. P-321 is a novel potent inhibitor of the epithelial sodium channel (ENaC) and a structural analog of amiloride with unique pharmacokinetic (PK) and pharmacodynamic characteristics designed for ocular topical administration, metabolic stability and limited systemic exposure. The formulation being studied is a sterile non-preserved aqueous solution of P-321.

ENaC is a transmembrane sodium channel important in the regulation of epithelial sodium absorption that is present in the eye and other tissues such as lung, kidney, colon, and sweat glands. ENaC has been extensively characterized in the lung where it plays a major role in the regulation of the composition of the airway surface liquid and is tightly linked to the maintenance of airway surface hydration and mucocilliary clearance (Barker, 1998). The absorption of sodium from the epithelial surface liquid via ENaC osmotically entrains water into the epithelium, decreasing the level of hydration of the mucosal surface.

The dynamics of the ocular tear film are maintained through an integrated system known as the lacrimal functional unit that consists of the ocular surface epithelium (cornea and conjunctiva), the lacrimal glands, the meibomian glands, and their neural and immunological components (Stern, 2004).

ENaC is a key ion channel in this process, mediating the absorption of sodium (and hence water) from the tear fluid through the ocular surface epithelium (cornea and conjunctiva) where ENaC is expressed (Levin, 2006; Thelin, 2012; Krueger. 2012; Yu, 2012a; Yu, 2012b). Therefore, ENaC provides an absorptive pathway for tear fluid loss, which regulates the composition and volume of tears under non-stimulated or “basal” conditions. The inhibition of ENaC with highly potent, small molecule channel blockers represents a unique ocular hydration strategy, working by preventing the absorption of tear fluid and thereby maintaining the protective and lubricating actions of tears on the ocular surface.

2.2 Non-Clinical Studies for P-321 Ophthalmic Solution

2.2.1 Pharmacology

***In vitro* studies:**

The inhibitory effect of P-321 on ENaC was studied in human and canine primary cultures of bronchial epithelial cells. Apical administration of P-321 produced a concentration-dependent inhibition of ENaC-mediated short-circuit currents (Isc) with a potency of 3.247 ± 1.231 nM (n=12) and 1.901 ± 0.7543 nM (n=3) for canine and human airway epithelial cells, respectively.

Primary cultures of human conjunctival epithelia grown on a permeable support at an air-liquid interface were used to assess the effect of P-321 on the absorption of fluid by the conjunctiva. Addition of fluid to the apical surface of these cells resulted in rapid absorption of fluid into the tissue. The presence of P-321 on the apical surface inhibited the "tear" fluid absorption of the conjunctiva, suggesting that ENaC-mediated sodium absorption plays an important role on fluid absorption and tear dynamics.

Among the unique characteristics built in the design of P-321 are the low systemic exposure following administration on the ocular surface, and the retention of the drug on the ocular surface to increase the duration of action. The penetration of P-321 into the corneal epithelium was measured by confocal microscopy imaging, taking advantage of the intrinsic fluorescence of P-321. *Ex vivo* imaging of mouse corneas treated with P-321 or amiloride indicated that in corneas treated with P-321, the drug remained associated with the mucosal surface of the cornea, whereas under identical conditions, amiloride had fully penetrated the epithelium, the stroma, and the endothelium, suggesting that P-321 is selectively retained by the epithelium of the ocular surface.

***In vivo* Studies**

The effect of P-321 on the activity of ENaC in the ocular surface was studied in C57BL/6 mice by *in vivo* measurements of the transepithelial potential difference (PD) driven by sodium movement through ENaC**.** Topical ocular instillation of P-321 produced a concentration-dependent inhibition of the ocular PD measurements with a potency of 57.2 nM, (95% Confidence Interval: 35.2 to 93.0 nM). These studies are consistent with *in vitro* results obtained in airway cells and suggest that P-321 is a potent inhibitor of the ocular ENaC-mediated epithelial sodium transport *in vivo*.

The ability of P-321 to increase tear volume was studied in the ExLac dry eye animal model in rats in which the main lacrimal glands have been surgically removed (Fujihara, 2001). ExLac rats exhibit approximately 50 to 60% reduction in the basal tear volume compared to normal animals. A single ocular instillation of P-321 produced a concentration-dependent increase of tear volume that reached tear volumes similar to those observed in normal animals and at maximally effective concentrations. The increase in tear levels was maintained for several hours following a single administration of P-321.

The effect of repeat dosing of P-321 on tear volume was also studied in the ExLac rat animal model.

Animals were treated with repeated administrations of 0.001% P-321 or vehicle control for five consecutive days at two dosing frequencies, twice daily (BID) and four times daily (QID). Animals treated with vehicle control showed no significant changes in tear volume throughout the five days of dosing. In contrast, in the group of animals treated with P-321 administered BID or QID, a gradual increase in tear volume was observed on each day of treatment reaching by the fifth day of treatment a steady state level similar to the tear volume of normal rats. On the first day of treatment, the animals receiving P-321 QID had a larger increase in tear volume than the animals treated BID, however, both groups had similar levels of tear volume by the end of treatment on Day 5. These results show that a maximum effect on tear volume can be achieved with low concentrations of P-321 (0.001%) when administered either BID or QID over five consecutive days.

In vivo pharmacology studies have shown that epithelial sodium channel inhibitors, including P-321 have a stimulatory effect on tear volume in an animal model of dry eye disease (Thelin, 2012).

These *in vivo* and *in vitro* pharmacological actions together with the PK properties of P-321 provide a strong scientific rationale for the use of P-321 Ophthalmic Solution for the treatment of diseases of impaired ocular hydration such as dry eye.

2.2.2 Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution, metabolism, and excretion profile of P-321 was characterized in *in vitro* and *in vivo* studies. P-321 was not metabolized in plasma from different species (rat, rabbit, dog and human) or by incubation with rat and dog hepatocytes.

Oral administration of P-321 to rats did not produce measurable plasma drug concentrations, suggesting that P-321 is not orally available.

The PK and systemic clearance of P-321 was characterized in rats following intravenous (IV) administration. P-321 displayed biphasic elimination from plasma with a long terminal half-life and a high volume of distribution. The PK of P-321 in plasma showed no difference among sex, and no accumulation was observed after multiple days of IV administration.

The renal elimination of P-321 and its potential for inhibition of ENaC in the kidney was studied in rats treated with P-321 by topical ocular administration or by IV infusion. The amount of P-321 recovered in urine over 24 hours following IV and ocular administration accounted for only a small fraction of the total dose administered corresponding to 0.57-1.3% and 0.19%, respectively. Ocular administration of P-321 Ophthalmic Solution in dogs given four times per day over multiple days also indicated that very low amounts of P-321 were excreted in the urine and these concentrations of drug were not associated with changes in urine electrolyte excretion, the most sensitive measurement of the effect of ENaC blockers in the kidney.

The ocular distribution and PK following topical ocular administration of P-321 was assessed in Dutch Belted rabbits following a single dose of 0.1% P-321 Ophthalmic Solution or during QID administration for 14 days. The ocular distribution of P-321 was limited only to the external surface of the eye. P-321 had a long terminal half-life in tears of approximately 24 hours. Sustained drug levels were also observed in the palpebral and bulbar conjunctiva and eyelids for up to 48 hours following a single administration. After multiple days of QID dosing, P-321

concentrations in these tissues, increased relative to the levels observed following a single administration, and reached a steady-state level after approximately 5 days of dosing. P-321 had minimal or no penetration into internal regions of the eye such as the retina and aqueous humor, or the main lacrimal glands. Furthermore, the systemic exposure of P-321 following ocular instillation was low, as evidenced by low or non-detectable drug levels in the plasma following a single dose or during 14 days of QID administration.

The toxicokinetics of P-321 were estimated in Good Laboratory Practices (GLP) studies conducted in rabbits and dogs following ocular administration four times per day for 28 days. The systemic exposure of P-321 in rabbits was low with only the highest dose tested (0.5% P-321, QID) exhibiting quantifiable plasma drug levels. P-321 was rapidly absorbed following ocular administration and rapidly cleared from plasma, with mean $t_{1/2}$ values ranging from 0.183 to 0.254 hours on Days 1 and 28, respectively. No accumulation was observed over 28 days of dosing based on plasma drug concentrations. Note, the nominal ocular dose that produced systemic exposure in rabbits is 120-fold larger than the initial dose to be given to the subjects in this study, and 40-fold larger than the high dose planned for this study (0.05%). The systemic exposure in dogs was also low, and only observed with the highest dose tested (0.05% QID) on Day 28. P-321 was rapidly absorbed following ocular administration and rapidly cleared from plasma, with mean $t_{1/2}$ values ranging from 2.28 to 2.68 hours. Note, the nominal ocular dose that produced systemic exposure in dogs is 12-fold larger than the initial dose to be given to the subjects in this study, and 4-fold larger than the high dose planned for this study (0.05%).

In summary, P-321 is metabolically stable. Following IV administration P-321 is rapidly cleared primarily via non-renal mechanisms, and is not orally available. The ocular tissue distribution of P-321 following ocular instillation is essentially limited to its site of action on the ocular surface (tears, palpebral conjunctiva, bulbar conjunctiva, and cornea) with no penetration to internal structures of the eye (aqueous humor, retina) or the main lacrimal glands. Topical ocular administration of P-321 Ophthalmic Solution at pharmacologically active concentrations results in minimal to no systemic exposure and also failed to produce any renal effect. Furthermore, in subjects with dry eye disease treated with 0.01% P-321 BID for 28 days, we were not able to detect any drug present in plasma or urine from these subjects.

2.2.3 Toxicology

Multiple toxicology studies have been conducted using P-321Ophthalmic Solution. Systemic administration of P-321 via IV, oral and ocular routes of administration, in both acute and repeat-dose nonclinical studies, demonstrated that P-321 Ophthalmic Solution at pharmacologically active concentrations is well tolerated. Extensive ocular toxicology studies for up to 28 days of administration at higher frequencies than those used in this study have been conducted in rabbits and dogs, with the dog being the most sensitive species. The primary finding associated with ocular administration of P-321 was a dose-dependent increase of ocular irritation, described as minimal or slight, that reversed upon discontinuation of treatment. No significant effects of P-321 Ophthalmic Solution were observed at any dose level for both species on electroretinograms, corneal thickness, and corneal endothelial cell density. For the dose limiting factor of ocular irritation, the no-observed-adverse-effect levels (NOAEL) of 0.8 mg/eye/day in rabbits and 0.024 mg/eye/day in dogs exceeds the human doses planned for of this study. The NOAEL of 0.05 mg/kg/day in rats administered P-321 IV exceeds the potential exposure from

the proposed initial ocular dose of $2.2661x10^{-4}$ mg/kg/day (assuming bilateral dosing of 0.017% P-321, QD with a 40 µl drop size in a 60 kg person) by 225-fold on a Human Equivalent Dose basis. For the high dose planned in this study, the dose of $6.666x10^{-4}$ mg/Kg/day (assuming bilateral dose of 0.05% P-321 QD in a 60 Kg person, the systemic safety margin is 75-fold on a Human Equivalent Dose basis.

No systemic effects at any dose level in dogs or rabbits were observed following ocular administration of P-321 Ophthalmic Solution.

In a battery of genotoxicity studies, P-321 did not cause mutations in the Ames or mouse lymphoma assay in vitro with or without S9 metabolic activation. Additionally, the compound was negative in a rat micronucleus study. Thus, P-321 can be considered to be non-mutagenic and non-clastogenic, with no evidence of disruption of the mitotic apparatus.

2.3 Clinical Experience with P-321 Ophthalmic Solution

There has been one study completed with P-321 Ophthalmic Solution in which 40 subjects with mild to moderate dry eye disease were treated with P-321 Ophthalmic Solution. In this study, P-321 Ophthalmic Solution was well-tolerated at concentrations of 0.0005%, 0.0015%, 0.005% instilled twice daily for up to 15 days and 0.01% instilled twice daily for up to 28 days. There were no clinically relevant adverse drug-related, dose-related or time of treatment-related effects on any safety measure. No serious adverse events (SAEs) were reported. No evidence of systemic exposure of P-321 was observed.

Although this study was not powered for effects on efficacy measurements, improvements in the frequency and severity of symptoms of dry eye disease that approached statistical significance compared with placebo were observed. In addition, consistent with the proposed mechanism of action of P-321, the treatment difference relative to placebo favored P-321 for the measurements of tear meniscus height (TMH) observed in this study.

2.4 Justification for Route of Administration and Dose Selection

P-321 Ophthalmic Solution is expected to exert its biological activity through direct interaction with ENaC on the corneal and conjunctival surface of the eye. Since the intended route of administration for P-321 is topical ocular, this route of administration will be used in this study. The dose planned for this study was selected based upon results of the first clinical study and preclinical safety studies.

This study is a single day crossover comparing P-321 Ophthalmic Solution and Placebo on the pharmacodynamic measure, tear volume. During Phase 1, a single dose of 0.017% will be administered and during Phase 2 and based on the recommendation of the Data Review Group, a single dose of either 0.05%, 0.017% or 0.01% will be administered. These doses on a single instillation frequency and are covered by the safety profile obtained in the most sensitive species (dog) with safety margins that range from 6-fold to 1.2-fold.

2.5 GCP Compliance

This clinical trial will be conducted in compliance with the protocol, International Conference on Harmonization (ICH) Good Clinical Practice guidelines, and other applicable regulatory requirements.

2.6 Population to be Studied

Dry eye disease is a multifactorial debilitating disease of the ocular surface characterized by ocular signs of corneal and conjunctival impairment and damage of the protective epithelial surface, as well as, decreased tear volume, decreased tear break-up time, and symptoms of discomfort that can range from mild to severe such as, burning, pain, stinging, itching, swelling, foreign body sensation, photophobia, and ocular fatigue. Frequent instillation of artificial tears is currently the most commonly used treatment for mild to moderate dry eye signs and symptoms.

According to recent prevalence estimates, dry eye disease can affect up to 20 million people in the US, with a disproportionate number of women and elderly being afflicted. However, with the spread use of digital technologies in recent years, increased incidence of dry eye in younger adults is being observed. There is a heterogeneous collection of diseases with many shared characteristics that can precipitate dry eye disease. Dry eye disease is one of the hallmarks of the Sjögren's syndrome (SS); however, they represent only a fraction of subjects who experience dry eye symptoms. Dry eye is also observed in approximately 40 to 60% of subjects who have undergone hematopoietic stem cell transplantation and develop graft versus host disease (Saboo, 2015). One core abnormality of the disease is the decrease in tear volume that can be originated by a deficiency of tear secretion or composition which in turn triggers the loss of volume and compromises the protective barrier of the ocular film. Additionally, dry eye symptoms can be the side effect of many commonly used medications such as anticholinergics, antidepressants, and environmental factors such as air conditioning and focusing on video displays for prolonged periods of time. These symptoms of dry eye can vary from mild to severe and in the most severe cases, can result in significant vision impairment and permanent damage of the ocular surface. In this study, subjects with tear deficient dry eye disease will be recruited for participation.

Approximately 24 subjects with tear deficient dry eye disease documented by low tear volume as measured by UHR-OCT will be completed in this single-center, randomized, cross-over, placebo controlled study to evaluate the changes in tear volume by P-321 Ophthalmic Solution or Placebo. The study consists of two Phases. Approximately 8 subjects participating in Phase 1 will receive 0.017% P-321 Ophthalmic Solution or Placebo, and the remainder of the 24 subjects participating in Phase 2 will receive either 0.05%, 0.017% or 0.01%. The decision on the dose administered in Phase 2 will be made following unmasked data review from subjects in Phase 1. In each Phase, subjects will receive sequentially two treatments: P-321 Ophthalmic Solution (Treatment A) and Placebo (Treatment B). Subjects will be randomly assigned to receive treatment sequence AB or BA, with each subject in Phase 1 and Phase 2 being treated with P-321 Ophthalmic Solution and placebo The Principal Investigator, Medical Monitor and Parion Sciences representatives not involved in the day to day conduct of the study will review unmasked data as part of the Data Review Group, which will decide the dose to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur during the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above.

3 TRIAL OBJECTIVES AND PURPOSE

The primary objective of this trial is to assess changes in tear volume by the non-invasive techniques Ultra High Resolution Optical Coherence Tomography (UHR-OCT) following the administration of P-321 Ophthalmic Solution or Placebo in subjects with tear deficient dry eye disease.

The secondary objectives are to:

- assess changes in lower tear meniscus height by Keratograph 5M
- compare lower tear meniscus height measurements between UHR-OCT and Keratograph 5M
- assess the duration of action of P-321 Ophthalmic Solution
- compare the pharmacodynamic activity of different strengths of P-321 in subjects with tear-deficient dry eye
- monitor safety and tolerability of P-321 Ophthalmic Solution

4 INVESTIGATIONAL PLAN

4.1 Overall Study Design

This is a single-center, randomized, cross-over, placebo-controlled, Phase 2a trial designed to evaluate the pharmacodynamic effects of tear volume of P-321 Ophthalmic Solution in tear-deficient dry eye subjects. Approximately 24 eligible subjects will complete the study which consists of two Phases: Approximately 8 subjects participating in Phase 1 will receive 0.017% P-321 ophthalmic Solution and Placebo, and the remainder of the 24 subjects participating in Phase 2 will receive either 0.05%, 0.017% or 0.01% P-321 and placebo. The decision on the dose administered in Phase 2 will be made following unmasked data review from subjects in Phase 1. In each Phase, subjects will receive sequentially two treatments: P-321 Ophthalmic Solution (Treatment A) and Placebo (Treatment B). Subjects will be randomly assigned to receive treatment sequence AB or BA, in a 1:1 ratio.

The pharmacodynamic measures of tear volume will be assessed by two methods: UHR-OCT and Keratograph 5M. Optical coherence tomography (OCT) has been used to image the anterior segment of the eye including tears on the ocular surface. The method is non-invasive and non-contact which is suitable for imaging the tear meniscus around both upper and lower lids. The system at Bascom Palmer Eye Institute is a custom made OCT system (UHR-OCT) with an ultra-high resolution (3 μm). Many previous studies have been done using UHR-OCT and its clinical application has been well documented. With the wide scan of the UHR-OCT, cross-sectional images of the tear menisci can be imaged and tear meniscus volume can be calculated. The Keratograph 5M is a corneal topographer with a built in camera optimized for external imaging. The Keratograph 5M has a feature that enables the non-invasive assessment of the tear meniscus height of the inferior eyelid from high resolution images obtained under infrared light. With the use of a built in measurement tool the height of the tear meniscus is obtained.

The study will consist of three study visits: a Screening Visit (Visit 1), and two treatment visits (Visit 2 and Visit 3). The duration of the study may be up to 23 days.

At Visit 1, three tear volume measurements will be conducted over 1 hour with measurements taken approximately 30 minutes apart (initial timepoint, and at 30 and 60 minutes later) and prior to any invasive ocular assessments. The average of these three measurements will be obtained and used as baseline tear volume and meniscus height and to determine the eligibility of the subject to participate in the study.

At Visits 2 and 3, tear volume will be assessed serially over 6 hours: Before administration of assigned study medication (time = 0, pretreatment), and at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after study medication administration. Subjects will be asked to arrive to the clinic for Visit 2 and Visit 3 between 8:00AM and 11:00AM and at approximately the same time as Visit 1.

Safety assessments including adverse events (AEs), biomicroscopy and external eye examination and an assessment of comfort immediately after taking the medication will be conducted following each in-clinic dose. Changes in medical conditions and medication, abbreviated physical exam changes and vital signs will be recorded periodically throughout the study. A post

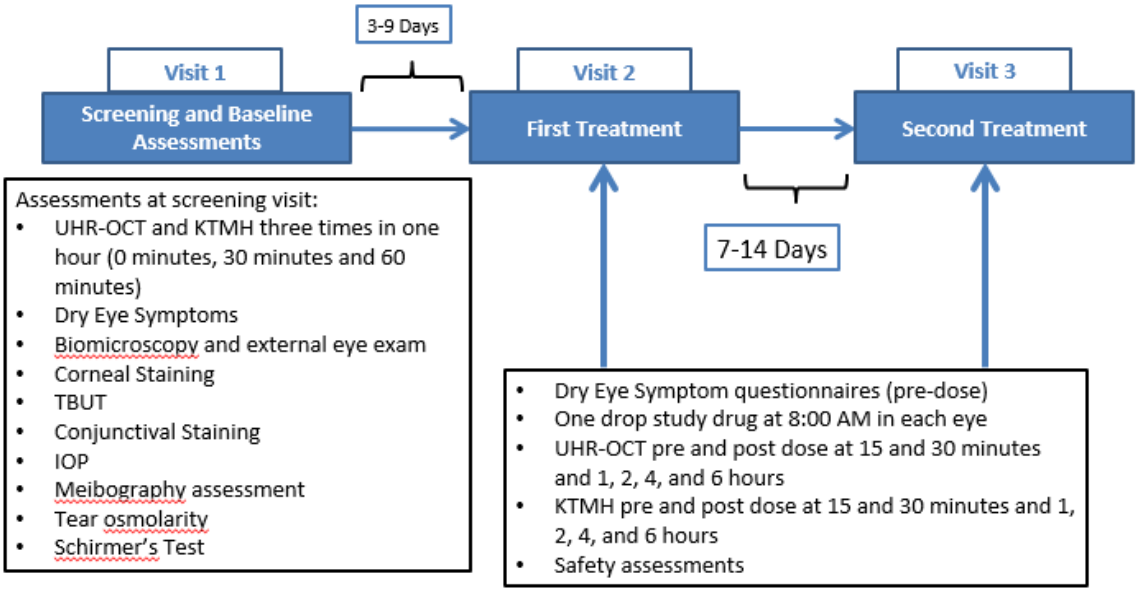
instillation dry eye symptom assessment will be conducted at Visits 2 and 3 immediately prior to the 2 hour post dose time point.

Subjects may be asked to stop or withhold certain medications, and on visit days and for at least 6 hours after dosing with study medication, withhold certain medications and not wear contact lenses on visit days. After the assessments of Visit 3 are completed, the subject will be discharged from the study.

A detailed schedule of the time and events for the study is provided in Appendix I.

A schematic of the study design is shown in Figure 1.

Figure 1: P-321-201 Study Schematic



4.2 Endpoints

4.2.1 Primary Endpoint

The primary endpoint for this study is the change in measurements of tear volume over time as measured by the UHR-OCT following the administration of P-321 Ophthalmic Solution and Placebo in subjects with tear deficient dry eye disease.

4.2.2 Secondary Endpoints

The secondary endpoints are to:

- assess changes in lower tear meniscus height by Keratograph 5M
- compare lower tear meniscus height measurements between UHR-OCT and Keratograph 5M

- assess the duration of action of P-321 Ophthalmic Solution
- compare the pharmacodynamic activity of different strengths of P-321 in subjects with tear-deficient dry eye
- monitor safety and tolerability of P-321 Ophthalmic Solution

4.2.3 Safety and Tolerability Endpoints

Safety endpoints include: AEs, biomicroscopy and external eye examination and comfort assessment (after each in-clinic dose).

4.3 Duration of Participation

The study will consist of three study visits: A Screening Visit (Visit 1), and two Treatment Visits (Visit 2 and Visit 3). The duration of the study may be up to 23 days. The start of each visit will take place between 8:00AM and 11:00 AM and at approximately the same time as Visit 1.

Visit 1 is expected to last approximately 2 hours and Visits 2 and 3 are expected to last approximately 7 hours.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Subject Inclusion Criteria

Subjects must meet the following criteria at Screening to be enrolled in the study:

1. Provide written informed consent
2. Male or female subjects aged 18 to 80 years
3. Are willing and able to follow instructions and can be present for the required study visits for the duration of the study
4. Have a history of predominantly tear-deficient dry eye of mild to moderate severity, supported by a previous clinical diagnosis
5. Subjects must have in both eyes an average tear meniscus height (TMH) of the lower eye lid of less than 175µm as assessed by UHR-OCT
6. Have a Schirmer's test >1mm and <10 mm
7. Have normal lid anatomy
8. Female subjects of child bearing potential must have a negative serum pregnancy test at Screening and agree to use a medically acceptable form of birth control (e.g., intrauterine device, birth control pill, patch or subcutaneous implant, condoms, diaphragm, or abstinence) throughout the duration of the study. Females who are breast feeding an infant are not eligible to participate in the study.
9. Subjects must:
    a. Remain on current medications for the duration of the study
    b. Be on the current medication regimen at least during the past 28 days
    c. Be able to withhold ocular topical medications, including artificial tears, topical steroids, Restasis®, autologous serum, and lid wipes and scrubs on visit days while in the clinic, and for at least 6 hours after dosing with study medication
    d. If they are contact lens wearers, be able to withhold wearing contact lenses on visit days, while in the clinic, and for at least 6 hours after dosing

5.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria at Screening or during the study will be excluded from the study.

1. Have an identifiable or suspected dry eye caused by pharmacologic, post-traumatic, or post-surgical condition
2. Have undergone refractive eye surgery (e.g., LASIK) in either eye during the past 12 months
3. Have undergone uncomplicated cataract surgery in either eye during the past 3 months
4. Have undergone previous eyelid surgery in either eye (e.g., blepharoplasty, ptosis repair)
5. Have lid irregularities or deformities
6. Have severe corneal surface irregularities
7. Have undergone botulinum toxin (Botox™ or equivalent) injection in the periocular area within 3 months prior to Visit 1

8. Have a history of glaucoma or IOP > 25 mmHg at the Screening Visit (Visit 1) or a history of elevated IOP within the past year
9. Subjects that have a systemic, multi-organ disease with the exception of subjects with SS or GVHD requiring active medical or surgical treatment are excluded, **except** if the following conditions are met:
    a. the condition is chronic (> 1 years' duration), stable, of mild severity and adequately controlled
    b. the condition does not or is unlikely to have ocular manifestations
    c. the condition is one of the following:
        i. essential hypertension
        ii. coronary artery disease
        iii. Type II diabetes mellitus without diabetic retinopathy
        iv. thyroid dysfunction without thyroid eye disease
        v. non-morbid obesity
        vi. remote history of cancer (> 5 years from diagnosis)
10. Have punctal plugs, punctal occlusion, or history of nasolacrimal duct obstruction
11. Stevens-Johnson syndrome, ocular cicatricial pemphigoid, or other ocular cicatrizing disorders
12. Past or present exposure keratopathy, neurotrophic keratopathy, lagophthalmos, or trichiasis
13. Subjects taking the following medications within 14 days of Visit 1 or during the study are excluded
    a. Oral, nasal and ocular antihistamines
    b. Oral or topical secretagogues such as pilocarpine and Evoxac
    c. Topical anti-glaucoma medications
14. History of allergies to any of the components of the study medication
15. Are pregnant or breast feeding an infant.
16. Have any significant illness that, in the opinion of the Principal Investigator (PI), could interfere with the study parameters
17. Use of any investigational product or device within 28 days prior to the Screening Visit or during the study
18. Are unable in the opinion of the PI to comply fully with the study requirements or to complete the study

5.3 Randomization Criteria

At Visit 2 (First Treatment Visit) and Visit 3 (Second Treatment Visit), an eligible subject must:

1. Continue to meet all clinical inclusion/exclusion criteria as defined in Section 5.1 and Section 5.2, with the exception of the inclusion criteria #5 (UHR-OCT measurement) and #6 (Schirmer's test measurement) which will not be reassessed for continuation at these visits.

A log will be maintained at the sites for those subjects that do not meet eligibility criteria. Minimally this log will include the subject's age, sex and race and the reason that they were not eligible. Screen failure data will not be collected in the clinical database.

5.4 Subject Withdrawal Criteria and Stopping Rules

Treatment may be discontinued and the subject withdrawn at any time during the study at the discretion of the investigator, Medical Monitor or Parion Sciences for any reason including but not limited to occurrence of an AE which precludes subsequent participation, withdrawal of Informed Consent, or requirement of an ocular surgery or intervention. Also in the event that a woman becomes pregnant while participating on the study, she will be withdrawn from the study. In the event that discontinuation of treatment is necessary, the investigator will make every attempt to complete all subsequent safety assessments and the Early Termination Visit. The reason for premature discontinuation should be entered onto the paper case report form (CRF). Subjects who withdraw from the study will be replaced.

Additionally, the trial or parts of the trial may be discontinued by Parion Sciences, Inc. or at the recommendation of the Investigator or Medical Monitor after consultation with Parion. This may be based on a significant number of AEs of a similar nature that warrant such action or at the request of Parion Sciences, Inc.

6 PROCEDURES

The procedures performed for this study are listed below. The times indicated in the following sections and Appendices are based on the visit starting in the AM with each visit starting at a consistent timeframe relative to Visit 1.

6.1 Visit 1: Screening (3 to 9 days prior to Visit 2 – Randomization)

The following procedures will be completed at Visit 1 which will start between 8:00AM and 11:00AM:

- Obtain written informed consent
- Review eligbility criteria
- Review of the medical history
- Review concomitant medication history in the past 28 days
- Administer ocular symptom questionnaires (SPEED, OSDI, and SANDE Part 1 only) before other procedures
- Biomicroscopy and external eye examination
- Visual acuity
- Abbreviated physical exam (including respiratory, cardiovascular, musculoskeletal, gastrointestinal, dermatological)
- Vital signs (pulse, blood pressure, temperature and respiration rate) and height and weight
- Conduct pretreatment measurements in both eyes by UHR-OCT and Keratograph 5M three times in one hour (at 0 minutes, 30 ± 10 minutes and 60 ± 20 minutes). NOTE: throughout the study, UHR-OCT will be conducted first, immediately followed by Keratograph 5M.
- Tear osmolarity
- TBUT
- Assessment of meibomian glands
- Corneal staining
- Conjunctival staining
- Schirmer's test
- IOP
- Collect blood for hematology, chemistry and pregnancy tests

Monitoring of adverse events will begin from the time the Informed consent is signed

Eligible subjects will be instructed to return to the clinic between 3 and 9 days for the first treatment (Visit 2, First Treatment Visit) with the visit occurring in the AM, at approximately the same time of day as Visit 1. Subjects will be reminded not to use ocular medications or wear contact lenses on the day of Visit 2.

6.2 Visit 2: First Treatment Visit (3 -9 days after Visit 1)

The following procedures will be completed at Visit 2 which will take place between 8:00AM and 11:00 AM and at approximately the same time as Visit 1:

- Subjects will be asked to remain on site through this visit and limit use of devices with video display screens.
- Confirm continued eligibility through review of inclusion and exclusion criteria as outlined in Sections 5.1 and 5.2 of the protocol with the exception of the inclusion criteria #5 (UHR-OCT dry eye) and #6 (Schirmer's test measurement) which will not be reassessed for continuation at this visit.
- Review of concomitant medications and medical changes since last visit
- Administer symptom questionnaires (SPEED, OSDI, and SANDE Part 1 and Part 2) before other procedures and before dosing
- Assess adverse events since the last visit and during this visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate) pre-treatment
- Conduct pretreatment measurements in both eyes by UHR-OCT and Keratograph 5M (at approximately the same time as conducted at Visit 1) immediately prior the administration of study medication. UHR-OCT will be conducted first, immediately followed by Keratograph 5M.
- Subjects will be assigned a treatment number at this visit.
- Administer one drop of assigned study medication in each eye immediately after the pretreatment assessment (the drug administration will be conducted by the PI or clinical site personnel). NOTE: Remove study medication from refrigerator at least 15 minutes prior to administration.
- At approximately 5 minutes after drug administration, administer drop instillation comfort assessment
- Repeat assessments by UHR-OCT and Keratograph 5M post dose at 15 ± 5 minutes, 30 ± 10 minutes minutes, 1 hour ± 20 minutes, 2 hours ± 30 minutes, 4 hours ± 30 minutes, and 6 hours ± 30 minutes.
- Immediately prior to the 2 hour time point for tear volume measurement, a post instillation dry eye symptom assessment will be completed
- After the 6-hour tear volume assessment, conduct a biomicroscopy and external examination
- At the end of the visit, the subject will be instructed to return to the clinic between 7 and 14 days for the second treatment (Visit 3, Second Treatment Visit) with the visit occurring in the AM, at approximately the same time of day as Visit 1
- The subjects will be instructed to maintain the same medications and study restrictions between visits to continue being eligible to remain in the study. Subjects will be reminded not to use ocular medications or wear contact lenses on the day of Visit 3

6.3 Visit 3: Second Treatment Visit (7-14 days after Visit 2)

- Subjects will be asked to remain on site through this visit and limit use of devices with video display screens.

- Confirm continued eligibility through review of inclusion and exclusion criteria as outlined in Sections 5.1 and 5.2 of the protocol with the exception of the inclusion criteria #5 (UHR-OCT dry eye) and #6 (Schirmer's test measurement) which will not be reassessed for continuation at this visit.
- Review of concomitant medications and medical changes since last visit
- Administer symptom questionnaires (SPEED, OSDI, and SANDE Part 1 and Part 2) before other procedures and before dosing
- Assess adverse events since the last visit and during this visit
- Collect vital signs pre-treatment (pulse, blood pressure, temperature and respiration rate)
- Conduct pretreatment measurements in both eyes by UHR-OCT and Keratograph 5M in the AM (at approximately the same time as conducted at Visit 1) immediately prior the administration of study medication. NOTE: throughout the study, UHR-OCT will be conducted first, immediately followed by Keratograph 5M.
- Administer one drop of assigned study medication in each eye immediately after the pretreatment assessment (the drug administration will be conducted by the PI or clinical site personnel). NOTE: Remove study medication from refrigerator at least 15 minutes prior to administration.
- At approximately 5 minutes after drug administration, administer drop instillation comfort assessment
- Repeat assessments by UHR-OCT and Keratograph 5M post dose at 15 ± 5 minutes, 30 ± 10 minutes minutes, 1 hour ± 20 minutes, 2 hours ± 30 minutes, 4 hours ± 30 minutes, and 6 hours ± 30 minutes.
- Immediately prior to the 2 hour time point for tear volume measurement, a post instillation dry eye symptoms assessment will be completed
- After the 6-hour tear volume assessment, conduct a biomicroscopy and external eye examination
- At this point, the subject will be discharged from the study.
- Subjects may resume previously used ocular medications when discharged from the study.

6.4 Early Termination Visit

The following assessments will be performed at the Early Termination Visit for randomized subjects who are withdrawn from the study prematurely:

- Assess adverse events since the last visit and during this visit
- Assess concomitant medications and medical changes since last visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate) and height and weight
- Conduct an abbreviated physical examination (including respiratory, cardiovascular, musculoskeletal, gastrointestinal, and dermatological)
- Conduct a biomicroscopy and external eye examination

6.5 Selecting Dosage Strength in Phase 2

The decision of dose strength for P-321 Opthalmic Solution in Phase 2 of the study will be the responsibility of the Data Review Group constituted by the Prinicipal Investigator (or a designee not directly involved in the conduct of the study), the Medical Monitor and representatives of Parion Sciences. Decision on the dose level to be given in Phase 2 will be made based on unmasked review of the study data from the first 8 subjects by the review committee. Data to be considered will include safety and efficacy measures. A detailed criterion for Dose selection for Phase 2 is described in Appendix XIV and outlined below.

The dose level may be increased to 0.05% in Phase 2 in the event that:

1) No cases of serious, suspected related, unexpected events are noted in Phase 1 OR
2) No cases of serious ocular safety events are noted in Phase 1
3) Lack of evidence that P-321 produced an increase in tear volume based on OCT and/or Keratograph 5M, or evidence that additional improvements in tear volume (or the duration of effect) may be obtained with a higher dose.

The dose level may be decreased to 0.01% in Phase 2 in the event that:

1) No cases of serious, suspected related, unexpected events are noted in Phase 1
2) No cases of serious ocular safety events are noted in Phase 1
3) There is evidence of a significant increase in tear volume and the duration of effect based on OCT and/or Keratograph 5M

The dose level may remain at 0.017% (the same as in Phase 1) in the event:

1) No cases of serious, suspected related, unexpected events are noted in Phase 1
2) No cases of serious ocular safety events are noted in Phase 1
3) There is not compelling data to either increase or decrease the strength for Phase 2.

The Data Review Group will make a recommendation to the Parion Sciences to increase the dose, decrease the dose or maintain the dose.

Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above.

6.6 Potential Toxicity Management

All preclinical evidence indicates that systemic exposure of P-321 via ocular topical administration is none or negligible at concentrations up to 10-fold higher than the concentration planned in this study (the estimated systemic safety margins for the doses anticipated in this study range from 75- to 375-fold). However, since P-321 is an ENaC inhibitor, a previous potential safety signal of concern following the administration of P-321, was drug-induced hyperkalemia. This possibility was carefully investigated in Study P-321-101. No changes in plasma or serum potassium were noted in this study in subjects administered 0.01% P-321 Ophthalmic Solution BID for up to 28 days.

No concerns for systemic toxicity in this single-dose administration study are based on the lack of safety findings from the P-321-101 study when treatment with P-321 was administered BID for up to 28 days. In addition, in the same study, the pharmacokinetic results demonstrated a lack of systemic availability of 0.01% P-321 Ophthalmic Solution given twice daily for 28 days. In this study, treatment with P-321 Ophthalmic Solution at doses up to 0.05% will be given only **once** to each participating subject. Thus, no post dose monitoring of serum potassium is planned for this trial.

Any additional questions regarding toxicity management should be managed by appropriate medical professional at the clinical site and directed to Parion Sciences Medical Monitor.

6.7 Collection of Data

Source documentation for data collected in the study will be maintained at the investigative site. In cases where no source documents will be used (i.e., data will be recorded directly onto the CRF without first being recorded on another document, such as the subject symptom questionnaires, for which data will be recorded directly on the CRF pages by the subject), the original data will be included in the CRF and this will be noted in the investigator files.

7 TREATMENT OF SUBJECTS

7.1 Treatment Administration

Study medication will be administered by the PI or clinical site personnel at Visits 2 and 3. At Visit 2, eligible subjects will be randomized (1:1 ratio) to receive one of two treatment sequences: P-321 Ophthalmic Solution at Visit 2, and Placebo at Visit 3, or Placebo at Visit 2 and P-321 at Visit 3. The order of the treatment is randomized, neither the subjects nor site personnel will know which treatment the subject receives at which visit. Assessments will be conducted before and at the indicated times after study medication administration in the indicated order in Section 6.

7.2 Trial Treatments

Subjects will receive P-321 Opthalmic Solution or Placebo in random order at Visit 2 and 3. The study treatment order will be determined by the assignment of the randomization code. P-321 Ophthalmic Solution and Placebo.

P-321 Ophthalmic Solution is a sterile, aqueous, non-preserved solution of approximately pH 5, and tonicity of approximately 290 mOsm/kg. Placebo solution has identical composition without the active ingredient. Both solutions are packaged in low density, polyethylene dropper bottles. Each bottle of study medication will contain sufficient volume of ophthalmic solution for the intended dosing (1 drop in each eye). Only one drop should be instilled in each eye.

For Phase 1, one pouch of study medication will be used at each clinical visit, the label on the pouch will indicate the visit number at which the drug will be administered (Visit 2 or Visit 3). The labels on the kit and the pouches will minimally contain the following information: Study ID, kit number, storage temperature, and "Caution: New Drug – Limited by Federal Law to Investigational use". The study medications will be stored at approximately 2-8°C in a secure area with limited access to study personnel.

For Phase 2, the dose selected of P-321 Ophthalmic Solution and Placebo will be provided by a compounding pharmacy in identical individual vials as those of the Phase1 based on a randomization code.No medication kits will be prepared for Phase 2. The pharmacy will be unmasked to distribute according to the randomization scheme. The study medications will be stored at approximately 2-8°C in a secure area with limited access to study personnel and should be labeled and "Caution: New Drug – Limited by Federal Law to Investigational use". Pharmacy records should include Study ID, randomization number, visit number, date, and subject identifiers.

The medication will be administered by the PI or clinical site personnel at each of Visit 2 and Visit 3. There will be no study medication administered at an Early Termination Visit.

7.3 Methods to Minimize Bias

To minimize bias, the treatment regimen will be randomized and masked. The randomization code will be generated by an independent designee of Parion Sciences, Inc. who is not involved in the day-to-day conduct of the clinical study. The randomization code will be provided to the designee of Parion Sciences, Inc. who is responsible for the manufacture, packaging and labeling

of the clinical supplies; further the randomization code will be maintained in a secure location separate from the clinical study site personnel.

The Medical Monitor and Principal Investigator and members of the Parion team not involved in the conduct of the study will be unmasked as part of the Data Review Group for decision making on the strength of P-321 Opthalmic Solution to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined in Section 6.5.

Subjects will be assigned the next available treatment number upon qualifying to be randomized.

7.4 Concomitant Medications

7.4.1 Medications Permitted

All medications including prescription, over-the-counter, and natural remedies, that the subject has taken within 28 days prior to Visit 1 or during the study will be recorded in the CRF and in the subjects' source documents. The name of the drug, dose, route of administration, duration of treatment (including start and stop dates), frequency, and indication will be recorded for each medication. The use of fluorescein and lissamine green do not need to be recorded on the concomitant medications page.

Subjects should use medications consistently throughout the study and for 28 days prior to Visit 1 with the exceptions outlined in Section 7.4.2.

7.4.2 Medications Not Permitted

Use of the following products on visit days, while in the clinic, and for at least 6 hours after dosing will not be allowed (use of these all other times during the study is permitted):

- Topical ocular medications including artificial tears, topical steroids, Restasis®, autologous serum, and lid wipes and scrubs.
- Contact lens wear

Use of the following medications during the study will NOT be allowed within 14 days of Visit 1 or during the study:

- Oral, nasal and ocular antihistamines
- Oral or topical secretagogues such as pilocarpine or Evoxac
- Topical anti-glaucoma medications

Use of the following medications during the study or within 28 days prior to Visit 1 will NOT be allowed:

- Investigational product or device

Use of the following medications during the study or within 3 months prior to Visit 1 will NOT be allowed:

- Botulinum toxin (Botox® or equivalent) injection in the periocular region

7.5 Treatment Compliance

Because this is a single day crossover, with study medication administered by the PI or clinical site personnel, treatment compliance will not be calculated. Time of administration of study medication and kit number will be captured in the CRF.

7.6 Drug Accountability

Study medication accountability will occur during monitoring visits by Parion Sciences, Inc, or its designee. Accountability will be ascertained by performing reconciliation between numbers of Kits/bottles of study medication received on site/compounded for distribution, number of bottles dispensed to subjects according to the protocol-specified dosing regimen, and the remaining unused study medication at the time of reconciliation.

Clinical trial materials and bulk supplies will be shipped to the investigational site/pharmacy under sealed conditions. Study medication shipment records will be verified by comparing actual quantity of drug received against the shipment inventory sheet accompanying the drug received at the site. Accurate records of receipt and disposition of the study medications (e.g., dates, quantity, subject number, dose dispensed, returned, etc.) must be maintained by the investigator or his/her designee. Study medication will be stored at the site in an area free of environmental extremes, at controlled temperatures between 2 – 8°C (36 - 46°F). This area should be limited and have controlled access.

At the end of the study, all study materials, including unused study medication bottles and bulk supplies will be returned to Parion Sciences, Inc. (or its designee) for disposal. The study monitor or designee should verify drug accountability at routine monitoring visits.

7.7 Maintenance of Randomization and Procedure for Breaking the Code

The treatment assignments will be masked to the investigator, medical monitor, study site personnel, subjects in the study and those involved in the conduct of the study.

Only in the case of a medical emergency, or occurrence of a suspected related, unexpected, SAE, may the randomization code be unmasked and made available to the investigator, medical monitor, subject, and/or other personnel involved in the monitoring or conduct of this study. In the absence of medical need, the randomization code will not be available to the above personnel until after the study is completed and the database is locked.

The Data Review Group will review unmasked data. The Medical Monitor and Principal Investigator (or designee) and members of the Parion team not involved in the conduct of the study will be unmasked as part of the Data Review Group for decision making on the strength of P-321 Opthalmic Solution to be used in Phase 2 of the study. The treatment assignments will remain masked to subjects in the study, the clinical monitor, and other site and Parion Sciences personnel involved in the conduct of the study and not involved in the Data Review Group. Additional unmasked reviews of subject data may occur throughout the study to make

recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined in Section 6.5.

For Phase 1, a a randomization list will be generated and the kits will be labeled in a masked fashion. The randomization assignments will be kept in a secure area at Parion Sciences, Inc. or designee. For Phase 2, a randomization list will be provided to the pharmacy and the pharmacy will distribute the assigned drug according to the randomization schedule with subjects remaining masked.

In case of a medical need, the investigator will treat the subject as needed regardless of randomized treatment. Since there is no specific antidote to P-321 Ophthalmic Solution, immediate emergency unmasking is not necessary. If the investigator feels it is necessary to unmask a subject's treatment assignment after an emergency situation, the investigator must call the medical monitor and notify Parion Sciences, Inc. The treatment assignment will be revealed on a subject-by-subject basis, leaving the masking of the remaining subjects intact.

8 STATISTICS

8.1 Statistical Methods

8.1.1 Subject Disposition, Demographic and Background Characteristics

Subject disposition, demographic, and background characteristics will be summarized by using descriptive statistics. Baseline homogeneity with respect to demographic and background characteristics will be assessed via an overall F-test from analysis of variance (ANOVA). For categorical variables, treatment differences will be assessed using the chi-square test or Cochran Mantel Haenszel (CMH) test with modified ridit scores for ordered categorical variables.

Background characteristics include: demographics, tear volume measurements by UHR-OCT and Keratograph 5M, visual acuity, symptoms upon entry, medical history, concomitant medication use, tear osmolarity, tear break up time, meibomian gland assessments, corneal and conjunctival staining, Schirmer and IOP.

8.1.2 Pharmacodynamic parameters

UHR-OCT will assess the following parameters in both eyes: UTMH, LTMH, UTMV, LTMV, and TTMV (UTMV + LTMV). These assessments, as appropriate, will be compared between each dose of P-321 Ophthalmic Solution and Placebo using a t-test for each eye separately and for the within-subject average. Analysis of covariance (ANCOVA) models will be used to adjust for the baseline tear volume and tear meniscus height for each eye separately and for the average of the two eyes. Generalized estimation equations (GEE) will also be used to account for the correlation between eyes. For the GEE analysis, an exchangeable correlation matrix will be assumed. Area under the curve (AUC) analysis will also be performed as an exploratory analysis to compare OCT assessments and Keratograph 5M LTMH for Placebo vs each dose of P-321.

8.1.3 Analysis of Efficacy

This is a pharmacodynamic and tolerability study of a single administration of P-321 Ophthalmic Solution or Placebo, and therefore, no primary or secondary efficacy endpoints will be formally assessed.

8.1.4 Analysis of Safety

All safety data collected on or after the date that study medication was first dispensed up through the date of the final post dose assessment will be summarized.

The objective of the statistical analysis of the safety parameters is to investigate the data for any effects of study medication on clinical tolerability. All such parameters will be summarized by treatment group and time point as indicated below. Because this study, is a cross over design and, at the cross over treatment, the subjects may no longer be treatment naive, analysis will check for sequence/period effects. If sequence/period effects are detected, the analysis of safety will use just the first period results. Provided that no sequence/period effects are seen, analysis will use the most-recent treatment received as the treatment group.

In the absence of any predefined hypotheses, the general strategy of the safety analysis will be to examine the data summaries for any trends amongst the treatment groups. No formal hypothesis testing will be carried out.

The incidence of AEs will be tabulated by treatment group overall, by treatment sequence, by severity, and relationship to study medication.

Change from baseline values (or shifts from baseline, if more appropriate) will be presented for other safety measures for the visits at which they are assessed.

8.1.5 Interim Reviews of Data

A Data Review Group will review unmasked data when approximately 8 subjects have completed Phase 1. The Data Review Group will include the Prinicpal Investigator (or designee), the Medical Monitor and representative of Parion Sciences not involved in the conduct of the study. Other study site personnel, subjects in the study and those involved in the conduct of the study will remain masked. Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined in Section 6.5.

8.2 Sample Size Estimation

An initial sample size of approximately 24 subjects is proposed for this study. The study design includes OCT measurements at time 0 (pretreatment) and post dose at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours. Shen et al. (Shen, 2009) reported that among the measures of tear volume and tear film thickness obtainable through OCT, the most sensitive and specific measure of dry eye is the low tear meniscus height (LTMH). Power estimations for a two-period, two-treatment, two-sequence crossover design (2x2x2),this study size when all subjects treated with P-321are analyzed, will provide 98% power to detect a difference of 60 μm at 1 hour or a power of 99% if the average increase in LTMH over the six hours is 60 μm (assuming within-subject SD of 71). In this study it is expected that the drug will reach its peak effect at 1 hour post dose and that the effect diminishes at hours 4 and 6 (to values still above pre-dose values).

8.3 Level of Significance

Each hypothesis will be tested at a significance level of 0.05.

8.4 Criteria for Termination of an Individual Subject

An individual subject may be terminated from the trial for any reason including, but not limited to the following:

- Subject withdrawing consent to continue in the trial
- Occurrence of an AE which precludes subsequent participation
- Requirement of an ocular surgery or intervention that is exclusionary for the trial
- Physician discretion based on the ability of the subject to continue schedule or treatment regimens according to protocol, or for the safety of the subject
- In the event that a woman becomes pregnant while participating on the study, she will be withdrawn from the study

8.5 Criteria for Termination of the Trial

The trial may be discontinued by Parion Sciences, Inc. or at the recommendation of the principal investigator after consultation with Parion. This may be based on a significant number of AEs of

a similar nature that warrant such action, or at the request of Parion Sciences, Inc. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies) and Institutional Review Board (IRB). In terminating the study, Parion Sciences and the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

8.6 Procedure for Accounting for Missing, Unused, or Spurious Data

All analyses will be based on observed data only. No attempts will be made to impute or estimate missing data.

8.7 Procedure for Reporting Deviations from the Statistical Plan

Any deviations from the statistical plan will be described and a justification given in the final clinical report.

8.8 Subjects to be Included in the Analysis

All safety analyses will include all subjects who were randomized and received at least one dose of study medication.

9 ASSESSMENT OF PHARMACODYNAMICS

Detailed measurements of tear volume before and after dose administration will be obtained using UHR-OCT (tear volume and tear meniscus height) and Keratograph 5M (tear meniscus height). Procedures for these measurements are located in Appendix X.

10 ASSESSMENT OF SAFETY

10.1 Safety Parameters

Subject safety will be evaluated by a medical professional during the study. Tolerance of study medication as well as general subject well-being will be assessed. Subjects may be discontinued from the study at any time by the investigator if this action is considered in the subject's best interest.

Post dose safety parameters are measured as described in Appendix I and include:

- Assessment of adverse events (Appendix II)
- Biomicroscopy and external eye examination (Appendix IV)
- Assessment of comfort in taking the medication will be conducted (Appendix XI)

10.2 Procedures for Adverse Events Reporting

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, which does not necessarily have to have a causal relationship with this treatment. AEs will be documented upon signing the informed consent. A treatment emergent AE (TEAE) is an AE that either commenced following initiation of study treatment or was present prior to study treatment but increased in frequency or severity following initiation of study treatment.

A non-serious AE is any AE that does not meet the definitions for SAEs as described in Section 10.3.

AEs that occur prior to subject randomization at Visit 2 will be documented in source documents. TEAEs will be monitored throughout the study and will be recorded on the CRF with the date and time of onset, date and time of resolution, intensity, seriousness, causality (relationship to study medication), treatment required and the outcome.

If an AE occurs, the investigator will institute support and/or treatment as deemed appropriate. If a non-serious AE is unresolved at the time of the last visit, a reasonable effort will be made to follow up until the AE is resolved or stabilized, the subject is lost to follow-up, or there is some other resolution of the event.

To elicit AEs simple questions with minimal connotations should be used as the initial questions at all evaluation points during the trial. For example:

- How have you felt since your last assessment?
- Have you had any health problems since your last assessment?

10.3 Serious Adverse Event Reporting

An SAE is any untoward medical occurrence occurring on this trial that results in any of the following outcomes:

- Death

- A life-threatening adverse drug experience (i.e., the subject is at immediate risk of death at the time of the event)
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

The Investigator or other study personnel must immediately inform Parion Sciences or designee by phone or email of any AE considered serious or otherwise significant, as described above. **In addition, a completed SAE report form must be submitted to Parion within 24 hours of initial awareness of the event.**

SAE Reporting
Dedicated Safety Email Address: safety@parion.com

It is the responsibility of the investigator or their designee to report any event of this nature to Parion Sciences, Inc. within 24 hours of the event being brought to the investigator's or their staffs' attention. It is also the responsibility of the investigator to report all SAEs to their IRB according to their requirements and provide a copy of this notification to Parion Sciences. The investigator should make every attempt to follow all SAEs to resolution.

Information to submit when reporting an SAE to Parion Sciences, Inc. is located in Appendix II.

10.4 Procedures for Reporting Pregnancy

Any subject found to be pregnant at any time during the study will be withdrawn from the study immediately. Any pregnancy outcome that meets the criteria for an SAE will be reported as an SAE. All pregnancies will be reported to Parion Sciences within 24 hours of the event being brought to the investigator's or their staffs' attention. It is also the responsibility of the investigator to report all pregnanciess to their IRB according to their requirements and provide a copy of this notification to Parion Sciences. The investigator should make every attempt to follow all pregnancies to resolution (i.e., birth or voluntary or spontaneous termination of the pregnancy).

11 DIRECT ACCESS TO SOURCE DATA and DOCUMENTS

The principal investigator will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to source data and documents.

12 QUALITY CONTROL AND QUALITY ASSURANCE

The progress of the study will be monitored by on-site, written, e-mail and telephone communications between personnel at the study center and Parion Sciences, Inc. (or designated monitor). The investigator will allow Parion Sciences, Inc. clinical monitors, designees, auditors, and regulatory authorities to inspect all CRFs; subject records (source documents), signed informed consent forms; records of study medication receipt, storage, and dispensation; and regulatory files related to this study.

At the time of database lock, the clinical database will be audited in order to ensure accuracy of all data as well as to provide an estimated error rate for the final, locked database. The results of the audit will be provided in the final study report. The audit will involve a comparison of CRF values with values from data listings generated from the clinical database. Values of all variables will be confirmed for the five randomly selected subjects. If the database fails to meet an accuracy rate of 99.5% (less than 1 error / 200 fields), a second set of 5 subjects will be selected, If a combined sample fails to meet an accuracy rate of 99.5%, the remainder of the database will be rechecked or take other action taken agreed with by Parion.

13 ETHICS

This protocol is written in accordance with the principles established by the 18th World Medical Assembly General Assembly (Helsinki, 1964) and amendments and clarifications adopted by subsequent General Assemblies. The investigator will make sure that the study described in this protocol is conducted in full conformance with those principles, the protocol, current FDA regulations, ICH Good Clinical Practices (GCP) guidelines, Good Laboratory Practices (GLP) guidelines, local ethical and regulatory requirements, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (CFR) (title 21), any Ethics Committee (EC) requirements relative to clinical studies. As required by the US FDA, the study drug may not be shipped to any participating investigator until the requisite study documentation has been submitted to the IND.

13.1 Institutional Review Board

The EC/IRB must be a properly constituted board or committee operating in accordance with 21 CFR Part 56, "Institutional Review Boards." This protocol, any protocol amendments and the associated informed consent forms and translations must be submitted to the EC/IRB for review and approved before the enrollment of any subject into the trial.

All subject recruitment materials and advertising information, including translated versions of these documents, must be submitted to Parion or its designee and to the EC/IRB for review and approval prior to implementation. EC/IRB approval of any protocol amendments must be received before any of the changes outlined in the amendments are put into effect, except when the amendment has been enacted to eliminate a potential hazard to study subjects. In such cases, the chair of the EC/IRB should be notified immediately and the amendment forwarded to the EC/IRB for review and approval.

In addition, the Clinical Investigator Brochure should be submitted to the IRB.

Written IRB approval must adequately identify the materials approved. Copies of all approved materials, all correspondence with the IRB, and written approval from the IRB must be made available to Parion Sciences, Inc. (or designated monitor) *prior* to shipment of study medication and the start of subject enrollment into the study.

SAEs will be reported to the IRB by the principal investigator as required by the IRB.

13.2 Informed Consent Requirements

Written informed consent will be obtained from each subject prior to any study related procedures being performed. A copy of the signed and dated consent document will be given to each subject. The original signed and dated informed consent document must be maintained in the study files at the investigative site and be available for Parion Sciences, Inc. or designee, and regulatory authorities for review.

Each informed consent form will contain contact information with a telephone number the subject should contact if they have medical concerns 24-hours a day.

13.3 Data Handling and Record Keeping

All procedures for the handling and analysis of data will be conducted using good clinical practices meeting ICH and U.S. Food and Drug Administration (FDA) guidelines for the handling and analysis of data for clinical trials.

13.4 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks and manual data reviews will be implemented and applied to the database. Query reports pertaining to data omissions and discrepancies will be forwarded to the clinical investigator and monitor(s) for resolution. The study database will be updated in accordance with the resolved query reports. All changes to the study database will be documented.

CRFs will be provided by Parion Sciences, Inc. All data relating to the trial must be recorded in the CRFs. The Investigator must verify that all data entries in the CRFs are accurate and complete. The CRF must be signed and dated by the Investigator upon subject withdrawal or completion of the study.

During monitoring visits, CRFs will be reviewed by study monitor(s) for completeness, accuracy, and legibility. CRFs will be compared with the source documents to ensure that there are no discrepancies. All CRF entries, corrections, and alterations must be made by an Investigator or his/her designee. Corrections must be made in such a way that the original entry is not obliterated. Correction fluid must NOT be used. The correct data must be inserted, dated and initialed by the Investigator or his/her designee. The study monitor is not allowed to make entries on the CRF pages.

The original CRF page will be retrieved by the monitor and returned to Parion Sciences, Inc. after complete review of the case. A copy must be maintained by the Investigator. If documented corrections to a CRF are needed after removal of the original CRF copy from the center, a Data Correction Form (DCF) will be used. The Investigator or a staff member must verify, sign, and date each DCF.

A log will be maintained at the sites for those subjects that do not meet eligibility criteria. Minimally this log will include the subject's age, sex and race and the reason that they were not eligible. Screen failure data will not be collected in the clinical database.

13.5 Records Retention

The study center will retain all records related to the study in compliance with ICH Good Clinical Practices Guidelines.

13.6 Publication Policy

The institution and investigators participating in this trial shall have no rights to publish, disclose or present the results of this study without prior written consent of Parion Sciences, Inc.

14 REFERENCES

Arita R, Itoh K, Inoue K, Amano S. Noncontact infrared meibography to document age-related changes of the Meibomian glands in a normal population. Ophthalmology. 2008;115:911–915.

Barker, P. M., Nguyen, M. S., Gatzy, J. T., Grubb, B., Norman, H., Hummler, E., Koller, B. (1998). Role of gammaENaC subunit in lung liquid clearance and electrolyte balance in newborn mice. Insights into perinatal adaptation and pseudohypoaldosteronism. The Journal of Clinical Investigation, 102(8), 1634–40. doi:10.1172/JCI3971.

Foulks G, Bron AJ. A clinical description of meibomian gland dysfunction. Ocul Surf. 2003;1:107–126.

Fujihara, T., Murakami, T., Fujita, H., Nakamura, M., Nakata, K. (2001). Improvement of corneal barrier function by the P2Y(2) agonist INS365 in a rat dry eye model. Investigative Ophthalmology & Visual Science, 42(1), 96–100.

Korb D.R., Herman J.P., Greiner J.V., Scaffidi R.C., Finnemore V.M., Exford J.M., Blackie C.A., Douglass T. (2005) Lid Wiper Epitheliopathy and Dry Eye Symptoms. Eye and Contact Lens, 31(1), 2-8.

Krueger, B., Schlötzer-Schrehardt, U., Haerteis, S., Zenkel, M., Chankiewitz, V. E., Amann, K. U., Korbmacher, C. (2012). Four subunits (αβγδ) of the epithelial sodium channel (ENaC) are expressed in the human eye in various locations. Investigative Ophthalmology & Visual Science, 53(2), 596–604. doi:10.1167/iovs.11-8581.

Lemp, M. A. (1995). Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. The CLAO Journal : Official Publication of the Contact Lens Association of Ophthalmologists, Inc, 21(4), 221–32.

Levin, M. H., Kim, J. K., Hu, J., & Verkman, A. S. (2006). Potential difference measurements of ocular surface Na+ absorption analyzed using an electrokinetic model. Investigative Ophthalmology & Visual Science, 47(1), 306–16. doi:10.1167/iovs.05-1082.

Ngo W., Situ P., Keir N., Korb D., Blackie C., Simpson T., (2013). Psychometric Properties and Validation of the Standard Patient Evaluation of Eye Dryness Questionnaire. Cornea, (32):1204–1210.

Saboo, U., Amparo, F., Abud, T., Schaumberg, D., Dana, R., (2015). Vision-Related Quality of Life in Patients with Graft-versus-Host Disease. Ophthalmology, 122 (8), 1669-1674.

Schaumberg, D. A., Gulati, A., Mathers, W. D., Clinch, T., Lemp, M. A., Nelson, J. D., Dana, R. (2007). Development and validation of a short global dry eye symptom index. The Ocular Surface, 5(1), 50–7.

Schiffman R.M., Christianson M.D., Jacobsen G., Hirsch J.D., Reis B.L. (2000). Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol., 118:615-621.

Shen M., Li J., Wang J., Ma H., Cai C., Tao A., Yuan Y., Lu F (2009). Upper and lower tear menisci in the diagnosis of dry eye. Invest. Ophthalmol. Vis. Sci., 50:2722-2726

Stern, M. E., Gao, J., Siemasko, K. F., Beuerman, R. W., & Pflugfelder, S. C. (2004). The role of the lacrimal functional unit in the pathophysiology of dry eye. Experimental Eye Research, 78(3), 409–16.

Thelin, W. R., Johnson, M. R., Hirsh, A. J., Kublin, C. L., & Zoukhri, D. (2012). Effect of topically applied epithelial sodium channel inhibitors on tear production in normal mice and in mice with induced aqueous tear deficiency. Journal of Ocular Pharmacology and Therapeutics : The Official Journal of the Association for Ocular Pharmacology and Therapeutics, 28(4), 433–8. doi:10.1089/jop.2011.0157.

Yu, D., Thelin, W. R., Randell, S. H., & Boucher, R. C. (2012a). Expression profiles of aquaporins in rat conjunctiva, cornea, lacrimal gland and Meibomian gland. Exp Eye Res. (103), 22–32.

Yu, D., Thelin, W. R., Rogers, T. D., Stutts, M. J., Randell, S. H., Grubb, B. R., & Boucher, R. C. (2012b). Regional differences in rat conjunctival ion transport activities. Am J Physiol Cell Physiol., 303(7), C767–780.

APPENDIX I: SCHEDULE OF VISITS AND PROCEDURES.

Table 1 Schedule of Visits and Procedures

Event	Visit 1	Visit 2		Visit 3	ET
	Screening Visit	First Treatment Visit (3 to 9 days after Visit 1)	Wash-out (7 to 14 Days)	Second Treatment Visit (7 to 14 days after Visit 2)	Early Termination Visit
Informed Consent	X				
Eligibility Criteria Reviewed	X	X		X	
Medical History/Changes	X	X		X	X
Concomitant Medication/Changes	X[a]	X		X	X
Abbreviated Physical Exam	X				X
Vital Signs	X	X[b]		X[b]	X
Tear Osmolarity	X				
Assessment of Meibomian Glands[c]	X				
Pregnancy Test	X				
Serum Chemistry	X				
Hematology	X				
Dry Eye Symptoms[d]	X	X		X	
Study Medication Administration	N/A	One drop per eye in AM[e]		One drop per eye in AM[e]	
UHR-OCT Assessments[f]	X[g]	X[h]		X[h]	
Keratograph 5M	X[g]	X[h]		X[h]	
Drop Instillation Comfort Assessment[i]		X		X	

Event	Visit 1	Visit 2		Visit 3	ET
	Screening Visit	First Treatment Visit (3 to 9 days after Visit 1)	Wash-out (7 to 14 Days)	Second Treatment Visit (7 to 14 days after Visit 2)	Early Termination Visit
Post Instillation Dry Eye Symptoms Assessment		X[j]		X[j]	
Visual Acuity	X				
Biomicroscopy and External Eye Examination	X	X[k]		X[k]	X
Corneal Staining	X				
Conjunctival Staining	X				
TBUT	X				
Intraocular Pressure	X				
Schirmer's Test	X				
Adverse Event Monitoring	X	X		X	X

a. Concomitant medications taken within 28 days of screening will be reviewed
b. To be completed pre-treatment
c. Meibomian gland assessment to include clinical exam and meibography
d. Dry eye symptom questionnaires (OSDI, SPEED, and SANDE with SANDE part 1 at visit 1, 2 and 3 and Part 2 at Visit 2 and Visit 3 only) will be administered pre-dose and before other procedures.
e. Remove study medication from refrigerator at least 15 minutes before administration
f. Measurementes include: UTMH, LTMH, UTMV, LTMV, and TTMV (UTMV + LTMV)
g. To be measured three times in one hour (at 0 minutes, 30 minutes, and 60 minute interval)
h. Timepoints: Pre dose and post dose at 15±5 minutes, 30±10 minutes, 1 hour±20 minutes, 2 hours±30 minutes, 4 hours±30 minutes, and 6 hours±30 minutes
i. To be completed approximately 5 minutes after dosing
j. To be completed immediately prior to the 2 hour post dose tear volume measurements
k. To be completed after 6 hour tear volume assessment

APPENDIX II: ADVERSE EVENT REPORTING

Adverse Event Definitions

Adverse Event (AE): Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product; which does not necessarily have a causal relationship with this treatment.

Treatment Emergent Adverse Event (TEAE): A treatment emergent AE (TEAE) is an AE that either commenced following initiation of study treatment or was present prior to study treatment but increased in frequency or severity following initiation of study treatment.

A **SERIOUS ADVERSE EVENT (SAE)** is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience (i.e., the subject is at immediate risk of death at the time of the event)
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

A **NON-SERIOUS ADVERSE EVENT** is any adverse event that does not meet the definitions for serious adverse events as described above.

Adverse Event Classification

Each **Adverse Event** will be classified as **SERIOUS** or **NONSERIOUS** using the definitions provided above.

The **INTENSITY** of each Adverse Event will be classified as **MILD, MODERATE, or SEVERE.**

The **CAUSALITY** of the Adverse Event will be classified as **NOT RELATED to study medication, POSSIBLY RELATED to study medication, or RELATED to study medication defined as follows:**

Not related: An event that does not follow a reasonable temporal sequence from administration to the suspected drug, is not a known response pattern to the suspect drug or due wholly to the subject's clinical state or other factors.

Possibly related: An event that follows a reasonable temporal sequence form administration of the study medication, follows a known or expected response pattern to the suspected drug, but that could be explained by the subject's clinical state or other factors.

Related: An event that follows a distinct temporal sequence from administration of the study medication, follows a known or expected response pattern to the suspected drug, and cannot be explained by subject's clinical state or other factors

Serious Adverse Event Reporting

The following information should be provided when an SAE is reported to Parion Sciences:

1. Protocol Number
2. Site ID
3. Subject Initials
4. Subject Number
5. Subject Demographic information, including:
    a. Date of Birth
    b. Sex
    c. Race
6. Start date of study medication administration
7. Date of last dose of study product
9. SAE information, including:
    a. SAE Term (diagnosis only; if known or serious signs/symptoms)
    b. Description of SAE/narrative
    c. Date of onset
    d. Outcome
    e. Date of resolution or death
    f. Duration of SAE if less than 24 hours
    g. Relationship to study medication
    h. Action taken with Study medication
    i. If ocular, which eye(s) were affected
10. Criteria for classifying the event as serious, including whether the SAE resulted in any of the following:
    a. Death

b. Life-threatening

c. In-patient hospitalization or prolongation of existing hospitalization

d. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

e. Congenital anomaly/birth defect

f. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

11. Concomitant medications
12. Relevant history
13. Possible causes of SAE other than study medication
14. Copy of the Adverse Event page from the Case Report Form if the subject has been randomized
15. Signed copy of the SAE form

APPENDIX III: VISUAL ACUITY ASSESSMENT

Visual acuity will be measured by the M&S Technologies Smart System II (Park Ridge, IL). This is a computer generated visual acuity test. The Smart System II comprises a computer processor, using a flat LCD screen monitor, and an interactive keypad controller. The LCD screen monitor is wall-mounted and manufactured to M&S Technologies' specifications, with high resolution and a 450:1 nominal contrast ratio. The examiner uses the keypad to access directly each primary acuity test, specific optotypes, randomization options, and to increase or decrease the size of the optotype on demand. The system allows the examiner to present random letter sequences to preclude subjects from memorizing the sequence to pass each line. The Smart System II can be calibrated for lane lengths of 6 to 22 feet.

APPENDIX IV: BIOMICROSCOPY/EXTERNAL EYE EXAMINATION

Slit lamp biomicroscopy will be performed and the observations will be graded as described below:

Lashes

0= Normal
1= Abnormal

Eyelid

Erythema

0 = Normal, without any redness
+1 = A low grade flushed reddish color
+2 = Diffused redness encompassing the entire lid margin
+3 = Deep diffused reddish color of lid margins and superior or inferior eyelid

Edema

0 = Normal, no swelling of the lid tissue
+1 = Slight diffuse swelling above normal
+2 = General swelling
+3 = Swelling sufficient to close the lid

Conjunctiva

Erythema

0 = Normal
+1 = A flush, pink color predominantly confined to the palpebral or bulbar conjunctiva; slight localized injection
+2 = More prominent red color of the palpebral or bulbar conjunctiva
+3 = Marked redness of the palpebral or bulbar conjunctiva

Edema

0 = Normal, no swelling of the conjunctiva
+1 = Slight diffuse or regional swelling of the conjunctiva
+2 = General swelling of the conjunctiva
+3 = Extensive swelling of the conjunctiva

Tear Film Debris

0 = None; absence of debris
+1 = Mild; presence of debris in inferior tear meniscus
+2 = Moderate; presence of debris in inferior tear meniscus and in tear film overlying cornea
+3 = Severe; presence of debris in inferior tear meniscus and in tear film overlying cornea
presence of mucus strands in inferior fornix or on bulbar conjunctiva
+4 = Very Severe; presence of debris in inferior tear meniscus and in tear film overlying cornea;
presence of numerous AND/OR adherent mucus strands in inferior fornix and on bulbar conjunctiva or filamentary keratitis

Cornea

Endothelial Changes

0 = Normal
+1 = Slight pigment, keratoprecipitates, guttata
+2 = Moderate pigment, keratoprecipitates, guttata
+3 = Dense pigment, keratoprecipitates, guttata

Edema

0 = None, transparent and clear
+1 = Mild, dull glassy appearance
+2 = Moderate, dull glassy appearance of epithelium with large number of vacuoles
+3 = Severe, epithelial bullae and/or stromal edema, localized or diffuse, with or without stromal striae.

Anterior Chamber

Cells

0 = No cells seen
+1 = + cells
+2 = ++ cells
+3 = +++ cells
+4 = ++++ cells, Hypopyon formation

Flare

0 = No Tyndall effect
+1 = Tyndall beam in the anterior chamber has a mild intensity
+2 = Tyndall beam in the anterior chamber is of strong intensity
+3 = Tyndall beam is very intense; the aqueous has a white, milky appearance

Lens Pathology

0 = Normal; no opacity in the lens
1 = Abnormal; existing opacity in the lens; aphakic or pseudophakic eyes or other abnormal findings.

APPENDIX V: FLUROSCEIN TEAR BREAK-UP TIME (TBUT)

Non-preserved fluorescein will be used to conduct this assessment. When conducting all assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

TBUT should be conducted as specified in Appendix I: Schedule of Visits and Procedures.

Fluorescein Tear Break-Up Time

- Perform the examination with the slit lamp at 10X magnification and cobalt blue illumination.
- Draw 5 µL of 2% sodium fluorescein into the micropipette, allowing a drop to form.
- Gently touch the drop at the tip of the delivery dropper to the lower palpebral conjunctiva of the right eye.
- In order to thoroughly mix the fluorescein with the tear film, ask the subject to blink several times and move his/her eye around.
- Using a stop watch, measure the time it takes for the appearance of the first dry spot after blinking.
- Ask the subject to blink again and repeat the measurement of the time it takes for the appearance of the first dry spot after blinking. Repeat this procedure once more for a total of three measurements.

NOTE: If this procedure is completed within two minutes, proceed to measure corneal staining in the same eye as described in Appendix VI without applying more fluorescein.

- Repeat these steps for the left eye.
- Using these three assessments calculate the mean time in seconds for each eye and record this time in the subject's chart as well as the case report form.

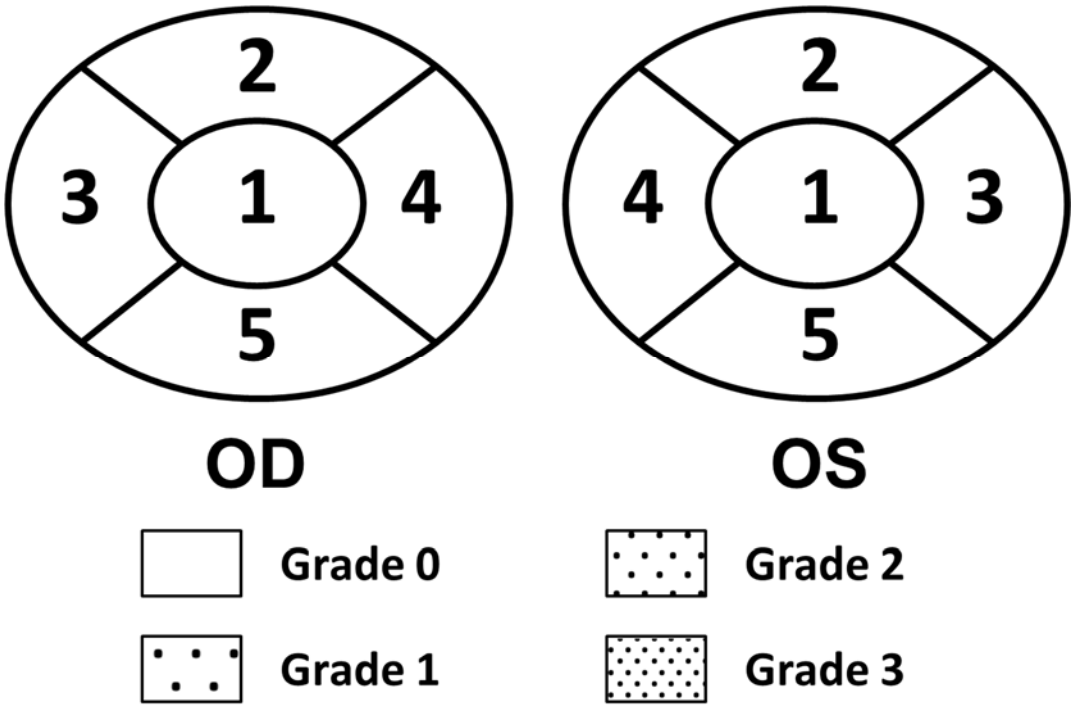
APPENDIX VI: FLUORESCEIN CORNEAL STAINING

The cornea will be stained with non-preserved, 2% fluorescein as described by Lemp et al. (Lemp, 1995). When conducting all assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

Corneal Staining should be conducted as specified in Appendix I: Schedule of Visits and Procedures.

Corneal Staining

- Using a yellow barrier filter (Wratten or Tiffen #11 or #12) and the slit lamp with cobalt blue illumination, with a 3 mm slit width and 10X or 16X magnification.
- Draw a 5 µL drop of fluorescein in the right eye.
- In order to thoroughly mix the fluorescein with the tear film, ask the subject to blink several times and move his/her eye around.
- Wait two minutes.
- Evaluate the cornea for staining referencing the diagram below.
- Punctate staining is recorded using a standard grading system of 0-3 for each of the five areas shown below.
- Repeat this procedure for the left eye.

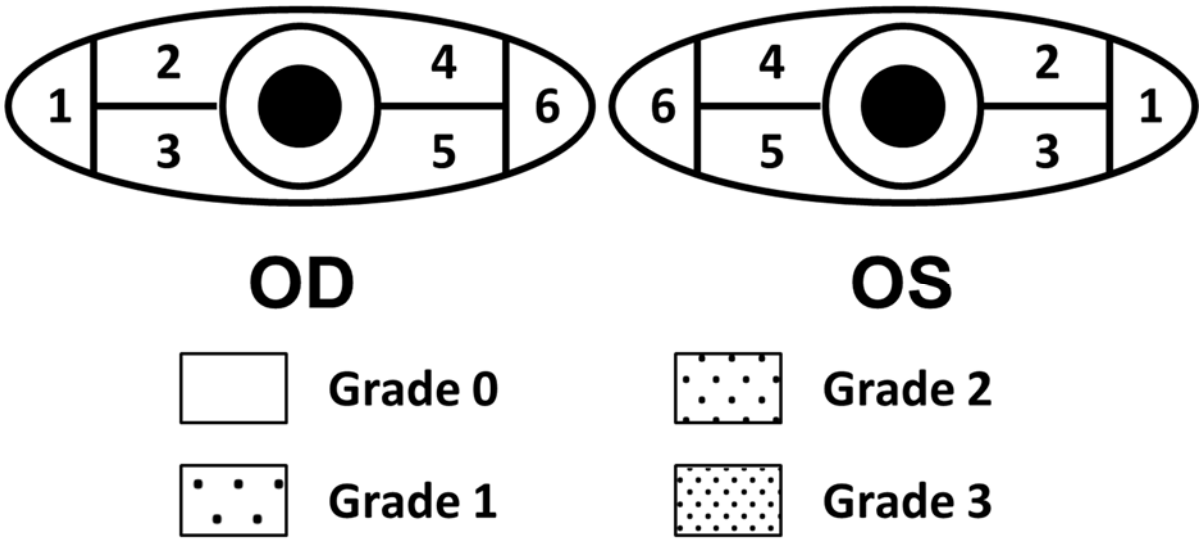


APPENDIX VII: LISSAMINE GREEN CONJUNCTIVAL STAINING

The conjunctiva will be stained with non-preserved, 1% lissamine green as described by Lemp et al. (Lemp, 1995). When conducting all assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

Conjunctival Staining should be conducted as specified in Appendix I: Schedule of Visits and Procedures.

- Without flushing the eye from the previous assessment, instill a 20 µl drop of lissamine green to the right eye using a micropipette, allowing a drop to form.
- Gently touch the drop at the tip of the delivery dropper to the lower palpebral conjunctiva of the right eye.
- In order to thoroughly coat the ocular surface with the lissamine, ask the subject to blink several times and move his/her eye around.
- After 1 minute and before 4 minutes have elapsed, using white light of moderate intensity, grade the areas of the conjunctiva of the right eye with the 0-3 scale shown below.
- Repeat this procedure for the left eye.



APPENDIX VIII: INSTILLATION OF MEDICATION

Study personnel will instill medication at Visit 2 (First Treatment Visit) and Visit 3 (Second Treatment Visit).

Instilling the Medication

- The study personnel will remove the study medication from the refrigerator at least 15 minutes prior to planned administration.
- The study personnel will record the kit number in the subject's records and the Subject ID number on the labels of the kit and vial pouches before proceeding with dosing the subject:
- Confirm that the visit number on the pouch corresponds to the subject's visit
- Open the pouch
- Instruct subject to slightly tilt his/her head back and look upward
- The study personnel should gently squeeze the bottle allowing one drop to coat the ocular surface.
- Repeat procedure for left eye using the same bottle.
- The subject should gently close his or her eyes for a few seconds.
- Use one pouch of assigned study medication for Visit 2 and one pouch for Visit 3.
- The used bottle should be returned to its pouch and the pouch returned to its kit box.
- After dose administration at Visit 2, return the kit containing one used, and one unused bottle to the refrigerator.
- After dosing at Visit 3, return the bottle into the pouch and place the pouch in the kit box. At this time, the kit containing two used bottles can be stored at room temperature before returning to the sponsor for disposal.

APPENDIX IX: DRY EYE SYMPTOM QUESTIONNAIRES

The following subject reported outcomes measurements will be collected as noted in Appendix I:

- SANDE
- SPEED
- OSDI

SYMPTOM ASSESSMENT IN DRY EYE QUESTIONNAIRE (SANDE) (Schaumberg, 2007)

Part 1 (administer at Visits 1, 2, and 3)

PLEASE COMPLETE THE FOLLOWING QUESTIONS REGARDING THE FREQUENCY AND SEVERITY OF YOUR DRY EYE SYMPTOMS

1. Frequency of symptoms:

Please place an '**I**' on the line to indicate how often, on average, your eyes felt **dry and/or irritated** in the past day:

Rarely ——————————————— **All the time**

2. Severity of symptoms:

Please place an '**I**' on the line to indicate how severe, on average, you feel your symptoms of **dryness and /or irritation** were in the past day:

Very Mild ——————————————— **Very Severe**

Part 2 (administer at only Visits 2 and 3)

PLEASE COMPLETE THE FOLLOWING QUESTIONS REGARDING THE FREQUENCY AND SEVERITY OF YOUR DRY EYE SYMPTOMS

1. Frequency of symptoms:

Please place an '**I**' on the line to indicate how often, on average, your eyes feel dry or irritated **now** *compared to at your last visit*

2. Severity of symptoms:

Please place an '**I**' on the line to indicate how severe, on average, you feel your symptoms of dryness and irritation are **now** *compared to at your last visit*

SPEED QUESTIONNAIRE (Ngo, 2013 and Korb, 2005)

1. Report the type of SYMPTOMS you experience and when they occur:

	At this Visit		Within the past 72 hours		Within past 3 months	
Symptoms	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Dryness						
Grittiness or Scratchiness						
Soreness or Irritation						
Burning or Watering						
Fatigue						

2. Report the FREQUENCY of your symptoms using the rating list below:

Symptoms	**0**	**1**	**2**	**3**
Dryness				
Grittiness or Scratchiness				
Soreness or Irritation				
Burning or Watering				
Fatigue				

0 = Never **1** = Sometimes **2** = Often **3** = Constant

3. Report the FREQUENCY of your symptoms using the rating list below:

Symptoms	**0**	**1**	**2**	**3**	**4**
Dryness					
Grittiness or Scratchiness					
Soreness or Irritation					
Burning or Watering					
Fatigue					

0 = No problems
1 =Tolerable; not perfect, but not uncomfortable
2 = Uncomfortable; irritating, but does not interfere with my day
3 = Bothersome; irritating and interferes with my day
4 = Intolerable; unable to perform my daily tasks

OCULAR SYMPTOM DISEASE INDEX (Schiffman, 2000)

Please answer the questions by checking the box that best represents your answer

HAVE YOU EXPERIENCED ANY OF THE FOLLOWING *DURING THE LAST WEEK:*

	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

HAVE PROBLEMS WITH YOUR EYES LIMITED YOU IN PERFORMING ANY OF THE FOLLOWING *DURING THE LAST WEEK:*

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

HAVE YOUR EYES FELT UNCOMFORTABLE IN ANY OF THE FOLLOWING SITUATIONS *DURING THE LAST WEEK:*

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

APPENDIX X: TEAR VOLUME ASSESSMENTS:

Three assessments of tear volume are used in the study: Ultra high resolution optical coherence tomography (UHR-OCT) measuring tear volume and tear meniscus height, lower tear meniscus height measured by Keratograph 5M, and Schirmer test. The sections below briefly describe procedures for each.

Tear meniscus imaged Ultra High Resolution Optical Coherence Tomography (UHR-OCT)

The custom-built ultra-high resolution (3 microns axial resolution) spectral domain OCT can image a full 15mm width scan at up to 48 images per second. The tear meniscus around the upper and lower eyelids can be imaged and measured with the OCT, a unique capability. The OCT images obtained with this system show the tear menisci around both eyelids (Figure 1).

Figure 1: Tear menisci imaged with UHR-OCT

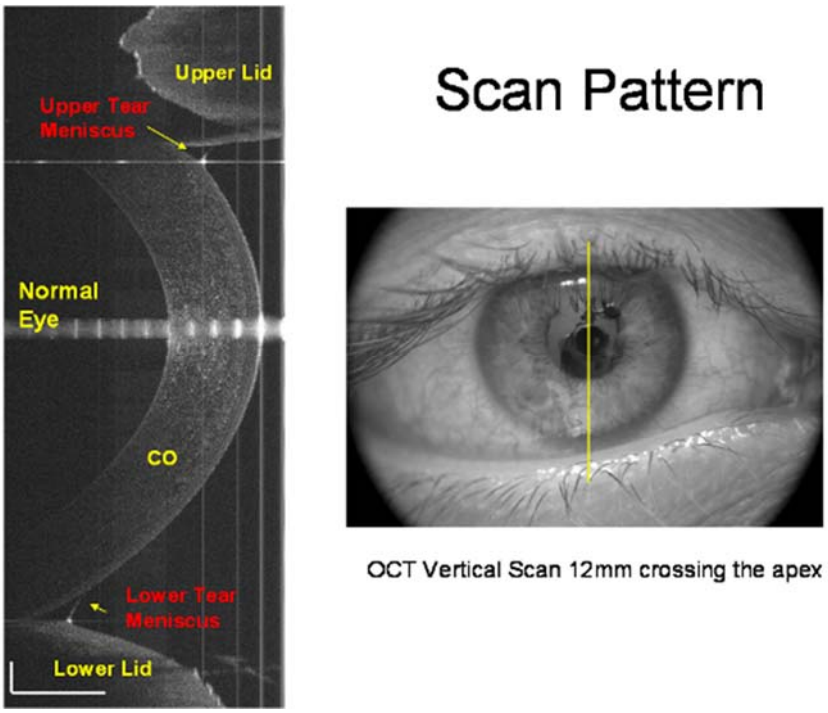


Figure 1: Upper and lower tear menisci imaged with ultra-high resolution optical coherence tomography. A custom built 3 µm resolution ultra-high resolution optical coherence tomography was used to image both upper and lower tear menisci. The boundaries of the tear meniscus were clearly visualized. The bars denote to 500 µm.

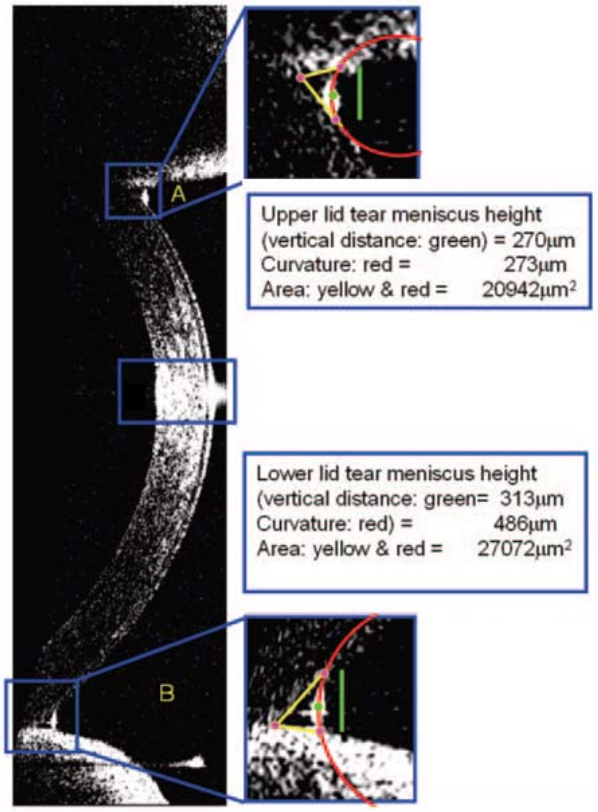
OCT imaging

Each subject will be asked to sit in front of a slit-lamp, on which an OCT probe is mounted. An internal fixation target will be provided to facilitate the subject to keep the eye on a straight forward direction. The central OCT beam as indicated as a green line on the OCT monitor is set to be on the corneal apex (Figure 1), which a specular reflection is normally detected. Immediately after eye opening, corneal images with the OCT will be recorded. A vertical OCT scan with a scan width of up to 13 mm will be used so that the entire cornea and both upper and lower eyelids will be imaged. The measurements will be acquired in one scan with 3 images in the same fashion. The first image will be processed for the measurement. If the first image is not good, the second or third image will be used. The measurements of the image analyzed will be collected in the CRF.

Image process to yield the results.

All meniscus variables of upper (**A**) and lower (**B**) tear menisci are measured with a custom software program with operator inputs (TDanalyer, ver. 1.0) (Figure 2). Touch points (*pink dots*) between two elements (eyelid, cornea, and tear meniscus) and a middle point (*green dot*) of the front edge of the tear meniscus are marked. After that, the software process the image, to yield the results. The three-point method is used to fit a circle to yield tear the meniscus height and area. Tear meniscus height and area of both upper and lower tear menisci will be used. In addition, the eye lid length will be photographied and measured to calculate tear meniscus volumes in both upper and lower tear menisci.

Figure 2: Measuring menisci variables with UHR-OCT



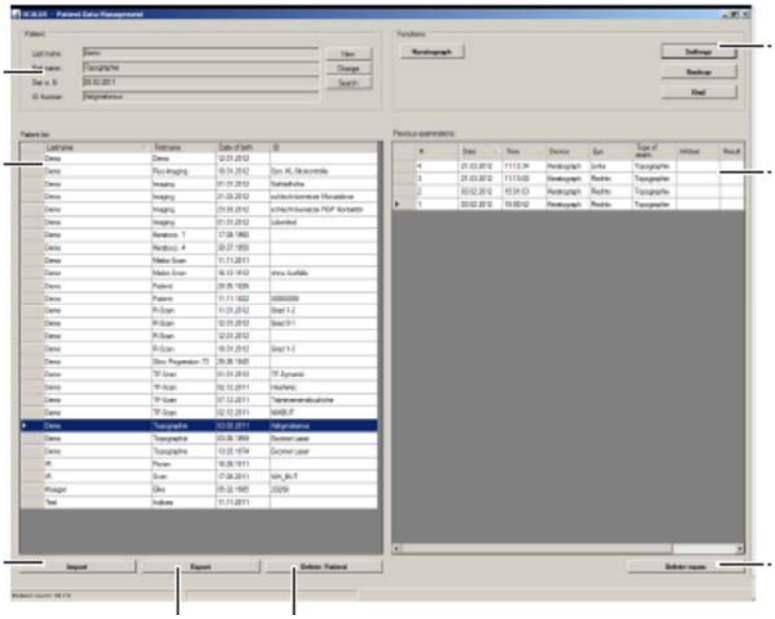
Tear Meniscus Height measured by Keratograph 5M

Evaluation of tear film volume will be assessed via tear meniscus height measurements using the Keratograph 5M. High resolution images of the lower tear meniscus from both eyes are obtained under infrared light as indicated in the Keratograph 5M user manual. Using integrated magnification and measurement tools, the tear meniscus height of the lower lid margin are estimated. Detailed description of the operation of the instrument and software is described in Keratograph 5M user manual provided by Oculus.

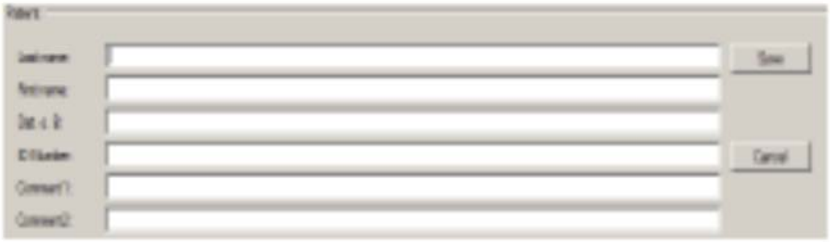
Instructions:

- Turn on the computer. After turning on the computer the operating system loads the Keratograph 5M program. If necessary click on the Keratograph 5M icon  .
- Turn on the Keratograph 5M

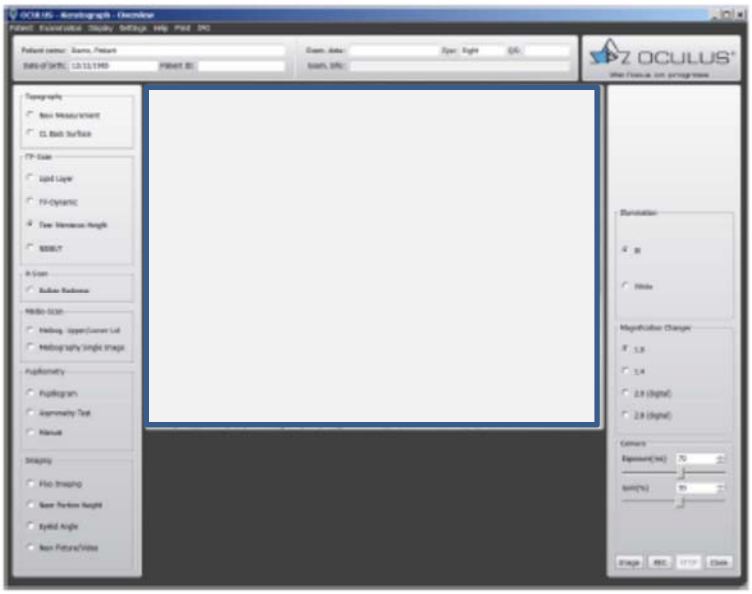
- Enter Subject information in the Patient Data Management Page.



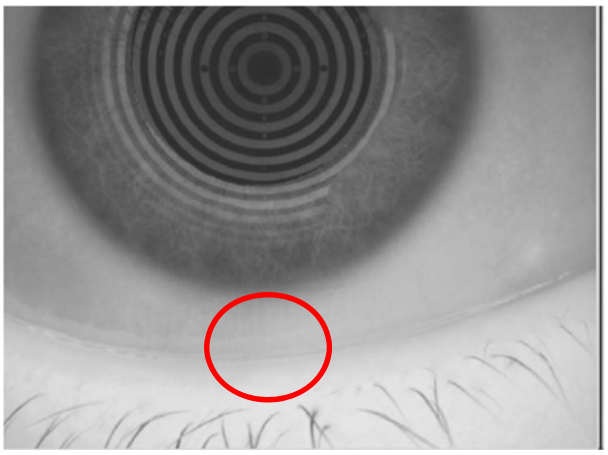
- In the "Patient" box on the top left, select **[New].** The following screen will appear:



- Enter the patient's last name, first name, and date of birth and click **[Save]**.
- Click **[Keratograph]** in the "Functions" box to transition from the Patient Data Management to the Keratograph Overview.
- In the "Examination" menu click **[New]** and the following screen will appear:



- Activate the **[Tear Meniscus Height]** function button on the "TF Scan" Box on the left.
- In the illumination box on the right-hand side of the screen, select **[IR]** lighting.
- If necessary, adjust the camera for optimal focusing on the tear meniscus as seen on the example in the image below (i.e. at approximately the 6:00 position).



- After a blink, focus the camera on the central part of the tear meniscus of the lower eyelid as indicated in the red circle above.
- Click on the **[Image]** button to record the image.
- The magnification changer and camera settings located at the right-hand side of the screen are set to optimal settings. No adjustments should be needed.
- Confirm that the image is in focus at the central area of the eyelid (below the center of the iris), if not, repeat the image.

Note: This procedure is repeated to obtain 3 quality images per eye

Repeat the procedure for the other eye.

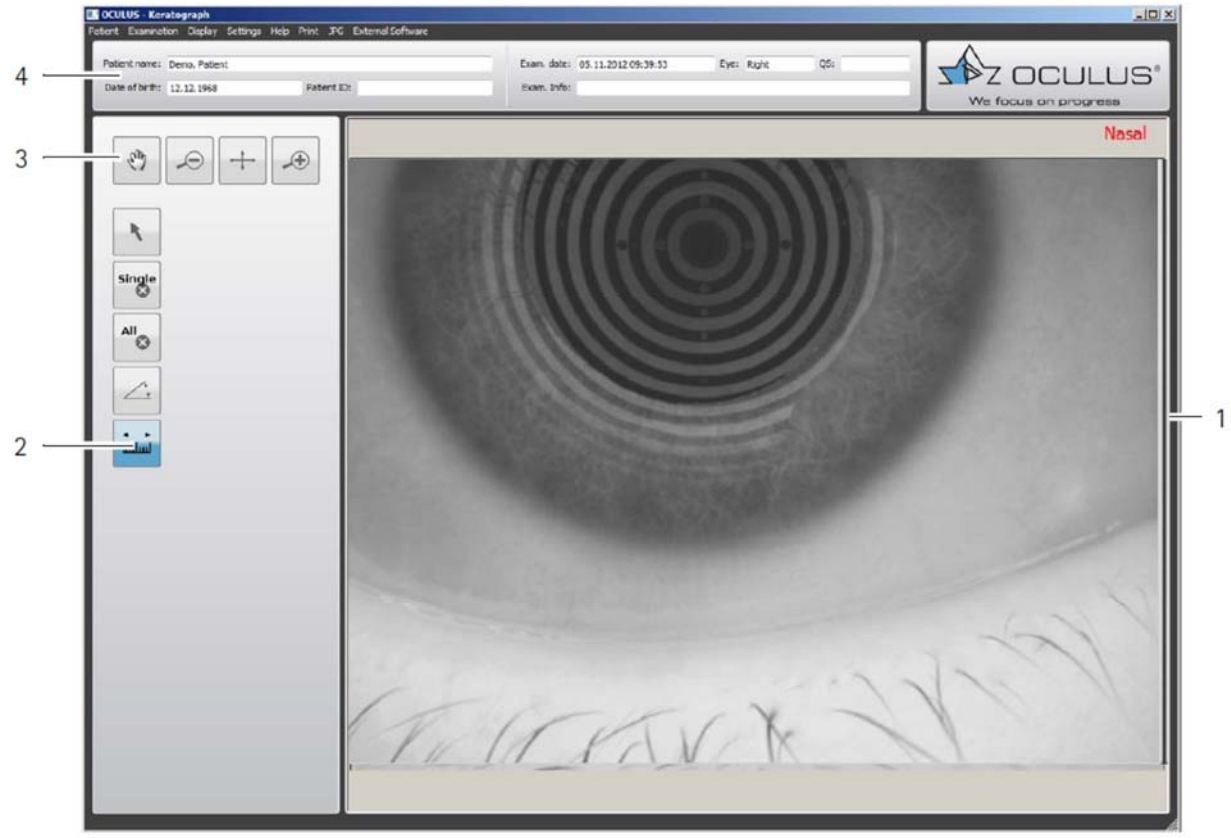
- Select **[Close]** after the three images have been taken.

Measurement of the Tear Meniscus Height (TMH)

- From the three images from each eye obtained above, the tear meniscus height is measured with a software-integrated ruler. The evaluations can be made at different magnification levels. Select a higher magnification if necessary.

If the TMH is not measured at the time the image is captured, select the patient and the assessment from the Patient Data Management Page by double clicking on the assessment on the right of the screen for the selected patient on the left.

The following screen will be displayed:

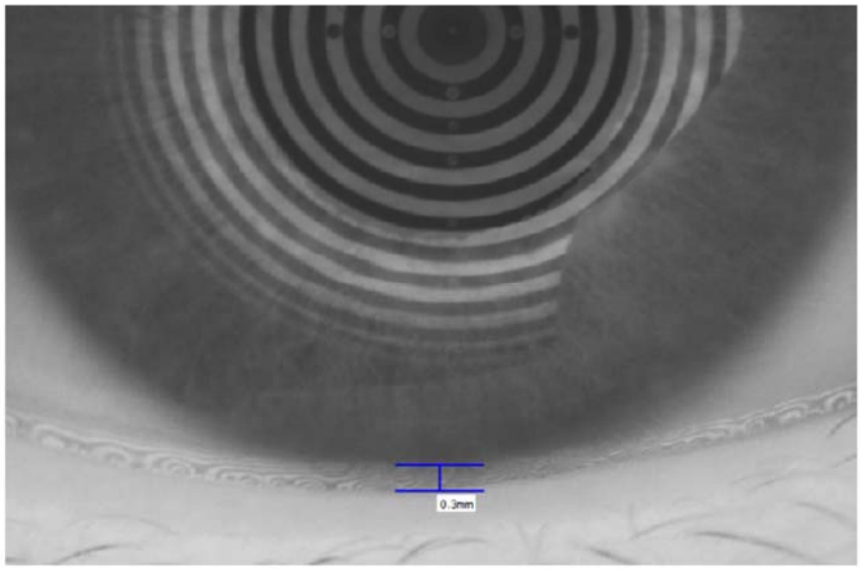


1 *Camera image*
2 *Buttons for tear meniscus measurement*
3 *Buttons to work in the camera image*
4 *Examination and patient data*

- Measure the tear meniscus height with the ruler option.
- Select the following button  from the left of the screen with the left mouse button.

- The button will be highlighted in blue.
- Click once with the left mouse button on the edge of the lower eyelid to position one of the edges of the measurement.
- Move the cursor vertically from this edge to the upper tear meniscus margin and
- click the left mouse button again.

- The measurement length will appear on the image (in millimeters) as indicated below:



- The system will display the real measurement regardless of the magnification used for the measurement.
- If the selection of the tear meniscus height is not accurate, delete the measurement and repeat the procedure.
- Record the distance of the TMH.
- Repeat the procedure for the all three images of each eye.
- Click **[Close]** and the image with the measurement will be saved in the patient file.

Schirmer Test

Schirmer strips will be supplied to the clinical site. When conducting assessments room temperature and humidity should be relatively consistent throughout each visit and throughout the study.

Schirmer Test without Anesthesia

- While still in the plastic sheath, fold the notched end of the Schirmer at the apex of "v". Additionally, fold a partial second fold at the halfway point of the strip so that the strip does not lie directly in the subject's line of sight.
- Remove the right eye strip from the sheath.

- Ask the subject to look up and gently draw the right lower lid in a downward and temporal direction.
- Place the rounded end of the strip toward the temporal one-third of the lower eyelid.
- Repeat this procedure in the left eye.
- Darken slightly the room, and instruct the subject to relax and look at an object in the room while blinking normally or have subjects gently close eyes.
- Strips are removed after five minutes.
- After removing the strips, with a sharp pencil draw a horizontal line across the leading edge of moisture and a second horizontal line across the lowest point of moisture.
- Using a ruler and/or the millimeters recorded on the strips, measure a point halfway between the two lines and record this as the amount of wetting.

APPENDIX XI: POST INSTILLATION QUESTIONNAIRES

DROP INSTILLATION COMFORT MEASUREMENT

Evaluation of the comfort of the eye drop will be conducted approximately 5 minutes following initial dosing at Visits 2 and 3 for subjects.

The subject will respond to the following question upon instillation of the study medication:

"Did you experience any discomfort when the drop was placed in your eyes?"

The response to the question will be graded using the following scale:

1 = None
2 – Mild
3 = Moderate
4 = Severe

Note: The drop instillation comfort response is NOT considered an AE regardless of severity unless it results in discontinuation of the subject from the study.

If the subject experiences discomfort symptoms within 15 minutes after the drop instillation comfort assessment is completed, then the site should record these symptoms as AEs.

POST INSTILLATION DRY EYE SYMPTOMS ASSESSMENT

The subject will respond to the following question immediately prior to the 2 hour post dose tear volume measurements at Visits 2 and 3:

Did you experience any change in your dry eye symptoms after instillation of the eye drop?

-1 = Worsening
0 = No change
+1 = Improvement

APPENDIX XII: MEIBOGRAPHY ASSESSMENT

Meibomian gland evaluation will be conducted in both eyes using the clinical grading scores indicated below for plugging, character of secretions, and ease of expression (modified from Foulks, 2003). In addition, meibomian gland loss as determined by non-contact Meibography as described by Arita (Arita, 2008).

Plugging (nasal third of lower lid)

0 no glands plugged
1 1-2 glands plugged
2 3- 4 glands plugged
3 5 glands plugged

Character of secretion

0 clear
1 turbid
2 turbid with clumps or granular
3 thick or paste-like

Ease of expression

0 easily expressed
1 light pressure
2 moderate pressure
3 heavy pressure

Noncontact meiboscopy (Arita et al[2]): Scored for LL and UL0 no gland loss
1 gland loss 33% of total area
2 gland loss, 33%–67% of total area
3 gland loss >67% of total area

Scores of upper and lower lid summed

Scale range: 0 – 6

APPENDIX XIII: SERUM CHEMISTRY AND HEMATOLOGY ANALYTES

Serum Chemistry Panel includes the following analytes:

- Sodium
- Potassium
- BUN/Urea
- Creatinine
- Glucose
- Calcium
- Phosphorus
- Total Protein
- Albumin
- AST(SGOT)
- ALT(SGPT)
- Alkaline Phosphatase

Hematology Panel includes:

- WBC
- Haemoglobin
- Haematocrit
- RBC
- MCV
- MCH
- MCHC
- RDW
- Platelet Count
- Differential (% and absolute)

Serum pregnancy test in women of child bearing potential.

APPENDIX XIV: DOSE SELECTION FOR PHASE 2 AND DATA REVIEW DURING STUDY

The decision on dosage strength to be used in Phase 2 will be the responsibility of the Data Review Group constituted by the Prinicipal Investigator (or designee), Medical Monitor and representative(s) of Parion Sciences not involved in the conduct of the study. The Data Review Group may request the participation of an independent statistician to assest in the assessment of the safety of the study medication and the dosage strength selection for Phase 2.

The Data Review Group will review unmasked data in order to make a decision on the dose to be administered in Phase 2. The Data Review Group will convene when approximately 8 subjects have completed the study,or earlier if needed. The trial will continue enrolling during the time the review by the Data Review Group is being prepared and being held. The Data Review Group will review the following to assist in making their recommendation for dose selection in Phase 2:

- Adverse events
- Biomicroscopy and external eye examination
- Drop instillation comfort assessments
- Abnormal alert laboratory results
- Tear volume via OCT
- Tear volume via Keratograph 5M

Recommendations by the Data Review Group may include:

1) The dose level may be increased to 0.05% in Phase 2 in the event that:
    a) No cases of serious, suspected related, unexpected events are noted in Phase 1 AND
    b) No cases of serious ocular safety events are noted in Phase 1
    c) Lack of evidence that P-321 produced an increase in tear volume based on OCT and/or Keratograph 5M, or evidence that additional improvements in tear volume (or the duration of effect) may be obtained with a higher dose
2) The dose level may be decreased to 0.01% in Phase 2 in the event that:
    a) There is evidence of a significant increase in tear volume and duration of effect in Phase 1 based on OCT and/or Keratograph 5M
    b) No cases of serious, suspected related, unexpected events are noted in Phase 1
    c) No cases of serious ocular safety events are noted in Phase 1
3) The dose level may remain at 0.017% (the same as in Phase 1) in the event that:
    a) There is no compelling data to either increase or decrease the strength for Phase 2.
    b) No cases of serious, suspected related, unexpected events are noted in Phase 1
    c) No cases of serious ocular safety events are noted in Phase 1
4) Discontinue the study.
5) Amend the protocol

The Data Review Group will make a recommendation to the Parion Sciences following the meeting and review of data and via a written memo. If additional data is required, the Data Review Group may request additional data for their review. If the Data Review Group requires

additional unmasked data in order to safeguard the safety of the study subjects, this data will be provided.

The Data Review Group will make every effort to arrive to a decision by consensus.
Upon receipt fo theData Review Group recommendation, Parion representatives will make a decision that is consistent with the safeguard of the interests of study subjects.

Additional Data Reviews

Additional unmasked reviews of subject data may occur throughout the study to make recommendations on further dose adjustments and for planning purposes. These reviews will follow the procedures outlined above.

Unmasking the study

In the event of a medical emergency where breaking the mask of the study is being considered, the Principal Investigator may request unmasking by contacting the Medical Monitor and the Parion Representative. If the Medical Monitor and Parion Representative grant the unmasking, the unmasking will be clearly documented with the date the code is broken indicated, who authorized the unmasking and other details regarding the unmasking. A copy of this unmasking memo will be provided to the sponsor for their records and a copy should remain in the source.