Clinical Protocol P-321-202

Project Number	**P-1003-I101**
Compound Number/ Name	**P-321 Ophthalmic Solution**
Protocol Number	**P-321-202**
Protocol Title	**Randomized, Double-Masked, Parallel Group Study of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Assessing Safety and Efficacy Over 28 Days**
Sponsor	**Parion Sciences, Inc.** **2800 Meridian Parkway** **Suite 195** **Durham, NC 27713**
Medical Monitor	[redacted]
Authors	
Issue Date	**Original: Version 1.0 released 29 April 2016** **Amendment 1.0: Version 2.0 Released 10 February 2017**

Sponsor Signature and Date _____

PARION SCIENCES, INC.
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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):

_____

Investigator Signature:

_____ _____
Date

Summary of Changes to the Protocol

The previous version of this protocol (Version 1.0, 29 April 2016) was amended to create the current version (Version 2.0, 10 February 2017). The protocol history is provided below.

Protocol History	
Version and Date of Protocol	**Comments**
Version 1.0, 29 April 2016	Original version
Version 2.0, 10 Feburary 2017	Current version

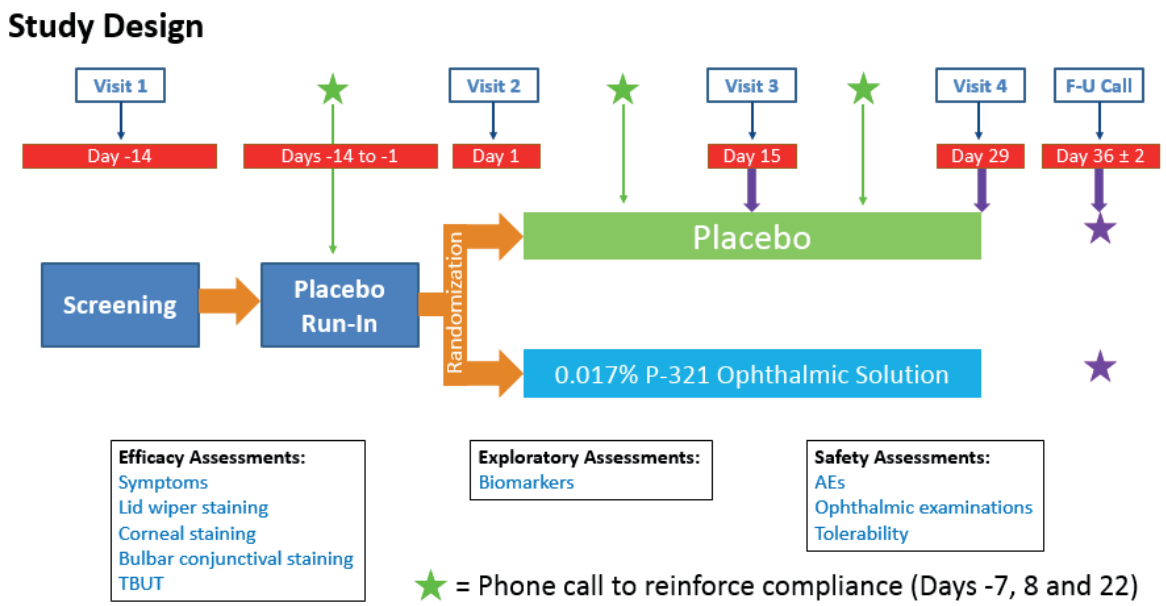
Key changes in the current version of the protocol are summarized below. Typographical and administrative changes were also made to improve the clarity of the document.

Change	**Affected Sections**
Updated the study period and anticipated date of first enrolled subject enrolled	Synopsis
Updated number of centers and number of screened patients. Clarified number of completed patients.	Synopsis, Sections 3.5, 5.1, 11.2
Modified the inclusion criteria to require Schirmer and Staining in at least one eye, not both	Synopsis, Section 6.1
Modified the exclusion criteria to exclude Meibomian Gland Dysfunction in the Study Eye	Synopsis, Section 6.2
Modified exclusion criteria to allow external blepharoplasty, not resulting in exposure or abnormal blinking.	Synopsis, Section 6.2
Added definition of qualifying eye, Study Eye, Fellow Eye.	Synopsis, Section 5.1, 6.4, and 11.1
Modified the statisitical analysis description to analyze the Study Eye as primary and supportive analyses with the qualifying eye	Synopsis, Sections 5.1, 6.4 and 11
Updated Other assements to be Exploratory as listed elsewhere.	Synopsis study design figure and Figure 1
Added clarification to exclusion of topical steroids to state topical ocular and intranasal steroids	Synopsis, Sections 6.2 and 8.9.2
Clarified that the Follow up phone call is made 7±2 days or 5-9 days after Visit 4	Synopsis, Section 5.3
Limited the exclusion of lid scrubs to the past 28 days and removed the exclusion of lid massage to treat MGD or anterior or posterior blepharitis	Section 6.2
Added lifitegrast (Xiidra) as an exclusion	Synopsis, Sections 6.2 and 8.9.2
Removed the requirement for “without anesthesia” from the IOP assessment	Section 7.1
Removed the requirement for height to be recorded at the Early Termination Visit.	Section 7.9
Clarified that all physical exams include an examination of eyes, ears, nose and throat and added EENT to list of abbreviations	Abbreviations, Sections 7.1, 7.9 and 10.5
Removed “Information to submit when reporting an SAE to Parion Sciences, Inc. is located in Appendix II”.	Section 10.1.1
Clarified that Drop Instillation Comfort Assessment should be performed in the Fellow Eye	Sections 7.1, 7.3, 7.5, 10.8, Appendix I, Appendix III, Appendix VI
Clarified that tears for prostaglandins and impression cytology will be done in the Study Eye and MMP-9 will be done in the Fellow Eye	Sections 7.3, 7.7, 9.2.4, and 11.4.4, Appendix I,
Modified the exclusion criteria to allow low dose (81mg) aspirin per day	Sections 6.2, 7.1.1, 8.9.2
Modified the randomization crtieria so that both must be met in the Study Eye only	Section 6.3
Modified exclusion criteria to prohibit medications with significant anticholinergic activity, however allow SSRIs If the subject is on a stable dose for 28 days prior to Visits 1 and during the study	Abbreviations, Sections 6.2 and 8.9.2
With regards to the treatment kit, the following clarifications were made : • Removed the wording “to cover visit window variability”. • Removed “treatment number” from the kit label	Section 8.2

1 PROTOCOL SYNOPSIS

Name of Sponsor: Parion Sciences, Inc.		**Study Medication:** 0.017% P-321 Ophthalmic Solution
Protocol Number: P-321-202	**Phase:** 2b	**Indication:** Treatment of dry eye disease
Title of the Study: Randomized, Double-Masked, Parallel Group Study of P-321 Ophthalmic Solution Compared to Placebo in Subjects with Dry Eye Disease Assessing Safety and Efficacy Over 28 Days		
Study Center: Approximately 7 centers in the United States		
Study period (FPFV – LPLV): Approximately 6 months Anticipated first subject enrolled: 4Q2016 Anticipated last subject complete: TBD		
Objectives: **Primary objective** • To evaluate the effect of treatment with 0.017% P-321 on Dry Eye Symptoms **Secondary objective** • To assess changes in conjunctival staining of the Lid Wiper area • To assess changes in corneal staining • To assess changes on the bulbar conjunctival staining • To assess changes in TBUT • To monitor safety and tolerability of P-321 Ophthalmic Solution through adverse events, biomicroscopy and external eye examination, and other safety assessments Exploratory objectives include expression of biomarkers in the bulbar conjunctiva, lid wiper, and in tears.		

Study Design:



This is a multi-center, randomized, double-masked, placebo-controlled, parallel group Phase 2b trial designed to evaluate symptoms and signs in subjects with mild to moderate dry eye disease. Subjects will be screened for inclusion in the study after signing written informed consent to participate. Those subjects who are eligible to continue, will have the first dose of study medication (single-masked [subject] placebo for all subjects) instilled into both eyes while at the clinic and then provided with sufficient study drug supplies for three times daily (TID) instillation into both eyes over the 2-week placebo run-in period. Subjects will return to the clinic for Visit 2 (Randomization and Treatment Day 1). Those subjects who meet randomization criteria will be randomly assigned in a double-masked, 1:1 ratio to either 0.017% P-321 Ophthalmic Solution or placebo (vehicle) TID for 28 days. The first dose of double-masked study drug will be instilled in the clinic once all assessments have been completed and the subject will be sent home with study drug for another 2-week period. Subsequent study visits will occur on Treatment Day 15 and after 28 days of treatment on Day 29 (Visits 3 and 4, respectively) followed by a post-treatment follow up phone call. Three phone calls to remind subjects of dosing regimen and to inquire about adverse effects will also be done during the study and will occur between Visits 1 and 2, Visits 2 and 3, and Visits 3 and 4.

This study is designed to evaluate the changes in symptoms, conjunctival lid wiper staining, corneal staining, bulbar conjunctival staining, and tear break-up time (TBUT) in subjects administered 0.017% P-321 Ophthalmic Solution or Placebo for 28 days. Subject-completed dry eye symptom questionnaires including the SANDE, SPEED, and a 7-item symptom questionnaire will be completed at each visit prior to other assessments. In addition, the expression of biomarkers of inflammation will be measured in tears, and in impression cytology samples collected from the lid wiper and the bulbar conjunctiva.

Safety will be assessed throughout the study by adverse events, best corrected visual acuity (BCVA), biomicroscopy and external eye examination, physical examinations, vital signs, drop instillation comfort assessments, and clinical laboratory tests. Subjects will be asked to stop or withhold certain medications and not wear contact lenses during the study.

Subject Population: It is anticipated that approximately 125 subjects with dry eye will need to be screened in order to randomize 65 subjects and complete approximately 60 subjects.

Key Inclusion and Exclusion Criteria: Male and female subjects 18-80 years of age who provide written informed consent and have a history of dry eye signs and symptoms supported by a previous clinical diagnosis will be included in the study. Additionally, eligible subjects will have documented daily use of topical lubricants for at least during the past 28 days, symptoms measured by the SANDE questionnaire of scale of >20 but less than 90 for both symptom severity and symptom frequency, Schirmer's test >1mm and <10mm in at least one eye, corneal staining of ≥2/15 and ≤10 on National Eye Institute (NEI) industry scale with a ≥2 in at least one region in at least one eye, bulbar conjunctival staining score of ≥2/18 and ≤10 on NEI industry scale with a ≥2 in at least one regionin at least one eye, normal lid anatomy, are able to comply with medication requirements for eligibility. Additionally, throughout the study, subjects must be able to withhold ocular topical medications (including artificial tears, topical ocular and intranasal steroids, ocular antihistamines, 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; must not have undergone refractive eye surgery (e.g., LASIK) in either eye during the past 12 months or cataract surgery in either eye during the past 3 months or 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, severe Meibomian Gland Dysfunction (MGD) in the study eye, a history of glaucoma or intraocular pressure > 25 mmHg at the Screening Visit (Visit 1) or a history of elevated intraocular pressure (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 permanent punctal occlusion, or history of nasolacrimal duct obstruction (punctal plugs are allowed, however, if a plug is lost, it must be promptly replaced), Stevens-Johnson syndrome, ocular cicatricial pemphigoid (OCP), or other ocular cicatrizing disorders, past or present exposure keratopathy, neurotrophic keratopathy, lagophthalmos, or trichiasis. Eligible subjects will have at least one eye that meets the entry criteria (Section 6.1 and Section 6.3). This will be referred to as the Study Eye. For the purpose of efficacy analysis in this trial, each subject will have only one Study Eye. If a subject has only one qualifying eye, this eye will be the Study Eye. If both eyes of the subject qualify, then the Study Eye will be the eye with the highest total corneal staining score. If the scores for total corneal staining are the same, the Study Eye will be the right eye. The non-Study Eye is referred to as the Fellow Eye.

Number of Subjects Randomized: Approximately 65 randomized to complete 60 subjects	**Number of Centers:** Approximately 7
Test Product and Doses: 0.017% P-321 Ophthalmic Solution	**Route of Administration:** Ocular instillation, one drop per eye TID for 28 days
Reference Product and Doses: Placebo (vehicle)	**Route of Administration:** Ocular instillation, one drop per eye TID for 14 days in the run-in period and for 28 days in the double-masked treatment period
Duration of Treatment: Eligible subjects will receive placebo TID in both eyes from Visit 1 and through the evening before Visit 2 (for 14 days). Starting at Visit 2 (Randomization – Treatment Day 1) and continuing through the day before Visit 4, subjects will receive 0.017% P-321 Ophthalmic Solution or Placebo TID in both eyes. A follow up call will be scheduled 5-9 days after the Visit 4. Total duration of the study is expected to be approximately 50 days including placebo-run-in, treatment and follow up. Three phone calls to remind subjects of dosing regimen and to inquire about adverse effects will also be done during the study and will occur between Visits 1 and 2, Visits 2 and 3, and Visits 3 and 4.	
Criteria for Evaluation:	

This study will evaluate the efficacy and safety of P-321 Ophthalmic Solution and Placebo in subjects with dry eye. Background characteristics will be evaluated to better characterize subjects at entry including: demographics, BCVA, dry eye symptoms upon entry, osmolarity, meibography (if available), meibomian gland evaluation, Screening IOP and Schirmer's test, medical history, and concomitant medication use.

Efficacy

Symptoms and signs will be assessed throughout the study. Symptoms will be assessed by subject-reported symptom questionnaires (SANDE and SPEED questionnaires and a 7-item symptom questionnaire). Signs will be assessed by staining of the cornea, bulbar conjunctiva, the lid wiper region of the upper eyelid and by TBUT. Exploratory assessments will include biomarkers of inflammation in tears and from impression cytology samples collected from the lid wiper and the bulbar conjunctiva.

Safety

Safety will be assessed throughout the study by adverse event monitoring, biomicroscopy and external eye examination, BCVA, physical examinations, vital signs, drop instillation comfort assessments, and clinical laboratory tests.

Statistical Analysis:

Sample Size

The sample size estimation was based on assumptions about the standard deviation and treatment effect for the change from baseline SANDE global score from a previous study, protocol P-321-101. At Day 28, the pooled standard deviation for the change from baseline was 22 and the difference in means was 20, representing a standard effect size of 0.9. If the true standard deviation is 22, a sample size of 30 subjects per group will provide at least 90% power to detect a true difference in means of 20 using a two-sided hypothesis test with Type 1 error of 5%. All else being equal, this sample size provides at least 70% power if the treatment difference is at least 15 units or if the standard deviation is no more than 29.

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 a two-sided t-test. 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.

Efficacy

Efficacy will be assessed by changes from baseline to Day 29 in symptom scores (SANDE, SPEED, and the 7-item symptom questionnaire) as well as changes from baseline to Day 29 in signs of dry eye including TBUT, bulbar conjunctival staining, corneal staining, and staining in the lid wiper region via analysis of covariance (ANCOVA) with fixed effects of treatment, study site, and respective baseline score. Analyses of efficacy will be conducted using the study eye. A supportive efficacy analysis will be also conducted utilizing all qualifying eyes.. Additional details for these analyses will be defined in the Statistical Analysis Plan. Analyses of efficacy conducted on all qualifying eyes will account for the correlation between eyes within subjects with two qualifying eyes.

Safety

All safety analyses will be conducted for all eyes of subjects who received at least one dose of study medication. The safety of the run-in and double-masked treatment periods will be analyzed separately.

The incidence of AEs will be tabulated by treatment group, by severity, and by relationship to study medication. Additionally, changes in BCVA, biomicroscopy and external eye examination, physical examinations, vital signs, drop instillation comfort assessments, and clinical laboratory tests will be examined.

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2 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of covariance
BCVA	Best Corrected Visual Acuity
BID	Two times daily
C	Celsius
CFR	Code of Federal Regulations
CRF	Case report form
EC	Ethics Committee
EENT	Eye, ear, nose, throat
ENaC	Epithelial sodium channel
ETDRS	Early Treatment of Diabetic Retinopathy Study
F	Fahrenheit
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
GLP	Good Laboratory Practices
ICH	International Conference on Harmonization
IOP	Intraocular pressure
IRB	Institutional Review Board
I_{sc}	Short-circuit current
IV	Intravenous
LASIK	Laser-Assisted In-Situ Keratomileusis
MGD	Meibomian Gland Dysfunction
mITT	Modified Intent-to-Treat
NEI	National Eye Institute

NOAEL	No-observed-adverse-effect level
NSAID	Non-steroidal anti-inflammatory drug
OCP	Ocular cicatricial pemphigoid
OD	Right eye (oculus dextrus)
OS	Left eye (oculus sinister)
OU	Each (both) eyes (oculus uterque)
OTC	Over-the-counter
PD	Potential difference
PI	Principal investigator
PK	Pharmacokinetic
PRN	As needed (pro re nata)
QID	Four times daily
SAE	Serious adverse event
SANDE	Symptom Assessment in Dry Eye questionnaire
SAP	Statistical Analysis Plan
SPEED	Standard Patient Evaluation of Eye Dryness questionnaire
SSRI	Selective Serotonin Re-uptake Inhibitor
TBUT	Tear break-up time

3 BACKGROUND INFORMATION

3.1 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.

3.2 Non-Clinical Studies for P-321 Ophthalmic Solution

3.2.1 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.

3.2.2 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 half-life ($t_{½}$) 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 40-fold larger than the dose to be given to the subjects in this study. 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 4-fold larger than the dose to be given to the subjects in this study.

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. The PK profile of P-321 is consistent with the long duration of its pharmacodynamic activity on the ocular surface.

3.2.3 Toxicology

Multiple toxicology studies have been conducted using P-321 Ophthalmic 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 human dose of this study (0.020 mg/eye/day) does not exceed the no-observed-adverse-effect levels (NOAEL) of 0.8 mg/eye/day in rabbits and 0.024 mg/eye/day in dogs . The NOAEL of 0.05 mg/kg/day in rats administered P-321 IV exceeds the potential exposure from the proposed ocular dose of $6.6x10^{-4}$ mg/kg/day (assuming bilateral dosing of 0.017% P-321, TID with a 40 µl drop size in a 60 kg person) by 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.

3.3 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 (Protocol P-321-101). 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 the above 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 observed in this study.

Additional details regarding the clinical and nonclinical experience with P-321 Opthalmic Solution can be found in the Investigator's Brochure.

3.4 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 favorable safety and tolerability of the first clinical study (including no evidence of systemic exposure) and the results of preclinical safety studies with the most sensitive species.

This study is a 28-day, multi-site, randomized, double-masked, parallel group study of the efficacy and tolerability of 0.017% P-321 Ophthalmic Solution compared to Placebo TID in subjects with dry eye disease. This dose and duration are covered by the safety profile obtained in the most sensitive species (dog).

3.5 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 of the 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. 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.

The objective of the current study is to evaluate the changes in symptoms (primary objective) and signs of dry eye as well as safety and tolerability (secondary objectives) in subjects with dry eye who are treated with either 0.017% P-321 Ophthalmic Solution or Placebo (vehicle) TID over 28 days. Approximately 65 subjects with dry eye disease will be randomized in this multi-center, randomized, double-masked, placebo controlled, parallel group study to complete 60 subjects.

4 TRIAL OBJECTIVES AND PURPOSE

The primary objective of this trial is to evaluate the efficacy of 0.017% P-321 Ophthalmic Solution taken TID in subjects with mild to moderate dry eye disease after 28 days of treatment. Secondary objectives are to evaluate the safety and tolerability of 0.017% P-321 taken TID in subjects with mild to moderate dry eye disease. In addition, exploratory objectives include expression of biomarkers of inflammation in tears and from impression cytology samples collected from the lid wiper and the bulbar conjunctiva.

5 INVESTIGATIONAL PLAN

5.1 Overall Study Design

This is a multi-center, randomized, double-masked, placebo-controlled, parallel group Phase 2b trial designed to evaluate symptoms and signs in subjects with mild to moderate dry eye disease. It is anticipated that approximately 125 subjects with dry eye will need to be screened in order to randomize about 65 subjects and complete approximately 60 subjects. Screened subjects will be enrolled in a single-masked placebo run-in period for up to 14 days. At the end of the run-in period, eligible subjects will be randomly assigned in a double-masked fashion to receive either 0.017% P-321 Ophthalmic Solution or Placebo TID for 28 days. The study will consist of four study visits and a follow up phone call: Visit 1 (Screening Visit), Visit 2 (Randomization and Treatment Day 1), Visit 3 (Treatment Day 15) and Visit 4 (Day 29, after 28 days of treatment) and a follow up phone call 5-9 days later. Three phone calls to remind subjects of dosing regimen and to inquire about adverse effects will also be done during the study and will occur between Visits 1 and 2, Visits 2 and 3, and Visits 3 and 4. The placebo-run-in is single-masked in that the subjects will remain masked to the placebo run-in, however the treatment is known to the investigator, medical monitor, study site personnel, and those involved in the conduct of the study. The randomized treatment assignments are double-masked in that the treatment will be masked to the investigator, medical monitor, study site personnel, subjects in the study and those involved in the conduct of the study.

This study is designed to evaluate the changes in symptoms and signs of dry eye disease including conjunctival staining of the lid wiper area of the upper eyelid (with photographs if technology is available), corneal staining, bulbar conjunctival staining, and TBUT. Dry eye symptom questionnaires including the Symptom Assessment in Dry Eye questionnaire (SANDE), the Standard Patient Evaluation of Eye Dryness (SPEED) questionnaire, and a 7-item symptom questionnaire will be completed at each visit prior to other assessments. Exploratory assessments of expression of biomarkers of inflammation in the tears and and from impression cytology samples collected from the lid wiper and the bulbar conjunctiva.

Safety will be assessed throughout the study by adverse event monitoring, biomicroscopy and external eye examination, best corrected visual acuity (BCVA), physical examinations, vital signs, clinical laboratory tests, and an assessment of comfort after taking the medication in-clinic.

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

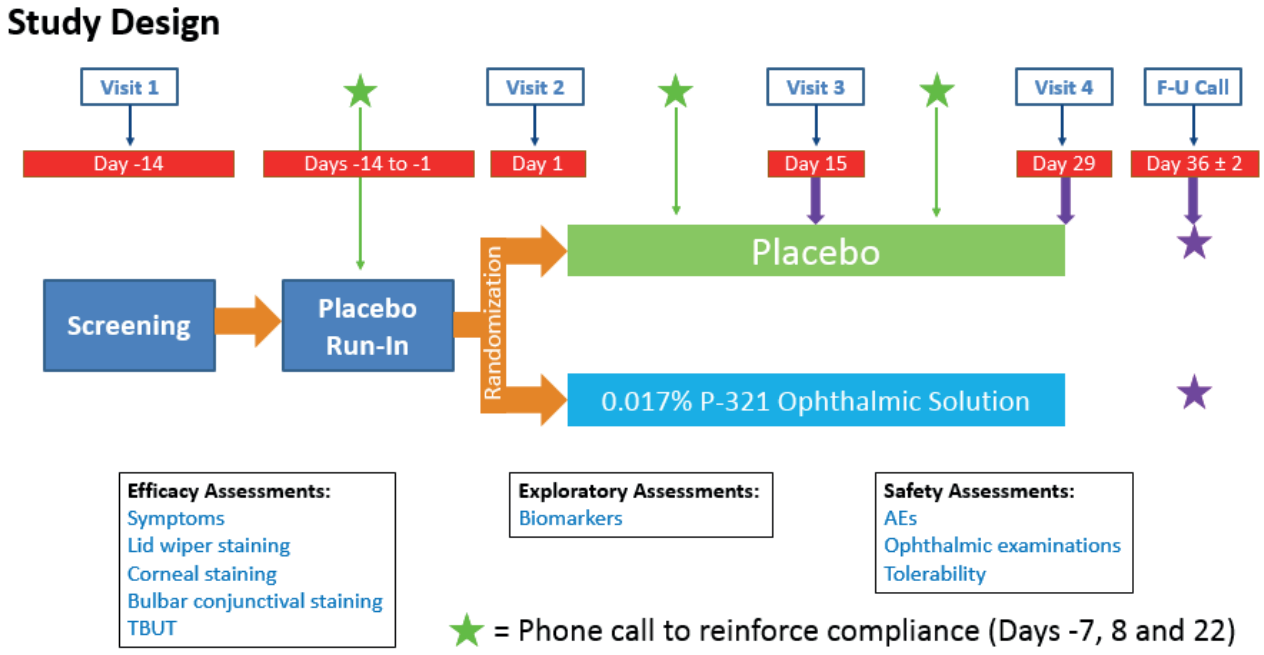
A schematic of the study design is shown in Figure 1.

Study Eye

Eligible subjects will have at least one eye that meets the entry criteria (Section 6.1 and Section 6.3). This will be referred to as the Study Eye. For the purpose of efficacy analysis in this trial, each subject will have only one Study Eye. If a subject has only one qualifying eye, this eye will be the Study Eye. If both eyes of the subject qualify, then the Study Eye will be the eye with the highest total corneal staining score at Visit 1. If the scores for total corneal staining are the same, the Study Eye will be the right eye. The non-Study Eye is referred to as the Fellow Eye.

If at Visit 2 the Study Eye does not qualify based on randomization criteria, but the subject had both eyes qualify at Visit 1 and the other eye continues to qualify based on randomization criteria, that eye will become the Study Eye.

Figure 1 Study Design Protocol P-321-202



5.2 Endpoints

5.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint for this study is the change from baseline (Visit 2) to Day 29 (Visit 4) in the subject-completed SANDE questionnaire global symptom score from Part 1.

5.2.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include:

- Change from baseline to Day 29 in the SPEED questionnaire total symptom score
- Changes from baseline to Day 29 in each of the 7-item symptom questionnaire scores (see Appendix)
- Change from baseline to Day 29 in staining of the Lid Wiper area
- Change from baseline to Day 29 in bulbar conjunctival staining total score
- Change from baseline to Day 29 in corneal staining total score
- Changes from baseline to Day 29 in TBUT
- Change in symptom frequency score and severity score as recorded by the subject using the SANDE Part 2 assessment

- Change from baseline to Day 29 in the SANDE symptom frequency and severity scores from Part 1
- The proportion of subjects with at least a 20% improvement from baseline to Day 29 in the SANDE global symptom score from Part 1 (and in the frequency and severity scores)
- The proportion of subjects with an improvement following 28 days of treatment in symptom frequency score and severity score using the SANDE Part 2 assessment

Changes from baseline to Day 15 will also be assessed.

Exploratory assessments include biomarkers of inflammation in tears and from impression cytology samples collected from the lid wiper and the bulbar conjunctiva.

5.2.3 Safety and Tolerability Assessments

Safety assessments include adverse events (AEs), biomicroscopy and external eye examination, BCVA, physical examinations, vital signs, and clinical laboratory tests.

5.3 Duration of Participation

The study will consist of four study visits and a follow up phone call: Visit 1 (Screening Visit, Day -14), Visit 2 (Randomization and Treatment Day 1; Day 1), Visit 3 (Treatment Day 15) and Visit 4 (Day 29, after 28 days of treatment) and a follow up phone call 5-9 days after Visit 4. The total duration of study participation is approximately 50 days.

5.4 Methods to Minimize Bias

The study will consist of a single-masked placebo run-in followed by a double-masked treatment period. During the single-masked run-in the subject will remain masked to treatment. To minimize bias, the treatment period regimen will be randomized and double-masked. In addition, all subject-rated symptom assessments will be completed at each visit prior to any other assessments so that the results and/or interaction with the sites will not bias the subject's responses on the symptom questionnaires.

6 SELECTION AND WITHDRAWAL OF SUBJECTS

6.1 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, inclusive
3. Willing and able to follow instructions for the duration of the study and can be present for the required study visits
4. Have a history of dry eye disease (DED) in both eyes supported by a previous clinical diagnosis or have a self-reported history of subjective complaints for at least 4 months prior to Screening
5. Have documented history of use of topical lubricants in the past 4 months with documented daily use in the past 28 days and be willing and able to refrain from use of these lubricants for the duration of the study.
6. Be on stable regimens of other needed medications, unless explicitly excluded in Section 8.9.2
7. Have a BCVA using corrective lenses, if necessary, in each eye of +0.7 or better as assessed by Early Treatment of Diabetic Retinopathy Study (ETDRS) at the Screening Visit
8. Have a score of >20 but less than 90 for both symptom frequency and symptom severity on the SANDE scale (Part 1) (Appendix IV).
9. Have the following signs in at least one eye:
    a. Schirmer without anesthesia of >1 and <10 mm at 5 min.
    b. Total corneal staining score ≥2/15 and ≤ 10 on the National Eye Institute (NEI)/Industry scale with a ≥2 in at least one region
    c. Total bulbar conjunctival staining score of ≥ 2/18 and ≤ 10 on the NEI/Industry scale with a ≥2 in at least one region
10. Have normal lid anatomy. Subjects with uncomplicated ptosis may be included at the Investigator's discretion.
11. If female, is either not of childbearing potential (defined as postmenopausal for at least 1 year or surgically sterile [e.g., bilateral tubal ligation, bilateral oophorectomy, or hysterectomy]) or is practicing one of the following medically acceptable methods of birth control throughout the study:
    a. Hormonal methods such as oral, implantable, injectable, or transdermal contraceptives for a minimum of one full cycle (based on the subject's usual menstrual cycle period) before study drug administration;
    b. Intrauterine device;
    c. Double-barrier method (condoms, sponge, diaphragm, or vaginal ring with spermicidal jellies or cream);
    d. Female subjects practicing abstinence or involved in stable same-sex relationships may have the birth control requirement waived;
    e. Partners of vasectomized males in stable relationship.

6.2 Subject Exclusion Criteria

Subjects meeting any of the following criteria at Screening will be excluded from the study:

1. Have identifiable or suspected dry eye caused by pharmacologic, post-traumatic, or post-surgical condition, Sjogren's syndrome disease, Graft-versus-host disease
2. Have anterior segment eye disease except primary dry eye and/or cataract.
3. Have undergone refractive eye surgery (e.g., Laser-Assisted In-Situ Keratomileusis [LASIK]) in either eye during the past 12 months
4. Have undergone cataract surgery in either eye during the past 3 months
5. Have undergone previous eyelid surgery in either eye (e.g., blepharoplasty, ptosis repair). External blepharoplasty, not resulting in exposure or abnormal blinking will be allowed.
6. Have lid irregularities or deformities
7. Have severe corneal surface irregularities
8. Received botulinum toxin (Botox™ or equivalent) injection in the periocular area within 3 months prior to Visit 1
9. Have a history of glaucoma, a history of an elevated IOP within the past year, or an IOP in either eye > 25 mmHg at the Screening Visit (Visit 1)
10. Have a serum potassium level at Screening Visit (Visit 1) of 1.1 times the normal upper limit.
11. Have any clinically significant, uncontrolled, or unstable medical or surgical conditions that could affect his or her ability to participate in the study or potentially compromise his or her well-being during the study. Subjects with medical conditions that meet the following may be enrolled:
    a. the condition is chronic (> 1 years' duration), stable, and adequately controlled
    b. the condition is of mild severity and does not cause functional impairment or end organ dysfunction
    c. the condition does not have and is unlikely to have ocular manifestations or require treatment that would affect vision, anterior segment health, or ocular surface function
    d. the condition does not have and is unlikely to have manifestations or require treatment that would affect compliance or cognition, or hepatic or renal function
    e. 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)
    f. Subjects with other conditions not listed above may be considered for the study if they meet conditions a. through d. and with Medical Monitor approval.
12. Have permanent punctal plugs, punctal occlusion, or history of nasolacrimal duct obstruction. Removable punctal plugs are allowed if they have been used regularly for at least 60 days

prior to the screening visit. However, if a plug comes out during the study it must be promptly replaced.

13. Have clinical findings of severe Meibomian Gland Dysfunction (MGD) in the Study Eye including any of the following:
    a. Evidence of plugging of the Meibomian gland of greater than five out of ten orifices in the nasal third of the lower lid
    b. Evidence of thickened or turbid expressible secretion in greater than five out of ten glands in the nasal third of the lower lid
    c. No expressible clear secretion from any of the glands of the lower lid
    d. Dilated vascularization or scarring of the lid margin
    e. Have required Meibomian gland probing within the past 3 months.
14. Have required treatment with LipiFlow®, MeiboFlow®, or Blephex™ use within the past 3 months
15. Past or present Sjogren's Syndrome or systemic autoimmune disorder. Subjects with diagnosis of rheumatoid arthritis or fibromyalgia may be considered for this study at the discretion of the investigator
16. Stevens-Johnson syndrome, ocular cicatricial pemphigoid (OCP), or other ocular cicatrizing disorders
17. Past or present exposure keratopathy, neurotrophic keratopathy, lagophthalmos, or trichiasis
18. Contact lens use within 28 days of Visit 1 or anticipated need for use of contacts during the study.
19. Use of lid scrubs (including baby shampoos) in the past 28 days
20. Known hypersensitivity or allergy to the study investigational medicinal product, or formulation excipients, including amiloride or related drugs. Subjects with a hypersensitivity to fluorescein or lissamine green should be excluded.
21. Have taken within 14 days of Screening or will need to take any of the following medications during the study. NOTE: See Section 8.9.2 for guidance about other excluded medications.
    a. Any topical prescription or over-the counter (OTC) ocular medication including topical cyclosporine (e.g. Restasis®), lifitegrast (Xiidra®), or topical anti-glaucoma medications. Use of lubricating ocular drops are to be discontinued at Screening and withheld for the duration of the study. Diagnostic solutions are exempt.
    b. Ocular, intranasal or systemic corticosteroids or other immunomodulatory or immunosuppressive medications
    c. Topical ocular or systemic antibiotics, including doxycycline or tetracycline analogs
    d. Oral or topical secretagogues such as pilocarpine and cevimeline (Evoxac®)
    e. Systemic medications with known significant anticholinergic pharmacologic activity as referenced in the Prohibited Medication List for the study, such as tricyclic antidepressants. Selective Serotonin Re-uptake Inhibitors (SSRIs) are permitted if patient is on stable dose for 28 days prior to Visit 1 and during the study.
    f. The following medications are explicitly excluded for 14 days prior to Randomization Visit and throughout the study:
        i. Nasal, ocular, or oral antihistamines

ii. NSAIDs (non-steroidal anti-inflammatory drugs) or aspirin use with the exception of low dose (81mg) aspirin per day
iii. Topical autologous serum
iv. Lubricant eye drops

22. Are pregnant or breast feeding
23. Use of any investigational product or device within 28 days prior to the Screening Visit or during the study
24. Be an employee of the site that is directly involved in the management, administration, or support of this study or be an immediate family member of the same.
25. Are unable in the opinion of the PI to comply fully with the study requirements or to complete the study

6.3 Randomization Criteria

At the Visit 2 (Randomization and Treatment Day 1), an eligible subject must continue to meet the following Randomization Criteria:

1. Have no clinically important changes in medical status or changes in concomitant medications since Visit 1 that would affect study participation or confound the evaluation of safety or efficacy.
2. Have a score of >20 but less than 90 for both symptom frequency and symptom severity on the SANDE scale (Part 1) (Appendix IV)
3. Continue to meet the following dry eye entry staining criteria, in the Study Eye that qualified at Visit 1. If both eyes qualified at Visit 1, either eye could be eligible to continue to qualify for randomization:
   a. Total corneal staining score ≥2/15 and ≤ 10 on the National Eye Institute (NEI)/Industry scale with a ≥2 in at least one region
   b. Total bulbar conjunctival staining score of ≥ 2/18 and ≤ 10 on the NEI/Industry scale with a ≥2 in at least one region

6.4 Definition of Study Eye

For the purpose of efficacy analysis in this trial, each subject will have only one study eye. If a subject has only one qualifying eye, this eye will be the Study Eye. If both eyes of the subject qualify, then the Study Eye will be the eye with the highest total corneal staining score. If the scores for total corneal staining are the same, the Study Eye will be the right eye. The non-study eye is referred to as the "Fellow Eye".

If at Visit 2 the Study Eye does not qualify based on randomization criteria, but the subject had both eyes qualify at Visit 1 and the other eye continues to qualify based on randomization criteria, that eye will become the Study Eye.

6.5 Subject Withdrawal Criteria

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 and followed to resolution (i.e., birth or voluntary or spontaneous termination of the pregnancy) (Section 10.2). In the event that discontinuation of treatment is necessary, the investigator will make every attempt to complete all subsequent safety assessments. The reason for premature discontinuation should be documented. Subjects who withdraw from the study will not be replaced.

7 PROCEDURES

The procedures required at each visit are described 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.

7.1 Visit 1: Screening Day -14 (14 days prior to Visit 2)

Procedures will be completed at Visit 1 in the following general order. The visit will start between approximately 8:00AM and 11:00AM. All subjects who sign an informed consent document will be assigned a unique 6-digit screening number (i.e., XXX-XXX, which will be a compilation of the site number (first 3 digits) and subject number (last 3 digits). This subject number will be unique within this trial and will be used to identify the subject throughout the study.

- Obtain written informed consent
- Administer ocular symptom questionnaires (SANDE Part 1 only, SPEED, and a 7-item symptom questionnaire) before other procedures
- Review of the medical history
- Review concomitant medication history in the past 28 days
- Vital signs (pulse, blood pressure, temperature and respiration rate), height and weight
- BCVA
- Biomicroscopy and external eye examination
- Tear osmolarity
- TBUT
- Assessment of Meibomian glands
- Staining procedures conducted in the following order (refer to Manual of Procedures):
    - Corneal staining
    - Bulbar conjunctival staining
    - Staining of the lid wiper area of the upper eyelid (with photographs taken if technology is available)
- Abbreviated physical exam (including eyes, ears, nose, throat (EENT), respiratory, cardiovascular, musculoskeletal, gastrointestinal, and dermatological)
- Collect blood for Hematology and Chemistry
- Urine pregnancy test (women of child-bearing potential only)
- Schirmer's test
- IOP)
- Review eligibility criteria
- Once all other procedures and assessments have been completed, review the qualification criteria and if the subject qualifies for the study, determine which eye is the Study Eye
- Instruct subject in study drug instillation technique and observe/correct technique using study drug dispensed at this visit.
- Complete drop instillation comfort questionnaire within 5 minutes after dosing for the Fellow Eye (the non-study eye)
- Assess adverse events during this visit

- At the end of the visit, the subject will be scheduled for Visit 2, 14 days from this visit, will receive their study medication to take home, and provided with the instructions in Section 7.1.1.

In the event that the subject does not qualify at Visit 1, the subject may be rescreened.

7.1.1 Subject Instructions

The following instructions will be provided to the subject before leaving the clinic.

- Store your study drug at room temperature and out of the reach of children. Only you should use your study drug.
- Instill your study medication into each eye three times daily. Study doses are to be taken approximately at the times of the meals. If you miss a dose, do not double-up or catch-up, but instead, resume taking your three times daily schedule as soon as possible.
- Open a pouch containing a single vial of study medication for every dose. Instill one drop of study medication from this newly opened vial into each eye, starting with the mid-day dose on the day of your clinic visit. Open a new pouch and vial for every dose.
- Continue to refrain from use of Aspirin with the exception of low dose aspirin (81mg) and NSAIDs such as ibuprofen (Advil®or Motrin®) or naproxen (Aleve®)
- Continue to refrain from use of contact lenses and any and all eye drops for dry eye or any other reason
- Continue to refrain from other disallowed medications
- Call the study clinic if you experience any problems, including more that your usual eye redness or discomfort
- On the morning of your next clinic visit, DO NOT TAKE your morning dose of study medication.
- Return to the clinic for your next appointment and remember to bring all used and unused study medication with you.

7.2 Phone Call: Day -7 (7 days ±1 day after Visit 1)

Site personnel will have an interim phone call to remind subjects to administer their medication and to assess if any adverse events have occurred since Visit 1. This phone call and attempts will be documented.

7.3 Visit 2: Randomization and Treatment Day 1

Procedures will be completed at Visit 2 in the following general order. This visit should take place between approximately 8:00AM and 11:00AM and at approximately the same time as Visit 1:. Subjects will be evaluated to determine if they are eligible for randomization to the double-masked treatment portion of the study.

- Administer symptom questionnaires (SANDE Part 1 and 2, SPEED, and 7-item symptom questionnaire) before other procedures and before dosing
- Collect previous study medication supplies from subject

- Document date and time of last dose of study medication
- Review concomitant medications and medical changes since last visit
- Assess adverse events since the last visit and during this visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate)
- BCVA
- Biomicroscopy and external eye examination
- Tear collection for biomarkers
- TBUT
- Staining procedures conducted in the following order (refer to Manual of Procedures):
    - Corneal staining
    - Bulbar conjunctival staining
    - Staining of the lid wiper area of the upper eyelid (with photographs taken it technology is available)
- Impression cytology (with anesthesia, in Study Eye only)
- Confirm continued eligibility for participation per Randomization Criteria (Section 6.3)

Subjects who continue to qualify for the study will be randomly assigned to double-masked treatment (Section 8.4) and will be provided with an identically appearing kit of study medication corresponding to their assigned treatment. Subjects who fail to qualify for this study will be considered screen failures and will be withdrawn from the study with the reason for disqualification documented.

For continuing subjects, the following will be done:

- Once all other procedures and assessments have been completed, observe/correct subject's study drug instillation technique using new study drug dispensed at this visit.
- Complete drop instillation comfort questionnaire within 5 minutes after dosing for the Fellow Eye (the non-study eye)
- At the end of the visit, the subject will be scheduled for Visit 3 in 14 days from this visit, will receive their study medication to take home, and provided with the instructions provided in Section 7.1.1.

7.4 Phone Call: Treatment Day 8 ±1 day

Site personnel will have an interim phone call to remind subjects to administer their medication and to assess if any adverse events have occurred since Visit 2. This phone call and attempts to contact the subject will be documented.

7.5 Visit 3: Treatment Day 15

Study procedures will be completed at Visit 3 in the following general order. The visit should take place between approximately 8:00AM and 11:00AM and at approximately the same time as Visit 1:

- Administer symptom questionnaires (SANDE Part 1 and 2, SPEED, and a 7-item symptom questionnaire) before other procedures and before dosing
- Collect previous study medication supplies from subject

- Document date and time of last dose of study medication
- Review concomitant medications and medical changes since last visit
- Assess adverse events since the last visit and during this visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate)
- Biomicroscopy and external eye examination
- Tear collection for biomarkers
- TBUT
- Staining procedures conducted in the following order (refer to Manual of Procedures):
    - Corneal staining
    - Bulbar conjunctival staining
    - Staining of the lid wiper area of the upper eyelid (with photographs if technology is available)
- Collect blood for serum potassium
- Once all other procedures and assessments have been completed, observe/correct subject's study drug instillation technique using study drug dispensed at this visit.
- Complete drop instillation comfort questionnaire within 5 minutes after dosing for the Fellow Eye (the non-study eye)
- At the end of the visit, the subject will be scheduled for Visit 4 in 14 days from this visit, will receive their study medication to take home, and provided with the instructions provided in Section 7.1.1.

7.6 Phone Call: Treatment Day 22 ±1 day

Site personnel will have an interim phone call to remind subjects to administer their medication and to assess if any adverse events have occurred since Visit 3. This phone call and attempts will be documented.

7.7 Visit 4: Day 29

Procedures will be completed at Visit 4 in the following general order. The visit should take place between approximately 8:00AM and 11:00AM and at approximately the same time as Visit 1:

- Administer symptom questionnaires (SANDE Part 1 and 2, SPEED, and a 7-item symptom questionnaire) before other procedures and before dosing
- Collect previous study medication supplies from subject
- Document date and time of last dose of study medication
- Review of concomitant medications and medical changes since last visit
- Assess adverse events since the last visit and during this visit
- Collect vital signs (pulse, blood pressure, temperature and respiration rate) and weight
- BCVA
- Biomicroscopy and external eye examination
- Tear collection for biomarkers
- TBUT
- Staining procedures onducted in the following order (refer to Manual of Procedures):
    - Corneal staining
    - Bulbar conjunctival staining

  - Staining of the lid wiper area of the upper eyelid (with photographs if technology is available)
- Impression cytology (with anesthesia, in Study Eye only)
- Abbreviated physical exam (including EENT, respiratory, cardiovascular, musculoskeletal, gastrointestinal, and dermatological)
- Collect blood for Hematology and Chemistry
- Urine pregnancy test (women of child-bearing potential only)

Once all the following assessments are complete, the subject will be discharged from the treatment portion of the study. Investigators will counsel the subject on the appropriate treatment regimen. The subjects will be reminded that they will receive a call from the site 5-9 days after this visit to follow up with them.

7.8 Follow up telephone call (7± 2days after Visit 4)

A follow-up telephone call will be conducted 7 ± 2 days after Visit 4 and the following will be assessed:

- Review of concomitant medications and medical changes since last visit
- Assess adverse events since the last visit

7.9 Early Termination Visit

The purpose of the Early Termination visit is to obtain critical information about the subject's participation, and should be scheduled preferably before there has been a substantial lapse in study medication usage. However, even if there has been a medication lapse, the subject should be encouraged to return to the clinic for this visit, and should be instructed to return all study medication. The following assessments will be performed at the Early Termination Visit for randomized subjects who are withdrawn from the study prematurely:

- Collect previous study medication supplies from subject
- Document reason for early termination
- Document date and time of last dose of study medication
- 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 weight
- Abbreviated physical exam (including EENT, respiratory, cardiovascular, musculoskeletal, gastrointestinal, and dermatological)
- Biomicroscopy and external eye examination
- Collect blood for Hematology and Chemistry
- Urine pregnancy test (women of child-bearing potential only)

Once these assessments have been complete, the subject may be discharged from the study, provided that there is no need for additional follow-up to continue to monitor an adverse event or other condition.

7.10 Unscheduled Visit

Though not anticipated, subjects may need to be seen at other times than the scheduled study visits for additional safety assessments or to follow-up, as medically necessary, on adverse event or other findings. If an additional study visit occurs, the date and nature of the visit will be documented in the CRF and in the source documents.

7.11 Potential Toxicity Management

There is no systemic toxicity expected in this study. All preclinical evidence indicates that systemic exposure of P-321 via ocular topical administration is minimal at doses up to 4-fold higher than the daily dose planned in this study (the estimated systemic safety margins for the maximal dose anticipated in this study is 75-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, a clinical trial evaluating multiple concentrations including 0.01%) P-321 Opthalmic solution taken BID for 28 days. In that study, pharmacokinetic results demonstrated a lack of systemic availability of 0.01% P-321 Ophthalmic Solution. No changes in plasma or serum potassium were noted in subjects administered 0.01% P-321 Ophthalmic Solution BID for up to 28 days. We will monitor serum potassium levels in this study.

Additional information is provided in the Investigator's Brochure.

8 TREATMENT OF SUBJECTS

8.1 Description of Study Drug

P-321 Ophthalmic Solution 0.017% is a sterile, aqueous, non-preserved solution with pH of approximately 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 is intended for a single administration on each eye.

Table 1 P-321 Ophthalmic Solution: 0.017% Investigational Product

	Investigational Product	
Product Name:	P-321 Ophthalmic Solution 0.017%	Matching placebo (vehicle with no active ingredient)
Dosage Form:	Sterile unpreserved ophthalmic solution for ocular instillation	Sterile unpreserved ophthalmic solution for ocular instillation
Unit Dose	Vial intended for a single-dose	Vial intended for a single-dose
Route of Administration	1 drop in each eye three times daily	1 drop in each eye three times daily
Physical Description	Aqueous solution, pH 5, approximately 290 mOsm/Kg	Aqueous solution, pH 5, approximately 290 mOsm/Kg
Manufacturer	Bio-Concept Laboratories, Inc.	Bio-Concept Laboratories, Inc.

8.2 Study Drug Packaging, Labeling, and Storage

Study drug for the placebo-run in phase of the study will be provided in a 14-day treatment kit. The treatment kit will include 42 pouches. Each pouch will contain one vial. Each vial is intended for dosing once (one drop in each eye and not used again). The label on the kit and pouch will minimally contain the following information: Study ID, kit number, storage temperature, and "Caution: New Drug – Limited by Federal Law to Investigational Use. Keep out of reach of children".

Study drug for the double-masked phase of the study will similarly be provided in two 14-day treatment kits which are labeled and packaged identically to supplies used in the run-in period. Each 14-day treatment kit will include 42 pouches. Each pouch will contain one vial. Each vial should be used for one dose (one drop in each eye) and not used again. The label on the kit and pouch will minimally contain the following information: Study ID, kit number, storage temperature, and "Caution: New Drug – Limited by Federal Law to Investigational Use. Keep out

of reach of children." There will be no labeling on the individual vials of study medication due to size.

Storage of study drug at the study site must be under locked and secure conditions with limited staff access. Study medication for the run-in and treatment phase must be stored at the site between 2 – 8°C (36 - 46°F). The subject may store the drug at ambient conditions (between 15°C and 25°C (59°F to 77°F), but they must be instructed to store study drug in a secure place at room temperature, away from environmental extremes, and out of the reach of children.

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

8.3 Treatment Administration and Dosing Instructions

Subjects will be instructed on how to instill study medication into each eye and to conduct three instillations on each eye daily.(Appendix III). At Visit 1, the first dose will be administered by the subject under the supervision of trained site personnel who may offer advice/correction on the instillation technique as necessary. At Visit 1 all subjects will receive single-masked placebo for up to 14 days . Only the site personnel and not the subject will know the study medication is placebo.

At Visit 2, eligible subjects will be randomized to receive either 0.017% P-321 Ophthalmic Solution, or Placebo in a 1:1 ratio for the duration of the trial. The first dose from the newly assigned treatment kit at Visits 2 and 3 will be self-administered by the subject in the clinic under the supervision of trained site personnel who may offer advice/correction on the instillation technique. During the double-masked treatment period, neither the subjects nor site personnel will know which treatment the subject receives at which visit. Subjects will administer treatment TID, and to enhance compliance should administer around an event so that it may be easier to remember, such as eating. However food is not required at administration.

At Visit 4, no new study medication will be dispensed and no further instillation of study drug is required.

8.4 Randomization, Study Masking, and Dispensing Instructions

8.4.1 Single-Masked Placebo for Placebo Run-In Period

For the single-masked placebo run-in period, subjects will be dispensed a two-week supply of placebo vehicle in a treatment kit that is identical in appearance to the double-masked treatment kit that will be dispensed at subsequent visits. Each treatment kit will contain 42 pouches with a single vial of study drug included in each pouch.

8.4.2 Double-Masked Treatment Period

Eligible subjects will be randomized in a double-masked manner once they have met all Randomization criteria (Section 6.3). Subjects will be randomized in a 1:1 ratio to P-321 Ophthalmic Solution 0.017% or Placebo (vehicle), stratified by clinical site. 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 packaging and labeling of the clinical supplies for each clinical site.

The study drug supplies will be labeled with sequential treatment numbers. Subjects will be assigned the next available treatment number upon qualifying to be randomized. Double-masked study medication for each subject will be packaged, labeled and supplied to the study site in one kit per subject. The carton will contain two boxes, each box will contain a two-week supply of study drug. Each 2-week supply box will contain 42 identical pouches with a single vial of study drug in each pouch.

The randomization codes will be maintained in a secure location separate from the study investigator. All study personnel directly associated with the conduct of the clinical study, including the investigator, medical monitor, study site personnel, subjects in the study and those involved in the conduct of the study will remain masked to double-masked study treatment assignments.

8.5 Release of Clinical Study Supplies to the Investigator

Parion or Parion's designee's standard operating procedures for releasing clinical trial supplies to the site will be followed.

8.6 Treatment Compliance

Treatment compliance will be assessed based on medication accountability performed by the site staff. Subjects will be asked to return used and unused study drug for this purpose. Study medication accountability will be recorded on a log and compliance will be calculated. Appropriate subject investigation and counseling should occur if discrepancies arise in expected vs returned study drug. The time and date of the in clinic study drug administrations, the screening and treatment number will be captured in the CRF.

Compliance will also be reinforced by study personnel during the visits and between visit phone calls.

8.7 Study 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/vials of study medication received on site, number of kits/vials dispensed to subjects according to the protocol-specified dosing regimen, and the remaining unused study medication at the time of reconciliation.

Clinical trial materials will be shipped to the investigational site 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.

At the end of the study, all study materials, including used and unused study medication vials and kits will be returned to Parion Sciences, Inc. or designee for destruction, once authorized. The study monitor or designee should verify drug accountability at routine monitoring visits.

8.8 Procedure for Breaking the Randomization Code

The randomization code may not be broken except in the case of a medical emergency, or occurrence of an SAE. 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 generally not warranted. 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.

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.

8.9 Concomitant Medications

8.9.1 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. Fluorescein, lissamine green, and topical ocular anesthetics used in study procedures do not need to be recorded on the concomitant medications page.

Subjects taking the following medications may continue to take these medications during the study provided they are taking them at stable doses (as needed [PRN] doses are not permitted) and have been on them for at least 28 days prior to the Visit 1. Subjects taking other concomitant medications at stable doses may be eligible for inclusion with Medical Monitor approval.

a. contraceptives (oral and transdermal)
b. antihypertensives
c. drugs for heart failure or angina
d. thyroid or hormone replacement therapy
e. anticoagulants
f. benzodiazepines
g. any nutritional supplement containing Omega-3 acids (eg, fish oil and Flax Seed oil)

8.9.2 Medications Not Permitted

The following medications are explicitly excluded for 14 days prior to Screening and throughout the study:

a. Any topical prescription or OTC ocular medication including topical cyclosporine (e.g. Restasis®), lifitegrast (Xiidra®), or topical anti-glaucoma medications. Diagnostic solutions are exempt.
b. Ocular, intranasal or systemic corticosteroids or other immunomodulatory or immunosuppressive medications

c. Topical ocular or systemic antibiotics, including doxycycline or tetracycline analogs
d. Oral or topical secretagogues such as pilocarpine and cevimeline (Evoxac®)
e. Systemic medications with known significant anticholinergic pharmacologic activity as referenced in the Prohibited Medication List for the study, such as tricyclic antidepressants. Selective Serotonin Re-uptake Inhibitors (SSRIs) are permitted if patient is on stable dose for 28 days prior to Visit 1 and during the study.

The following medications are explicitly excluded for 14 days prior to Randomization Visit and throughout the study:

a. Nasal, ocular, or oral antihistamines
b. NSAIDs and aspirin use with the exception of low dose (81mg) aspirin per day
c. Topical autologous serum
d. Lubricant eye drops

9 Assessment of Efficacy

Efficacy will be assessed by evaluation of symptoms and signs. Specific procedures for conducting these assessments will be provided in the Study Manual. Where relevant, it is imperative that ocular assessments be conducted in a specified order, first in the right eye and then in the left eye.

9.1 Symptom Assessments

The primary efficacy endpoint and several of the secondary efficacy endpoints in this study are derived from subjective subject-completed symptom questionnaires. Therefore, it is critical that these questionnaires are completed in the specified order, according to specific instructions, and in a setting where the subject has minimal distractions and sufficient time to complete them. After completion of these questionnaires, the study coordinator, unless specifically prohibited by instrument instructions, should review the responses for completeness only.

Symptoms will be assessed at every visit (Visits 1-4 and/or Early Termination) before any other study procedures have been conducted. The symptom questionnaires are commonly used instruments in dry eye trials and are described in Appendix IV.

The subject will be given the questionnaires to complete in the the following order:

- SANDE (Part 1 completed at Visits 1-4 and Part 2 completed at Visits 2-4). SANDE Part 1 scores must be >20 but less than 90 for both symptom frequency and symptom severity to be eligible for randomization (Section 6.3)
- SPEED
- 7-item symptom questionnaire

If collected on paper, original subject responses from these symptom questionnaires will be retained in the source documents and results will be transcribed from these source documents onto the CRF and verified that it has been transcribed correctly.

9.2 Assessment of Signs

9.2.1 Tear Collection for Biomarkers of Inflammation

Tear collection for biomarkers of inflammation will be assessed in both eyes at Visits 2, 3, and 4. Tear collection must be done under the slit lamp by the Investigator before any staining of the eye has occurred. Tears collected will be assessed for prostaglandins (from the Study Eye) and MMP-9 activity (from the Fellow Eye). Information on collection procedures, sample handling, labeling and storage is provided in the Study Manual.

9.2.2 TBUT

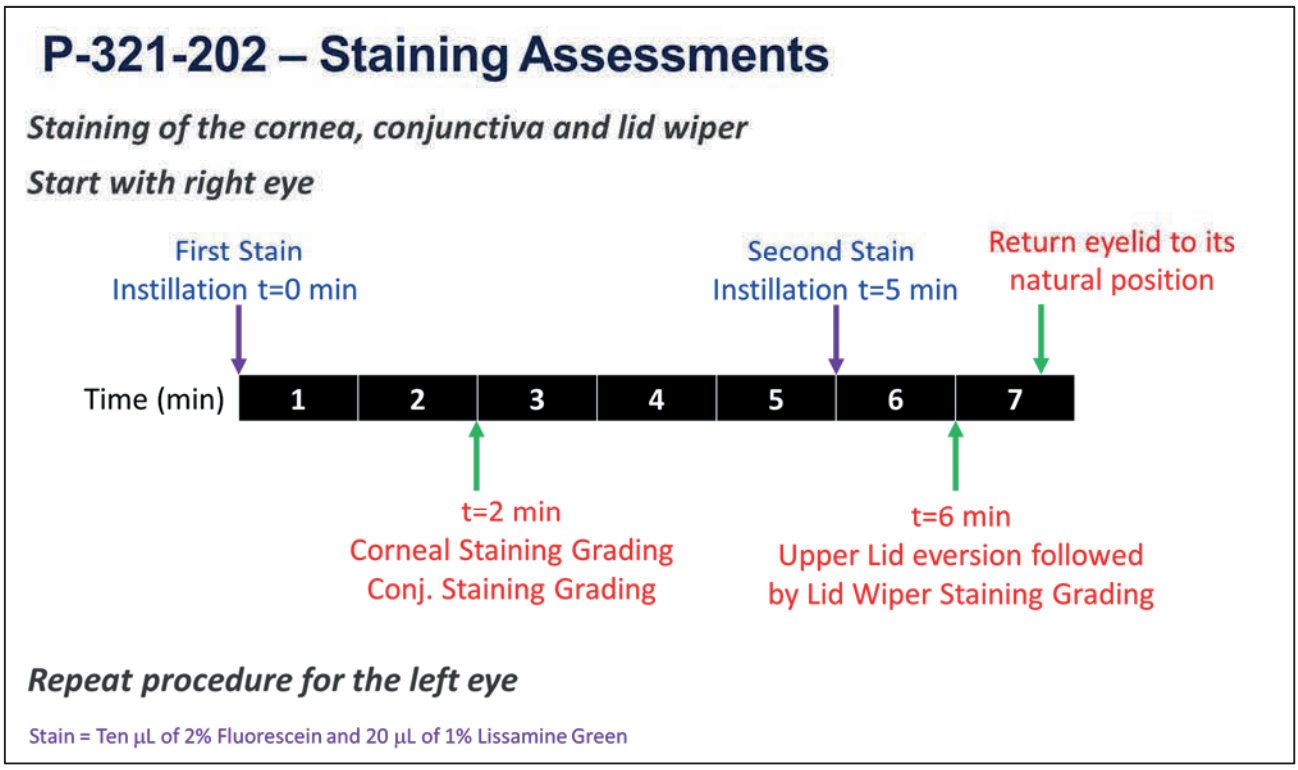
TBUT will be assessed in both eyes at Visits 1-4 in OD first, then OS. The procedure and information on staining is provided in the Study Manual.

9.2.3 Corneal Staining, Bulbar Conjunctival Staining, and Lid Wiper Staining

The assessment of corneal staining, bulbar conjunctival staining and staining of the lid wiper region of the upper eyelid will be assessed first in the right eye and then the left eye using

fluorescein 2% and lissamine green 1%. Corneal and conjunctival staining scores must be within certain limits at Visit 1 and Visit 2 in order for the subject to be eligible for randomization (Section 6.3). Timing of these assessments is specified in Figure 2. Photographs of lid wiper staining will be taken if technology is available. Additional detailed specific instructions related to each of these assessments will be provided in the Study Manual.

Figure 2 Sequence of Ocular Assessments



9.2.4 Impression Cytology

Impression cytology (with anesthesia) will be performed on the Study Eye only at Visits 2 and 4 per the procedures outlined in the Study Manual.

9.2.5 Other Assessments

In addition of the asssessments indicated in Sections 6.1 and 6.2, additional assessments will be done at Screening Visit 1 only to characterize the study population, including IOP, Schirmer's test, assessment of tear osmolarity and clinical assessment of the Meibomian glands, consisting in evaluation of plugging, character of secretion, and expressibility of the Meibomian glands complemented with meibography to assess Meibomian gland loss if technology is available at the site. Additional information about the procedures for these assessments are provided in the Study Manual.

10 ASSESSMENT OF SAFETY

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.

Safety will be assessed by:

- Adverse events
- BCVA
- Biomicroscopy and external eye examination
- Physical examinatons
- Vital signs
- Clinical laboratory tests

10.1 Adverse Event 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 double-masked study treatment or was present prior to double-masked study treatment but subsequently increased in frequency or severity following initiation of the double-masked treatment.

All AEs will be collected from the time ICF is signed and study procedures have started until the following time points:

- For subjects who are not randomized until time of screen failure (e.g., screen failure, withdrawal of consent)
- For enrolled subjects who have the Follow-up Telephone Call: through the Follow-up Telephone Call
- For subjects who Early Terminate from the study: through the Early Termination Visit.

All subjects will be queried, using non-leading questions, about the occurrence of AEs at each study visit. When possible, a constellation of signs and/or symptoms will be identified as 1 overall event or diagnosis. All AEs for enrolled subjects (those subjects who receive single-masked run-in and or double-masked medication) will be recorded in the CRF and source document. The following data will be documented for each AE:

- Description of the event
- Classification of “serious” or “nonserious”
- Date of first occurrence and date of resolution (if applicable)
- Severity
- Causal relationship to study drug(s)

- Action taken
- Outcome
- Concomitant medication or other treatment given

If an AE occurs, the investigator will institute and/or support 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.

There are many symptoms associated with dry eye that can vary in intensity and frequency over time. Only symptoms that worsen or become more frequent, in the opinion of the subject and outside of their normal experience, should be reported as an adverse event.

10.1.1 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 Institutional Review Board (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.

10.2 Pregnancy

Any subject found to be pregnant at any time during the study will be withdrawn from the study immediately. 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). Any pregnancy outcome that meets the criteria for an SAE will be reported as an SAE.

10.3 BCVA

BCVA will be assessed in both eyes at Visits 1, 2, and 4 per the procedures outlined in the Study Manual. At Visit 1, subjects must have a BCVA using corrective lenses, if necessary, in each eye of +0.7 or better as assessed by ETDRS to be eligible for the study. Changes in BCVA will not be reported as adverse events unless the change is deemed clinically significant compared to screening by the Investigator.

10.4 Biomicroscopy and External Eye Exam

Biomicroscopy and external eye exams will be conducted in both eyes per the procedures outlined in the Study Manual at every visit (Visits 1-4), and at Early Termination, if applicable. Changes in biomicroscopy and external eye exam findings will not be reported as adverse events, unless the change is deemed clinically significant by the Investigator.

10.5 Physical Examination

An abbreviated physical examination to include evaluation of EENT, respiratory, cardiovascular, musculoskeletal, gastrointestinal, and dermatological systems will be conducted by the PI or a trained designee at Visit 1, 4 and/or Early Termination. Changes in physical exam findings will not be reported as adverse events, unless the change is deemed clinically significant compared to screening by the Investigator.

10.6 Vital Signs and Weight

Vital signs including sitting pulse and blood pressure, temperature and respiration rate will be assessed at every visit (Visits 1-4), and at Early Termination, if necessary. Weight will be assessed without shoes at Visits 1 and 4, and at Early Termination, if applicable. Changes in weight or vital signs will not be reported as adverse events unless the change is deemed clinically significant by the Investigator.

10.7 Clinical Chemistry and Hematology

Appendix V defines the clinical chemistry and hematology analytes that will be analyzed at Visits 1 and 4. A urine pregnancy test must be performed on all women of child-bearing potential at Visits 1, 4, and/or Early Termination. With the exception of the urine pregnancy test, all clinical laboratory evaluations will be analyzed via a central clinical laboratory, and information regarding appropriate sample volume, collection tubes, sample labeling and handling, and shipment will be

provided in the clinical laboratory manual. Changes in clinical laboratory findings will not be reported as adverse events unless the change is deemed clinically significant by the Investigator.

10.8 Drop Instillation Comfort Assessment (performed on the Fellow Eye only)

After subject instillation of study medication at Visits 1, 2, and 3, subjects will be asked to complete an assessment of the comfort of the drop **on the Fellow Eye only** within 5 minutes after the instillation of study drug into both eyes (Appendix VI).

11 STATISTICAL CONSIDERATIONS AND ANALYSES

A complete description of the statistical analyses to be performed will be provided in a statistical analysis plan (SAP), which will be finalized prior to database lock and the unmasking of study treatments.

Baseline will be defined as those values recorded closest to, but prior to administration of the first dose of double-masked study drug.

Unless otherwise noted, significance tests of treatment differences will be tested at the two-sided 0.05 level.

11.1 Populations for Analysis

The following populations will be defined for analysis:

The Safety Population will include all subjects who receive at least one dose of double-masked study medication in either eye. Safety analyses will be conducted on the Safety Population, analyzed as treated.

Additional safety analyses will be conducted on the Run-in Population, defined as all subjects who receive at least one dose of single-masked placebo in either eye during the run-period.

The modified Intent-to-Treat Population (mITT) will include all randomized subjects who have at least a baseline and one post-baseline SANDE part 1 assessment. This is the primary population for efficacy analyses and subjects will be analyzed based on their randomized treatment.

For the efficacy measurements, analyses will be conducted using the Study Eye. Each subject will have only one Study Eye determined at the time of Randomization. If a subject has only one qualifying eye, this eye will be the Study Eye. If both eyes of the subject qualify, then the Study Eye will be the eye with the highest total corneal staining score. If the scores for total corneal staining are the same, the Study Eye will be the right eye. The non-Study Eye is referred to as the Fellow Eye. Additional analyses may be conducted using all qualifying eyes that met entry criteria.

11.2 Estimate of Sample Size

A sample size of approximately 65 randomized subjects is proposed. Assuming a dropout rate prior to Visit 3 of 10%, this provides approximately 60 subjects with post-baseline data. The sample size estimation was based on assumptions about the standard deviation and treatment effect for the change from baseline SANDE global score (Part 2 assessment) from a previous study, protocol P-321-101. At Day 28, the pooled standard deviation for the change from baseline was 22 and the difference in means was 20, representing a standard effect size of 0.9. If the true standard deviation is 22, a sample size of 30 subjects per group will provide at least 90% power to detect a true difference in means of 20 using a two-sided hypothesis test with Type 1 error of 5%. All else being equal, this sample size provides at least 70% power if the treatment difference is at least 15 units or if the standard deviation is no more than 29.

11.3 Demographic and Baseline Characteristics

Demographic, and baseline characteristics such as age, gender, race, ethnicity, weight, dry eye and other ocular history including MGD, BCVA, IOP, symptoms upon entry, corneal and conjunctival staining, staining in the lid wiper region, TBUT, tear osmolarity, and Schirmer tests will be summarized by treatment group and overall using descriptive statistics. Baseline homogeneity with respect to demographic and baseline characteristics will be assessed via hypothesis testing at the two-sided alpha level of 0.05.

11.4 Analysis of Efficacy

11.4.1 Efficacy Endpoints

Analyses of efficacy will be conducted using the Study Eye. A supportive efficacy analysis will be also conducted on all qualifying eyes, as defined in the Statistical Analysis Plan. Analyses of efficacy conducted on all qualifying eyes will account for the correlation between eyes within subjects with two qualifying eyes.

Primary Efficacy Endpoint:

The mean change from baseline (Visit 2) to Day 29 (Visit 4) in the SANDE questionnaire global symptom score from Part 1. The primary efficacy comparison will be the mean change from baseline in the SANDE global symptom score for the 0.017% treatment arm compared to placebo.

Secondary Efficacy Endpoints:

- Change from baseline to Day 29 in the SPEED questionnaire total symptom score
- Changes from baseline to Day 29 in each of the 7-item symptom questionnaire scores
- Change from baseline to Day 29 in staining of the Lid Wiper area
- Change from baseline to Day 29 in bulbar conjunctival staining total score
- Change from baseline to Day 29 in corneal staining total score
- Changes from baseline to Day 29 in TBUT
- Change in symptom frequency score and severity score as recorded by the subject using the SANDE Part 2 assessment
- Change from baseline to Day 29 in the SANDE symptom frequency and severity scores from Part 1
- The proportion of subjects with at least a 20% improvement from baseline to Day 29 in the SANDE global symptom score from Part 1 (and in the frequency and severity scores)
- The proportion of subjects with an improvement over 28 days in symptom frequency score and severity score using the SANDE Part 2 assessment
- Changes from baseline to Day 15 will also be assessed for all efficacy measurements.

Exploratory Efficacy Endpoints:

Changes from baseline in biomarkers of inflammation in tears and from impression cytology samples collected from the lid wiper and the bulbar conjunctiva.

11.4.2 Primary Efficacy Analysis

The mean change from baseline (Visit 2) in SANDE Part 1 global symptom score for the Study Eye after 28 days of treatment in the 0.017% P-321 and placebo arms will be analyzed via analysis of covariance (ANCOVA). The dependent variable will be the observed change from baseline to Visit 4 (Day 29) in the global symptom score from SANDE Part 1, with treatment, study site, and baseline SANDE Part 1 global score as fixed effects. A significance test of the treatment difference between the 0.017% arm and placebo will be tested at the two-sided 0.05 level and corresponding 95% confidence intervals will be calculated. The analysis will be based on the mITT population only. Details regarding the handling of missing data will be described in the SAP.

11.4.3 Secondary Efficacy Analyses

The proportion of subjects with a ≥20% improvement from baseline to Day 29 in the global symptom score from SANDE Part 1 in the 0.017% and placebo arms will be analyzed via logistic regression. The model will include all subjects in the mITT population, and subjects with a ≥20% improvement will be considered responders. Subjects with a <20% improvement will be considered non-responders. Responder status (yes/no) will be the dependent variable in the model, and the model will include the treatment, study site, and baseline SANDE Part 1 global symptom score as fixed effects. A significance test of the treatment difference will be tested at the two-sided 0.05 level, and odds ratios and their corresponding 95% confidence intervals will be calculated. Details regarding the handling of missing data due to early withdrawals or missed assessments will be described in the SAP. The proportion of subjects improving as evaluated using the SANDE Part 2 assessment will be analyzed in a similar manner.

Continuous secondary endpoints including the change from baseline in: the total SPEED symptom score, each of the items from the 7-item symptom questionnaire, total corneal staining score, total bulbar conjunctival staining score, lid wiper area staining score, and TBUT will be analyzed in a similar manner as the primary endpoint. The model will be changed to include the baseline value of the secondary endpoint instead of the baseline SANDE Part 1 global symptom score. Other endpoints, such as the change from baseline in the staining score of certain regions of the cornea, will be explored and analyzed in a similar manner. Further details will be provided in the SAP, along with details on how the change from baseline over the entire 28 day treatment period using the SANDE Part 2 assessment and the definition of improvement over 28 days using the SANDE Part 2 assessment will be defined.

The primary evaluation of interest for secondary endpoints measuring signs of dry eye will utilize the Study Eye. Additional analyses will be performed for all qualifying eyes. Analyses utilizing all qualifying eyes will account for the correlation between eyes within subjects. Total conjunctival and corneal staining scores will be defined as the sum of the scores from all six and five regions, respectively. The staining score of the lid wiper area will be defined as noted in the Study Manual.

Analyses of the change from baseline (Visit 2, Day 1) to Visit 3 (Day 15) for the primary and secondary efficacy endpoints may also be performed as secondary analyses. Further details of the secondary efficacy analyses, and details regarding multiplicity adjustments for the secondary endpoints will be provided in the SAP.

11.4.4 Exploratory Efficacy Analyses

Exploratory efficacy analyses will be based on the mITT population. To evaluate biomarkers of inflammation, tears collected from the Study Eye will be assessed for prostaglandins and tears from the Fellow Eye for MMP-9 activity. Impression cytology samples from the lid wiper and bulbar conjunctiva will be assessed for the expression of biomarkers including HLA-DR, IL-1β, IFN-γ, Cox-2, CK-10, and IL-8. Descriptive statistics will be provided by treatment group. Additional analyses will be described in further detail in the SAP.

11.5 Analysis of Safety

Safety analyses will be run on the Safety Population and the Run-in Population. The analysis of safety assessments will include summaries of the following safety and tolerability data collected for each subject:

- Adverse Events
- Clinical Laboratory Tests
- Biomicroscopy and external eye examination
- BCVA
- Vital Signs
- Physical Examinations
- Drop Instillation Comfort Assessments

Summaries of incidence rates (frequencies and percentages) of individual AEs by MedDRA system organ class and preferred term will be prepared. Such summaries will be displayed for all TEAEs, TEAEs by maximum severity, and TEAEs by strongest relationship to study drug. Serious AEs and AEs leading to discontinuation of study drug will also be summarized. Separate summaries will be given for those AEs occurring during the placebo run-in period.

All AEs will be displayed in data listings. AEs will be flagged by run-in or double-masked treatment period as appropriate.

Actual values and changes from baseline for clinical laboratory test results, BCVA, and vital signs will be summarized by study visit using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Laboratory values will be displayed in the data listings and those that are outside the normal range will be flagged, along with corresponding normal ranges.

Biomicroscopy and eye examination data will be presented by treatment group and visit for each area on both eyes. The number and percentage of subjects with observed values of each category

will be tabulated. Shifts from baseline will be presented for each eye separately. Physical examination data will be presented in data listings.

The frequency and percentage of subjects experiencing drop instillation discomfort will be tabulated by treatment group and severity (None, Mild, Moderate, Severe).

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

Procedures for handling missing data will be described in detail in the SAP.

11.7 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.

11.8 Interim Analyses

No interim analyses are currently planned.

12 DIRECT ACCESS TO SOURCE DATA and DOCUMENTS

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

Parion's designated representative (the monitor or auditor) will contact the investigator and conduct regular visits to the clinical site. The monitor will be expected and allowed to verify the investigator's qualifications, to inspect clinical site facilities, and to inspect study records, including proof of EC/IRB review, with the stipulation that subject confidentiality will be maintained in accordance with local and federal regulations, including HIPAA requirements. The monitor will also be responsible for confirming adherence to the study protocol, inspecting CRFs and source documents, and ensuring the integrity of the data. CRFs will be checked for accuracy, completeness, and clarity and will be cross-checked for consistency with source documents, including laboratory test reports and other subject records. Instances of missing or uninterpretable data will be resolved in coordination with the investigator.

The monitor/auditor will also investigate any questions concerning adherence to regulatory requirements. Any administrative concerns will be clarified and followed. The monitor will maintain contact with the site through frequent direct communications with the study site by e-mail, telephone, facsimile, and mail. The investigator and all other site personnel agree to cooperate fully with the monitor and will work in good faith with the monitor to resolve any and all questions raised and difficulties detected by the monitor.

13 QUALITY CONTROL AND QUALITY ASSURANCE

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.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. Study data for each randomized subject will be entered into a CRF by study site personnel using a secure, validated web-based electronic data capture (EDC) application. Parion Sciences will have read-only access to site-entered clinical data in the EDC application.

Instances of missing, discrepant, or uninterpretable data will be queried with the investigator for resolution. Any changes to study data will be made to the CRF and documented in an audit trail, which will be maintained within the clinical database.

14 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 Food and Drug Administration (FDA) regulations, International Conference on Harmonization (ICH) Good Clinical Practices (GCP) guidelines, 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.

14.1 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 PI as required by the IRB.

14.2 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.

15 Procedures for Modifying the Protocol or Terminating the Study

15.1 Protocol Modifications and Deviations

The investigator will make all reasonable efforts to comply with the written protocol and protocol amendments. All protocol modifications must be reviewed and approved by the appropriate EC/IRB before the revised protocol can be implemented. Emergency revisions that eliminate an apparent hazard to subjects do not require preapproval by the EC/IRB. However, the EC/IRB must be notified, in writing, as soon as possible after the modification has been made. A copy of this communication must be forwarded to Parion.

15.2 Study Termination

The trial or parts of this trial may be discontinued by Parion Sciences, Inc. or at the recommendation of the Medical Monitor and/or the PI(s) 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, all parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies) and the IRBs. In terminating the study, Parion Sciences and the investigators will assure that adequate consideration is given to the protection of the subjects' interests.

16 Data Handling and Record Keeping

16.1 Privacy and Confidentiality

In order to maintain subject privacy, all CRFs, study drug accountability records, and other documents, including communications between the study site and Parion or its designee, will identify subjects only by their initials and their assigned study identification number. If required, the investigator will grant monitors and auditors from Parion or its designee and/or regulatory authorities access to subjects' original medical records for verification of the data gathered on the CRFs and to audit the data collection process. Subject confidentiality will be maintained and will not be made publicly available unless mandated by applicable laws and regulations.

16.2 Maintaining Documents

Study site files for the retention of regulatory documents will be established at the beginning of the study, maintained for the duration of the study, and retained according to FDA and ICH/GCP guidelines and applicable regulatory requirements. The records maintained must be adequate to fully document appropriate protection of study subjects, the validity of the study, the integrity of the data, and the manner in which the study was conducted.

The investigator's site file, copies of protocols, CRFs, originals of test result reports, drug disposition logs, correspondence, records of written informed consent, and other documents pertaining to the conduct of the study must be kept on file by the investigator and in readily accessible order for at least 2 years after the last approval of a marketing application, until at least 2 years have elapsed after formal discontinuation of the clinical development of the investigational product, or according to local regulatory requirements. No study document may be destroyed without prior written consent from Parion or its designee.

16.3 Data Quality Control and Reporting

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

Parion Sciences will provide the study sites with secure access to and training on the EDC application sufficient to permit study site personnel to enter or correct information in the CRFs on the subjects for which they are responsible. Laboratory reports will be maintained by the site and data from central vendor transferred to data management outside the EDC system.

A CRF will be completed for each enrolled study subject. It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data will indicate the subject's participation in the study and will document the dates and details of study procedures, AEs, other observations, and subject status.

The investigator, or designated representative, will complete the CRF as soon as possible after information is collected. 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.

The audit trail entry will show the user's identification information and the date and time of any correction. The investigator will provide formal approval of all the information in the CRFs, including any changes made to the CRFs, to endorse the final submitted data for the subjects for whom the investigator is responsible. The CRF must be signed and dated by the Investigator upon subject withdrawal or completion of the study.

Parion Sciences will retain the CRF data and corresponding audit trails. A copy of the final archival CRF in the form of a CD or other electronic media will be placed in the investigator's study file.

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. A copy of the CRF must be maintained by the Investigator.

Screen failure data will be collected in the clinical database. Minimially demographics and reason for failure will be collected for all subjects who fail to qualify for the placebo run-in period. For subjects who fail to qualify for randomization but who have entered the placebo run-in period, additional information will be collected, including demographics, ophthalmic test results for completed measurements, reason for failure to qualify for randomization, and reported adverse events.

16.4 Clinical Laboratory Certification

A central clinical laboratory will be used to analyze all hematology and clinical chemistry samples in this study, with the exception of the urine pregnancy test. The investigator must maintain, on file, written evidence that the central clinical laboratory to be used is certified under the Clinical Laboratory Improvement Act or equivalent certification (depending on local regulations). Further, the investigator will maintain a copy of the certification, the range of normal values, the effective dates for the ranges, and the units of measurement for all laboratory tests requested in the protocol. If any of the laboratory measurements will be transformed and/or categorized in any way, a description of the procedures(s) used should be included. The investigator is expected to receive these documents before the shipment of clinical supplies.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.

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APPENDIX I: SCHEDULE OF VISITS AND PROCEDURES.

Event	Visit 1	Phone call	Visit 2	Phone Call	Visit 3	Phone call	Visit 4	ET	Follow up
	Screening Visit Day -14	Day -7 ± 1 to Day 1	Randomization -Treatment Day 1	Day 8 ± 1	Treatment Day 15	Day 22± 1	Day 29	Early Termination Visit	Follow up call 7 days ± 2 after Visit 4
Informed Consent	X								
Eligibility criteria reviewed	X		X						
Medical History/Changes	X		X		X		X	X	X
Concomitant Medication/Changes	X[a]		X		X		X	X	X
Abbreviated Physical Exam	X						X	X	
Vital Signs	X		X		X		X	X	
Urine Pregnancy Test	X						X	X	
Serum Chemistry, Hematology	X				X[b]		X	X	
Dry Eye Symptoms[c]	X		X		X		X		
Study Medication Instillation Technique Training/Observation in Clinic	X[d]		X[e]		X[e]				
Dispense Study Medication	X[d]		X[e]		X[e]				
Drop Instillation Comfort Assessment[f]	X		X		X				

Event	Visit 1	Phone call	Visit 2	Phone Call	Visit 3	Phone call	Visit 4	ET	Follow up
	Screening Visit Day -14	Day -7 ± 1 to Day 1	Randomization -Treatment Day 1	Day 8 ± 1	Treatment Day 15	Day 22± 1	Day 29	Early Termination Visit	Follow up call 7 days ± 2 after Visit 4
Subject reminder for when to take medication		X		X		X			
Collect Returned Study Medication			X[d]		X[e]		X[e]	X[e]	
Best Corrected Visual Acuity	X		X				X		
Biomicroscopy and External Eye Examination	X		X		X		X	X	
Tear osmolarity & Meibomian gland assessment[g]	X								
Tear Collection for Biomarker[h]			X		X		X		
TBUT	X		X		X		X		
Corneal Staining	X		X		X		X		
Bulbar Conjunctival Staining	X		X		X		X		
Lid wiper staining of the upper eyelid[i]	X		X		X		X		
Impression Cytology[j]			X				X		
Schirmer's Test (unanesthetized)	X								
Intraocular Pressure	X								
Adverse Event Monitoring	X	X	X	X	X	X	X	X	X

a. Concomitant medications taken within 28 days of screening will be reviewed
b. Only serum potassium

c. Dry eye symptom questionnaires (SANDE, SPEED, and 7-item symptom questionnaire) will be completed as ordered before any other study assessments are performed at each visit. SANDE Part 1 will be completed at Visits 1-4, and Part 2 will be completed at Visits 2, 3, and 4.
d. Placebo run-in study medication only
e. Double-masked study medication per assigned subject treatment number
f. To be assessed within 5 minutes after dosing in clinic for the Fellow Eye only
g. Meibomian gland assessment to include clinical gland evaluation ± meibography (if available). If available, photographs will become part of the source and electronic data.
h. Must be completed before any ocular staining has occurred. Tears from the Study Eye for prostaglandins and from the Fellow Eye for MMP-9 activity
i. Lid wiper staining may include photographs if technology is available. If available, photographs will become part of the source and electronic data.
j. Must be conducted after corneal, conjunctival and lid wiper staining and will be done in the Study Eye only

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.

An 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

The investigator will classify the study drug action taken with regard to the AE. The action taken will be classified according to the categories shown below.

Classifications for Study Drug Action Taken with Regard to an AE

Classification	Definition
Dose not changed	Study drug dose not changed in response to an AE
Drug interrupted	Study drug administration interrupted in response to an AE
Drug withdrawn	Study drug administration permanently discontinued in response to an AE
Not applicable	Action taken regarding study drug administration does not apply. "Not applicable" will be used in circumstances such as when the investigational treatment had been completed before the AE began and no opportunity to decide whether to continue, interrupt, or withdraw treatment is possible.

An AE will be followed until the investigator has determined and provided the final outcome. The outcome will be classified according to the categories shown below.

Classifications for Outcome of an AE

Classification	Definition
Recovered/Resolved	Resolution of an AE with no residual signs or symptoms
Recovered/ Resolved With Sequelae	Resolution of an AE with residual signs or symptoms
Not Recovered/Not Resolved (Continuing)	Either incomplete improvement or no improvement of an AE, such that it remains ongoing
Fatal	Outcome of an AE is death. "Fatal" will be used when death is at least possibly related to the AE.
Unknown	Outcome of an AE is not known (e.g., a subject lost to follow-up)

The investigator ensures adequate medical care is provided to subjects for any AEs, including clinically significant laboratory values related to study drug. In addition, the investigator will describe whether any treatment was given for the AE. "Yes" is used if any treatment was given in response to an AE, and may include treatments such as other medications, hospitalization, surgery, or physical therapy. "No" indicates the absence of any kind of treatment for an AE.

APPENDIX III: INSTILLATION OF MEDICATION

At Screening Visit 1, study personnel will instruct subjects who are eligible to proceed into the single-masked placebo run-in period on how to instill medication into their eyes using the study medication that subjects will be taking home. At subsequent Visits 2 and 3 after all in clinic assessments have been completed, study personnel will observe the subject's technique in self-instilling study medication using new supplies dispensed at these visits. As necessary, study personnel will re-instruct the subject on proper instillation technique and reinforce the importance of taking study medication three times daily.

Instillation Technique

- Open a pouch and remove the vial. Both eyes will be dosed with that vial
- Subject should slightly tilt his/her head back and look upward
- The subject should gently squeeze the bottle allowing one drop to coat the ocular surface on the right eye.
- Repeat instruction for left eye using the same bottle.
- The subject should gently close his or her eyes for a few seconds.
- Recapp the used vial, place it back into its pouch, and return it to the clinic for later reconciliation.

Within 5 minutes following instillation of study drops, subject should complete the Drop Instillation Comfort Assessment (Appendix VI) for the fellow eye only.

APPENDIX IV: DRY EYE SYMPTOM QUESTIONNAIRES

The following subject-completed symptom assessments will be collected as noted in Appendix I prior to any other clinic assessments to minimize bias.

- SANDE, Part 1 will be conducted at Visits 1, 2, 3, and 4, and Part 2 at Visit 2, 3, and 4.
- SPEED will be conducted at Visits 1, 2, 3, and 4
- 7-item symptom questionnaire will be conducted at Visits 1, 2, 3, and 4

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

Part 1

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** over 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 over the past day:

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

Instructions for scoring; Part 1. Measure the distance (in mm) between the left end of the line and the mark scored by the subject.

Part 2 (administer at only Visits 2, 3, and 4)

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 approximately 2 weeks ago*

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 approximately 2 weeks ago*

Instructions for scoring; Part 2. Measure the distance (in mm) between the "Last Visit" anchor at the center of the scale and the mark scored by the subject. If the mark is placed to the right of the anchor, the score will be assigned a positive value (e.g. +10). If the mark is to the left of the anchor, the score will be assigned a negative value (e.g. -15)

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 SEVERITY 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

7-ITEM SYMPTOM QUESTIONNAIRE

Please rate each of the following symptoms by drawing a line "I" on the scale where:
0 = no discomfort
100 = maximal discomfort

Eye dryness

0 ——————————————————————— 100

Burning/Stinging

0 ——————————————————————— 100

Photophobia

0 ——————————————————————— 100

Foreign Body Sensation

0 ——————————————————————— 100

Blurred Vision

0 ——————————————————————— 100

Itching

0 ——————————————————————— 100

Pain

0 ——————————————————————— 100

Note: For copying purposes, each line should be 100mm or 3.937 inches.

APPENDIX V: LABORATORY 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)

Urine pregnancy test in women of child bearing potential (Visits 1, 4 and Early Termination, if applicable).

A serum potassium test will also be done at Visit 3.

APPENDIX VI: DROP INSTILLATION COMFORT MEASUREMENT

An evaluation of the comfort of the instillation of eye drops will be assessed for the Fellow Eyeonly within 5 minutes following in-clinic dosing at Visits 1, 2, AND 3.

The subject will be asked the following question upon instillation of the study drug:

'Did you experience any discomfort when placing the drops in your right/left eye?"
Note: Personnel should indicate which eye is the Fellow Eye when asking this question.

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 an interruption of study drug treatment or discontinuation of the subject from the study.

If the subject is experiencing discomfort symptoms 5-15 minutes after the drop comfort assessment is completed, then the site should record these symptoms as AEs.