CLINICAL STUDY PROTOCOL

A Randomized, Placebo-Controlled, Double-Blind, Multicenter, Proof-of-Concept Study of Brimonidine Eye Drops for the Treatment of Dry Eye Disease (DED)

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Study Products:
Brimonidine 0.2%
Brimonidine 0.2% plus Loteprednol 0.2%
Sodium carboxymethylcellulose 0.25%(placebo)

Protocol Number:
OCU-310-DED-2017

Version:
2.0 (25 October 2017)

DOCUMENT HISTORY

Version Date	Version Number	Description
21 July 2017	1.0	Original Version
25 October 2017	2.0	Amendment 1

SCHEDULE OF VISITS AND PROCEDURES

	Day -7-0 (Screening)*	Day 1*	Day 28 (+/- 7 days)	Day 56 (+/- 7 days)	Day 84 (+/- 7 days)	Day 105 (+/- 7 days)
Informed consent	X					
Demographic information	X					
Medical history	X					
Ocular/ Dry Eye history	X					
Symptom Assessment iN Dry Eye (SANDE) questionnaire	X	X	X	X	X	X
Diary Review			X	X	X	
Vital signs		X			X	X
Visual Acuity (via Snellen)		X	X	X	X	X
Ocular Redness Score (ORS) (via Keratograph)		X	X	X	X	X
Noninvasive Tear Breakup Time (NITBUT) (via Keratograph)	X	X			X	
Validated Bulbar Redness (VBR) Scale (via Slit Lamp)		X	X	X	X	X
Ophthalmic examination (via Slit lamp)	X	X	X	X	X	X
Lissamine Green staining (Corneal and Conjunctival) (via Slit Lamp)	X	X			X	
Schirmer I test	X	X			X	
Intraocular Pressure (IOP) via Goldmann	X	X	X		X	X
Clinical Global Impression (CGI)			X	X	X	X
Subject Global Assessment (SGA)			X	X	X	X
First study medication		X				
Drug dispensation, with instruction on study medication self-administration		X[a]	X[b]	X[b]		
Adverse Events (AEs)		X[c]	X	X	X	X
Concomitant medications	X	X	X	X	X	X
Tolerability Visual Analogue Scale (VAS)		X[d]	X	X	X	

*Day 1, by definition, is when the first dose of study drug is administered. The Screening Visit and Day 1 Visit may be combined into a single visit; in this case, do not duplicate any of the Screening/Day 1 procedures. Subjects taking steroid-containing eye drops at the Screening Visit must discontinue those drops and wait a full 7 days before attending the Day 1 Visit.

[a] After administration of the first dose of study medication, subjects will be trained on how to self-administer the eyedrops and will be given a 1-month supply

[b] If required
[c] AE collection begins after the first dose of study medication on Day 1
[d] Tolerability VAS should be completed after the first dose of study medication on Day 1

ABBREVIATIONS

AE	Adverse Event
APC	Antigen-presenting cells
BAK	Benzalkonium chloride
BID	Two times per day
CNL	Corneal Neurobiology Laboratory
CRF	Case Report Form
CGI	Clinical Global Impression
DED	Dry Eye Disease
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IOP	Intraocular pressure
IRB	Institutional Review Board
KCS	Keratoconjunctivitis sicca
LLT	Lipid Layer Thickness
MAO	Monoamine Oxidase
MGD	Meibomian Gland Disease
NEI	National Eye Institute
NIKBUT	Non-invasive Keratograph Tear Film Break-Up Time
ORS	Ocular Redness Score (ORS)
OSDI	Ocular Surface Disease Index
OTC	Over the counter
SANDE	Symptom Assessment iN Dry Eye
SAE	Serious Adverse Event
SGA	Subject Global Assessment
SP	Substance P
TCA	Tricyclic Anti-depressant
TMH	Tear Meniscus Height
VA	Visual Acuity
VBR	Validated Bulbar Redness
VAS	Visual Analog Scale

Study Summary

Title	A Randomized, Placebo-Controlled, Double-Blind, Multicenter, Proof-of-Concept Study of Brimonidine Eye Drops for the Treatment of Dry Eye Disease (DED)
Short Title	Brimonidine treatment for Dry Eye Disease (DED)
Protocol Number	OCU-310-DED-2017
Phase	Proof-of-Concept
Methodology	Double-blind, placebo controlled, with 1:1:1 randomization
Study Duration	15 weeks (105 days)
Study Center(s)	Multicenter
Objectives	To evaluate the tolerability and preliminary efficacy of Brimonidine eye drops (with and without loteprednol ophthalmic suspension) for the treatment of DED.
Number of Subjects	90 (30 per arm)
Diagnosis and Main Inclusion Criteria	Men and women ≥ 18 years of age with a diagnosis of DED
Study Product, Dose, Route, Regimen	Study Drug Arm #1 (combination therapy): Brimonidine (0.2%) administered as eye drops, followed by loteprednol ophthalmic suspension (0.2%), two times a day (BID) for 12 weeks Study Drug Arm #2 (monotherapy): Brimonidine (0.2%) administered as eye drops, followed by placebo, two times a day (BID) for 12 weeks Control Arm (placebo): Lubricant Eye Drops (sodium carboxymethylcellulose, 0.25%) followed by a second application, two times a day (BID) for 12 weeks All treatment arms will contain benzalkonium chloride (BAK) preservative.
Duration of administration	12 weeks of double-blind randomized treatment. An additional End of Study visit will be performed at 15 weeks (Day 105), to provide post-treatment follow-up data.

Statistical Methodology	Unit of Analysis The unit of analysis for efficacy will be the study eye. Each subject will have a single eye identified as the study eye as follows: (i) if only 1 eye meets inclusion criteria, this eye will be the study eye and the other eye will be considered the non-qualified fellow eye; (ii) if both eyes meet inclusion criteria, the eye with the higher Ocular Redness Score (ORS) will be the study eye and the other eye will be considered the qualified fellow eye; (iii) if both eyes have the same Ocular Redness Score (ORS), then the eye with the lower Schirmer I score will be the study eye and the other eye will be considered the qualified fellow eye; (iv) if both eyes have same Schirmer 1 score, the right eye will be the study eye and the other eye will be considered the qualified fellow eye. Safety and Efficacy analyses will be primarily performed on the study eyes and secondarily on the qualified fellow eyes (for efficacy) and all fellow eyes (for safety). General Considerations The sample size was not calculated to have pre-determined power for efficacy in this proof of concept study. All continuous study assessments will be summarized by treatment and visit (as applicable) and change from baseline to each post-baseline visit using descriptive statistics (n, mean, median, standard deviation, minimum, and maximum). All categorical study assessments will be summarized by treatment and visit (as applicable) using frequency counts and percentages. Safety Tolerability will be summarized by treatment group at each visit using continuous summary statistics; p-values from pairwise two-sample t-tests and 90% and 95% CIs will be presented to test the difference in mean tolerability among the three treatment groups Efficacy The efficacy parameters, SANDE, CGI, SGA, Lissamine Green corneal and conjunctival staining, Schirmer's Test I, Keratograph Ocular Redness Score (ORS), and validated bulbar redness (VBR) scales will be summarized by treatment group at each visit and for change from baseline to each visit using continuous and discrete summary statistics as appropriate and using pairwise two-sample t-tests or Pearson's chi-squared test (or Fisher's exact test if expected cell count is <5) and corresponding asymptotic 90% and 95% CI around the difference in means or proportions. Lissamine Green corneal and conjunctival staining will be summarized by region and overall.

1 Background & Rationale

1.1 Introduction

Ocugen Inc. is developing brimonidine tartrate 0.2% eye drops (with or without loteprednol etabonate) for the treatment of Dry Eye Disease (DED). Brimonidine is an imizadoline compound that acts as a specific alpha 2 adrenergic agonist. Brimonidine tartrate ophthalmic solution is currently an FDA approved product and is available at different concentrations and is indicated for the reduction of intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension. Loteprednol etabonate ophthalmic suspension (0.2%) is a topical anti-inflammatory corticosteroid. The compound is FDA approved for the temporary relief of the signs and symptoms of seasonal allergic conjunctivitis.

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA CFR Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.2 Dry Eye Disease: Definition and Pathogenesis

Dry eye disease (DED) is a common ocular disorder involving the aberrant production and stability of tear film, which results in damage to the ocular surface and is correlated with symptoms of ocular discomfort. DED is also recognized as keratoconjunctivitis sicca (KCS), sicca syndrome, keratitis sicca, xerophthalmia, dry eye syndrome (DES), dysfunctional tear syndrome (DTS), ocular surface disease (OSD) or dry eye. DED is caused by chronic instability of preocular tear film. Tear film instability can be triggered by insufficient tear production, or by poor tear film quality that results in increased tear evaporation (Phadatare et al 2015). Furthermore, dry eye is typically categorized into two groups:

1) Aqueous tear deficient dry eye disease
2) Evaporative dry eye disease

DED is a result of changes to the lacrimal functional unit (LFU). The LFU is composed of the lacrimal glands, cornea, eyelids, meibomian glands, conjunctiva, goblet cells, and ocular nerves. The LFU is responsible for the sustained production of adequate tear film to consistently lubricate the ocular surface. Structural changes to the LFU can induce tear film instability and insufficiency, which in turn can lead to tear hyperosmolarity. Chronic osmotic stress from tear film can activate stress associated pathways in ocular surface epithelial cells, thereby triggering a pro-inflammatory response that involves a mix of chemokines, cytokines, and matrix metalloproteinases. The subsequent maturation of antigen-presenting cells (APC's) on the ocular surface leads to the migration, activation, and expansion of autoreactive T cell lymphocytes as well as other leukocytic classes in the LFU. The constant recruitment of pro-inflammatory leukocytes onto the ocular surface inflicts epithelium damage in the form of small abrasions and epithelium barrier defects (Pflugfelder et al 2008; Stevenson et al 2012). These abrasions can eventually progress to superficial punctuate keratitis, squamous metaplasia, extracellular matrix deposits, decreased goblet cell differentiation, increased epithelial cell turnover (epitheliopathy), and significant ocular surface nerve damage and neuropathy.

As DED progresses, lacrimal gland obstruction, meibomian gland orifice obstruction, thickened eyelid margins, cloudy, solid, or granular meibum secretion, eyelid telangiectasia, and meibomian gland dysfunction become common clinical features. In advanced cases, dry eye can cause fibrotic thickening of the cornea and conjunctiva, filamentous keratitis, mucoid clumping, trichiasis, symblepharon, keratinization of the eyelids and meibomian glands, corneal and conjunctival erosion and thinning, corneal and conjunctival neovascularization, corneal and conjunctival scarring, corneal ulceration, and

corneal perforation. In addition, prolonged ocular surface inflammation can lead to moderate or absolute loss/atrophy of the meibomian glands, lacrimal glands, and conjunctival goblet cells, and subsequently a dramatic reduction in tear film production and the onset of permanent DED (Messmer et al 2015).

DED prevalence increases with age. The most common causes of dry eye are contact lens usage, autoimmune disorders, systemic drug effects, and refractive surgeries, particularly in middle-aged and older adults. DED also occurs in a higher percentage of women than men, especially in women entering menopause or pregnancy; hormone imbalances during menopause or pregnancy can cause lacrimal gland and ocular surface inflammation and tear film abnormalities (Phadatare et al 2015).

1.3 Dry Eye Disease: Clinical Signs and Symptoms

Common signs and symptoms of DED include: eye redness, ocular pain, burning and stinging sensation, foreign body sensation, pruritus, itchy or scratchy eye sensation, tired eyes, enhanced eye pressure, photophobia, painful mucous discharge, and in some cases epiphora. DED typically affects eyes bilaterally. Dry eye can heavily impact visual function especially during visually intensive activities and can overall decrease quality of life. (Phadatare et al 2015; Messmer et al 2015).

1.4 Dry Eye Disease: Diagnostic Testing

Clinicians use several diagnostic tests to diagnose DED and to assess disease severity. These tests fall into two groups: signs (objective) and symptoms (subjective). For DED signs, there are several quantitative tests, including: Schirmer Test, Schirmer I Test, Schirmer II test, tear film breakup time (TBUT), non-invasive tear film breakup time (NITBUT) epithelial staining scores (rose bengal, lissamine green, fluorescein) via slit-lamp examination, tear function index (TFI), tear fluid protein immunoassays, fluorophotometry, meibography, meibometry, meiboscopy, meniscometry, lacrimal gland biopsy, impression cytology, hypolysozyme measurement, hyperosmolarity measurement, and lipid layer analysis, ocular redness scoring, automated blink analysis, and meniscus evaluation utilizing Lipiview II, Keratograph 5M, or OCT equipment (Phadatare et al 2015).

Symptom measurements utilizing physician and/or patient disease scoring include: extensive dry eye questionnaire (DEQ), visual analog scale (VAS), ocular surface disease index (OSDI), national eye institute visual function questionnaire (NEI-VFQ-25), symptom assessment in dry eye questionnaire (SANDE), and standardized patient evaluation of eye dryness questionnaire (SPEED). While most physicians use OSDI as the primary diagnostic questionnaire for dry eye, the SANDE visual analog scale-based questionnaire offers significant advantages, such as simplicity and brevity, while maintaining a high degree of diagnostic accuracy. In addition, in two recent review articles, the SANDE questionnaire showed a significant correlation to OSDI in terms of DED scoring (Amparo, et al, 2015; Saboo, et al, 2015).).

Many of the above tests have been used in recent DED studies. In this proof-of-concept study, there will be multiple exploratory endpoints based on conventional dry eye assessments, subjective and objective. Each study site will use the Oculos Keratograph 5M to assess the Ocular Redness Score (ORS), and these results will be compared to the Validated Bulbar Redness (VBR) scale (Section 3.4.5. Based on the results of this study, Ocugen will decide which sign and/or symptom to be used as the primary (or co-primary) endpoint(s) in later stage studies.

1.5 Dry Eye Disease: Current Management

Typically, clinicians prescribe artificial tear eyedrops and topical corticosteroids for short-term relief of DED. Antibiotics (tetracyclines and macrolides), non-steroidal anti-inflammatory agents, autologous serum drops, omega fatty acids, mucin secretagogues, and anti-inflammatory agents are also used to

combat DED symptoms. In addition, prosthetic scleral lenses (i.e. PROSE) that also serve as supplemental tear reservoirs are increasingly being prescribed to enhance ocular surface hydration in patients with chronic DED. Hot eyelid compresses are often utilized to treat meibomian gland dysfunction, a primary driver of evaporative dry eye disease. In advanced cases of DED, punctual plugs can be installed to block tear drainage. In severe cases of dry eye, tarsorrhaphy surgery, tear duct cauterization, or amniotic membrane transplant might be required to reduce tear evaporation (Phadatare et al 2015; Lin et al 2014).

Currently there are only two pharmaceutical agents that are FDA approved for the treatment of dry eye: cylcosporine A ophthalmic emulsion (Restasis®) and lifitegrast ophthalmic solution (Xiidra™). Restasis® 0.05% is a topical immunomodulator indicated to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca (Restasis® Prescribing Information). Xiidra™ 5% is a lymphocyte function-associated antigen (LFA-1) antagonist indicated for the treatment of signs and symptoms of dry eye disease (Xiidra™ Prescribing Information). Given the complexity, severity, and frequency of DED, and given the limited modes of action by which these two compounds treat dry eyes, there is a medical need for other dry eye therapies, particularly those with multiple modes of action that target the wider dry eye population and are effective and safe for long-term daily use.

1.6 Scientific Rationale for Brimonidine Therapy in Dry Eye Disease

The scientific rationale to establish a medically plausible basis for the use of local brimonidine tartrate for the treatment of DED include the potential following mechanisms:

1. Reduction of ocular surface blood flow: As an alpha agonist, brimonidine tartrate can significantly cause vasoconstriction leading to the reduction of blood flow to the ocular surface, which, in turn, reduces local pressure, edema and inflammation (Piwnica, 2014). In this manner, brimonidine has the potential to reduce redness of the ocular surface.
2. Disruption of leukocyte extravasation to the ocular tissue: brimonidine can inhibit the infiltration of activated leukocytes by modulating endothelial cell activity (Herrera-Garcia, 2014).
3. Suppression of leukocytes activation: Although inhibition of T lymphocytes has not been directly studied with brimonidine tartrate, activation of alpha 2 receptors (with another alpha 2 agonist) has been shown to suppress the reactivation of T lymphocytes (Felsner, 1995). In addition, induction of neutrophil apoptosis with brimonidine has been reported in acute inflammation (Herrera-Garcia, 2014).
4. Analgesic properties: Brimonidine antagonizes or suppresses the excitatory response of phenylephrine and noradrenaline (Bradshaw, 1984). As an alpha agonist, brimonidine may attenuate pro-inflammatory cytokine release from leukocytes, which in turn, attenuates neuritis-induced pain (Romero-Sandoval, 2007; Romero-Sandoval, 2005).
5. Reduction of fibrosis and suppression of excessive extracellular matrix (ECM) formation: Fibrosis is observed in ocular Graft-vs. Host Disease (oGVHD), and this is associated with severe dry eye. oGVHD is characterized by excessive number of CD34+ fibroblasts, excessive fibrosis, and over accumulation of ECM in the lacrimal glands leading to dysfunction (Ogawa, 2010). Brimonidine has been shown to attenuate the TGF-β1-induced production of ECM proteins, which in turn leads to the decrease in the synthesis of fibronectin and collagens in human fibroblasts (Hong, 2015).

In summary, brimonidine, through its ability to vasoconstrict, has the potential to reduce redness of the ocular surface. In addition, by attenuating chronic inflammation and excessive fibrosis, brimonidine tartrate allows the ocular surface and tear film producing glands to avoid further damage from DED. Moreover, brimonidine tartrate can alleviate heightened ocular pain through its analgesic and anti-inflammatory properties, which maximizes tolerability when delivered to the surface of the eye. Brimonidine tartrate is an FDA approved product that has demonstrated a robust safety profile via

topical ocular delivery for the treatment of open-angle glaucoma. Brimonidine tartrate 0.2%, which includes the preservative benzalkonium chloride (BAK), is generally safe and well tolerated.

1.7 Scientific Rationale for Brimonidine Combination Therapy in Dry Eye Disease

As mentioned, loteprednol etabonate ophthalmic suspension (0.2%) is a topical anti-inflammatory corticosteroid that is FDA approved for the temporary relief of the signs and symptoms of seasonal allergic conjunctivitis when given as eye drops 4 times a day. In addition, loteprednol, as both an ophthalmic suspension and gel, has been used to relieve some signs and symptoms of DED, though without specific FDA approval for this indication. Currently, a new formulation of loteprednol etabonate, designated KPI-121 0.25% ophthalmic suspension, is being assessed in two phase 3 studies in subjects who have a documented clinical diagnosis of DED (NCT02813265; NCT02819284).

Loteprednol, like other ophthalmic steroids, has the potential to raise intra-ocular pressure (IOP). Among the patients studied in the development program for loteprednol etabonate ophthalmic suspension, 0.2% (Alrex™), the incidence of clinically significant increases in IOP ($\geq$ 10mmHg) was 1% (1/133) and 1% (1/135) with placebo (Alrex™ Prescribing Information). Data from published literature indicate that topical loteprednol has a minimal effect on IOP when used for a variety of ocular surface and intraocular inflammatory disorders, including DED, ocular allergies, anterior uveitis, keratoplasty, and postoperative pain/inflammation following cataract surgery (Sheppard, 2016). Nevertheless, for safety purposes, IOP will be measured at Screening, and only subjects with an IOP $\geq$5 mmHg and $\leq$ 22mmHg are eligible for this study. In addition, IOP will be measured at Days 28, 84, and 105. Finally, loteprednol will be given twice per day (not 4 times per day) in this study, and only to subjects on the combination arm, who will also be receiving brimonidine.

As described, brimonidine and loteprednol, when given together, may enhance efficacy in treating DED, relative to either product alone, given their different modes of action. Moreover, these two compounds may act in synergy to enhance the safety profile compared to either product alone. For example, brimonidine is FDA approved to reduce IOP in patients with glaucoma. Therefore, when used in combination, brimonidine, which can lower IOP, can counteract loteprednol, which can elevate IOP. Conversely, loteprednol, like other corticosteroids, can treat allergic reactions, including those caused by other eye drops. Indeed, allergic reactions have occurred in patients receiving brimonidine, and these reactions could be treated (or prevented) by loteprednol.

In summary, the combination of brimonidine and loteprednol makes sense from both an efficacy and safety standpoint. Accordingly, in one arm of this study, loteprednol etabonate ophthalmic suspension (0.2%) will be given 5-10 minutes after brimonidine tartrate ophthalmic solution (0.2%). If the results are positive, a true combination formulation may be developed for use in subsequent clinical trials.

1.8 Specific Agents in this Study

The primary agent used in this study is a commercial alpha-2 adrenergic receptor agonist, brimonidine 0.2%, applied as eye drops in subjects with DED. Brimonidine tartrate ophthalmic solution is a relatively selective alpha-2 adrenergic agonist for ophthalmic use. The chemical name of brimonidine tartrate is 5-bromo-6-(2-imidazolidinylideneamino) quinoxaline L-tartrate.

The other active agent is a commercial topical anti-inflammatory corticosteroid, loteprednol etabonate ophthalmic suspension (0.2%), approved for the temporary relief of the signs and symptoms of seasonal allergic conjunctivitis. Loteprednol etabonate is structurally similar to other ester-based corticosteroids except that the position 20 ketone group is absent. It is lipid soluble, which enhances cell penetration, and undergoes extensive metabolism to inactive carboxylic acid metabolites. It has a

potent anti-inflammatory effect but decreased potential to raise IOP relative to other corticosteroids, (Alrex™ Prescribing Information).

The placebo in this study is Lubricant Eye Drops (sodium carboxymethylcellulose, 0.25%).

All three agents in this study are commercially available and preserved with benzalkonium chloride (BAK).

1.9 Brimonidine Preclinical Data

1.9.1 Published Literature: In Vitro Studies

1.9.1.1 Vasoconstriction Properties

Brimonidine is a potent vasoconstrictor. In an *ex vivo* human skin biopsy neural inflammation model, brimonidine significantly inhibited vasodilation of human blood vessels exposed to capsaicin. Further, in an *ex vivo* model, brimonidine has been shown to attenuate the vasodilation of subcutaneous vessels with a diameter of less than 200 micrometers (Piwnica, 2014) that is approximately 13-fold larger than typical capillaries of the conjunctiva, suggesting brimonidine has vasoconstriction effects on conjunctiva vessels (Shahidi, 2010). Supowit et al 1998 determined in a separate study that brimonidine attenuates the release of calcitonin gene-related peptide (CGRP, a key neurotransmitter) in adult rat dorsal root ganglia neurons that in turn mediates inflammation and vasodilation (Supowit, 1998).

1.9.1.2 Inhibition of Activated Leukocyte Migration

Alpha 2 adrenergic agonists disrupt leukocyte extravasation into inflamed tissue by modulating endothelial cell activity. Herrera-Garcia et al 2014 demonstrated that brimonidine significantly decreased neutrophil trans-endothelial migration by restricting macromolecular and ion transport in HUVEC endothelial cells (Herrera-Garcia, 2014). Herrera-Garcia et al also noted that brimonidine decreased ICAM 1- VE-cadherin phosphorylation on HUVEC cells; ICAM1 and VE-Cadherin play crucial roles in leukocyte extravasation via the destabilization of endothelial cell adherens junctions. The authors additionally proved that brimonidine restricted L-selectin shedding and CD11b up-regulation, both critical steps in neutrophil extravasation into inflamed tissue (Herrera-Garcia, 2014).

1.9.1.3 Alpha 2 Adrenergic Agonists Potently Disrupt Leukocyte Activation

Bao et al proved that alpha -2 adrenergic agonists (e.g., clonidine) markedly diminished rat leukocyte proliferation, specifically T lymphocytes, and interfered with the production of pro-inflammatory cytokines IFN-gamma and IL-4. Clonidine is an adrenergic agonist that binds with nanomolar affinity to alpha 2a receptor subtypes (analogous to brimonidine's binding affinity to alpha-2 adrenergic receptors; Bao, 2007), suggesting that both brimonidine and clonidine can inhibit the activation of leukocytes.

1.9.1.4 Mediation of Fibroblast Activity and Production of Extracellular Matrix Proteins

Brimonidine can potentially alleviate ocular surface fibrosis and stimulate corneal wound healing. Hong et al demonstrated that brimonidine down-regulated TGF-beta activity in human Tenon's fibroblasts suggesting that brimonidine acts an anti-fibrotic compound (Hong, 2015). It is possible that brimonidine can alleviate hyperkeratinization and fibrotic conditions found in the meibomian glands, lacrimal glands, conjunctiva, and cornea. It has also been shown that fibroblasts, excessive fibrosis, and over accumulation of ECM in the lacrimal glands can lead to dysfunction of the lacrimal gland (Ogawa, 2010).

1.9.2 Published Literature: In Vivo Animal Studies

1.9.2.1 Potential Ocular Analgesic Activity

Brimonidine is believed to act as an ocular analgesic via its antagonistic effects on nociceptive receptor signaling. The compound has been shown to significantly suppress the stimulation of nociceptive receptors in the murine cerebral cortex by antagonizing the pro-inflammatory effects of norepinephrine and phenylephrine (Bradshaw, 1984). Nociceptive neuron activation in ocular surface tissue is linked to elevated ocular pain, photophobia, ocular discomfort, and ocular surface inflammation (Belmonte, 2004). CGRP and substance P are two neuropeptides linked to chronic ocular surface inflammation and hyperemia; nociceptive corneal nerves release CGRP and substance P when the cornea is wounded. The neural inflammatory peptides spur the recruitment of immune cells onto the ocular surface and induce up-regulation of pro-inflammatory cytokines and hyperalgesia (Belmonte, 2004). Nociceptive neurons that release CGRP are found in the conjunctiva and eyelid, including the meibomian glands (Luhtala, 1991; Simons, 1994). An immunohistochemical study on primate meibomian tissue confirmed the presence of CGRP and substance P releasing nociceptive nerves (Seifert, 1996).

Moreover, a study in guinea pig demonstrated that brimonidine counteracts the bronchoconstriction effects of CGRP as well as the release of key peptides under inflammatory conditions (Lou, 1992). Therefore, it is likely that brimonidine's inhibition of nociceptive receptors prevents the release of neural-inflammatory peptides, including CGRP, on the ocular surface and reduces ocular surface pain sensitivity. Furthermore, recent evidence has suggested that tear film hyperosmolarity increases ocular surface nociceptive sensitivity to pain. Tear film hyperosmolarity is a key pathological sign of ocular surface disease and severe dry eye (Hirata, 2014). Brimonidine's analgesic properties may prevent the hyper-activation of ocular nociceptive neurons to inflammatory stimuli. In addition, alpha 2 adrenergic agonists, including brimonidine and clonidine, lower pro-inflammatory cytokine release from leukocytes, which in turn attenuate neuritis-induced pain (Romero-Sandoval, 2005).

1.9.2.2 Potential Suppression of Activated Leukocytes

Alpha 2 adrenergic agonists suppress leukocyte reactivity and activation, and can cause leukocyte apoptosis at high concentrations. This effect is particularly seen with T cell lymphocytes and neutrophils. Notably, clonidine (an alpha agonist) markedly diminishes leukocyte proliferation and interferes with the release of TNF alpha, IFN-gamma, IL-1a, IL-1beta, IL-4 and IL-6. Clonidine shifts leukocyte cytokine activity to an anti-inflammatory profile (Felsner, 1995; Romero-Sandoval, 2007; Romero-Sandoval, 2005). Herrera-Garcia et al (2014) demonstrated that brimonidine significantly lowered the recruitment and activation of neutrophils in an acute inflammation mouse model, suggesting that suppression of activated leukocytes may be one of the key modes of action of brimonidine.

1.9.2.3 In Vivo Inhibition of Local Edema

In an in vivo mouse ear inflammation model, brimonidine inhibited ear edema by up to 76% (Piwnica, 2014).

1.10 Brimonidine Clinical Data to Date

In two small studies, brimonidine has been used to treat ocular Graft-vs-Host Disease (oGVHD), which shares some of the features of severe Dry Eye Disease (DED).

In an exploratory retrospective investigator-led study of meibomian gland dysfunction (MGD) in 18 patients with chronic oGVHD, one drop of brimonidine tartrate ophthalmic solution, 0.15% (Alphagon® P) was instilled twice per day in each eye for at least 6 months. A beneficial effect was observed in 16/18 (89%) of patients, who in turn showed no progression of disease and no significant side effects. Of the 18 patients, 15 (83%) had moderate to marked improvement in the physician's clinical evaluation (Sponsor data, unpublished). After this hypothesis-generating study, plans were made for a small prospective, controlled study using objective endpoints.

A phase I/II randomized, placebo-controlled, double-blind, single-center, tolerability and preliminary efficacy study of use of brimonidine eye drops (0.075% and 0.15%) for treatment of ocular Graft-vs-Host Disease (oGVHD) was conducted at the University of Illinois (Chicago). Given the slow pace of identifying and recruiting oGVHD patients, the study was paused after 40% enrollment. An interim analysis was performed to access tolerability to avoid any unethical continuation of this trial, if any harm was found. The scope of this analysis was limited to tolerability only and imposed p-value adjustments on the analysis to alleviate inflated type I error. Specifically, the study evaluated if the categorical type of tolerability measure was different between control (Artificial Tears) and intervention (Brimonidine, including both 0.15% and 0.075% dose groups), by using Fisher's exact test. The type I error was adjusted by using an alpha spending function approach with O'Brien-Fleming type boundaries. There was inadequate power to assess an efficacy benefit.

In this tolerability analysis, the valid sample size was 11 patients at 12 weeks. The table below showed the frequency/distribution of tolerability at week 12 by randomization group. The numbers indicated that the distributions of two groups were similar, where most participants [4 out of 6 (67%) in Artificial Tears, and 4 out of 5 (80%) in Brimonidine group] had 100% tolerability. The other two tolerability categories, 95% and 90%, only had 1 or 2 responses. The investigator then grouped 90% and 95% tolerability together, and conducted a Fisher's exact test to see if statistically there was a distribution difference between the two groups. The result was negative with a p-value =1, meaning no statistical difference was found. Overall, the results showed similar tolerability between the Brimonidine and Artificial Tears groups. In summary, there was no specific tolerability concern for brimonidine (Sponsor data, unpublished).

Table 1. Distribution of Tolerability by Randomization

	Artificial Tears (N=6)	Brimonidine: low+high (N=5)	Total (N=11)
Tolerability (%):			
100	67% (4)	80% (4)	73% (8)
95	17% (1)	0% (0)	9% (1)
90	17% (1)	20% (1)	18% (2)

1.11 Dose Rationale and Risk/Benefits

Brimonidine 0.2% is currently used topically as eye drops, 2-3 times a day, for the lowering of intraocular pressure in patients with open-angle glaucoma or ocular hypertension. The other active agent is a commercial topical anti-inflammatory corticosteroid, loteprednol ophthalmic suspension (0.2%), used topically as eye drops 4 times a day, for the temporary relief of the signs and symptoms of seasonal allergic conjunctivitis. The placebo control group is Lubricant Eye Drops (sodium carboxymethylcellulose, 0.25%) eye drops. All three agents are considered safe and well tolerated when used multiple times per day over extended time periods. In this proof of concept study, all three agents will be preserved with benzalkonium chloride (BAK).

Regardless of study arm, a total of two drops will be applied to each eye, two times a day in a double blinded fashion over 12 weeks. An End of Study visit will be performed at 15 weeks (day 105) for post-treatment evaluation.

Several studies of brimonidine for the treatment of glaucoma have reported the overall long-term safety and efficacy of brimonidine 0.2% and 0.15% after 1, 3 and 4 years. One study demonstrated a reduction in adverse effects with brimonidine 0.15% (Katz, 2002), but another has shown no difference between brimonidine 0.2% and 0.15% (Mundorf, 2003). The most common systemic side effects include dysguesia, fatigue, eye pain, dry mouth and headache (Adkins, 1998; Chew, 2014; Melamed, 2000; LeBlanc, 1998; Schuman, 1997). The incidence of blepharitis and blepharoconjunctivitis has been reported as 9 – 12.7% (Schuman, 1997; Katz, 1999; Schuman, 1996); follicular conjunctivitis has been found in 7.8 – 12.7% of patients (Schuman, 1997; Katz, 1999); and conjunctival hyperemia has a reported incidence of 5 – 30.3% (Nguyen, 2013; Rahman, 2010).

Brimonidine tartrate ophthalmic solution 0.2% is contraindicated in patients with hypersensitivity to any component of this medication, and in neonates and infants under the age of 2 years in whom side effects associated with CNS depression have been observed (Carlsen, 1999; Korsch, 1999; Soto-Perez-de-Celis, 2007; Vanhaesebrouck, 2009). These effects may occur because children have a less mature blood-brain barrier (BBB), and hence, a limited capacity to impede brimonidine uptake and prevent CNS effects. In addition, there is laboratory evidence that alpha-2-adrenoceptor agonists may potentiate smooth muscle vasoconstriction in arteries (Chotani, 2000), and brimonidine is, therefore, contraindicated in cerebral or coronary insufficiency, orthostatic hypotension, thromboangitis obliterans and Raynaud's phenomenon (Alphagan® and Alphagan® P Prescribing Information).

An allergic reaction to brimonidine cannot be predicted beforehand. In event of an allergic reaction, the drug will be stopped immediately, and symptoms will be managed appropriately depending on the severity of the reaction. As mentioned, in the combination arm of the study, loteprednol could prevent or attenuate hypersensitivity reactions and is approved for the treatment of allergic conjunctivitis.

No psychological, social, legal, or financial risk is expected from participating in the research.

2 Study Objectives

The main objective of this study is to establish whether subjects with dry eye disease (DED) can tolerate receiving Brimonidine 0.2% eye drops, alone or in combination with loteprednol, two times a day for twelve weeks (primary tolerability objective). The other objective is to investigate the preliminary efficacy of Brimonidine 0.2% topical eye drop solution, alone or in combination with loteprednol 0.2%, in treating DED.

3 Study Design

3.1 General Design

This will be a randomized, placebo-controlled, double-blind study, in which 90 subjects will be enrolled at up to four clinical sites. Subjects will be randomly assigned to one of three groups, with 30 subjects per group, as follows:

> Study Drug Arm #1 (combination therapy): Brimonidine (0.2%) administered as eye drops, followed by loteprednol ophthalmic suspension (0.2%), two times a day (BID) for 12 weeks
>
> Study Drug Arm #2 (monotherapy): Brimonidine (0.2%) administered as eye drops, followed by placebo, two times a day (BID) for 12 weeks
>
> Control Arm (placebo): Lubricant Eye Drops (sodium carboxymethylcellulose, 0.25%), followed by a second application, two times a day (BID) for 12 weeks

Enrolled subjects will receive the study treatment for 12 weeks and will be asked to return for a final visit at 15 weeks to evaluate safety and efficacy post-treatment.

Patients with DED will be approached by a member of the research staff to determine if the patient might be interested in participating in this study. If the patient is interested, the research staff member will describe the study, and thereafter the patient will be asked to sign the informed consent form. Screening procedures include documentation of DED using currently accepted criteria. Eligible patients who are willing to participate and meet study criteria will be enrolled in the study.

All enrolled subjects will receive their first dose of the test medication (Brimonidine 0.2% with or without loteprednol ophthalmic suspension 0.2%), or placebo (sodium carboxymethylcellulose, 0.25%) on study Day 1 in the doctor's office, and will self-administer all subsequent doses. See Section 5.4 *Preparation and Administration of Study Drug* for details.

Subjects will be given diaries to record the time of each dose. In addition, they will be asked to make a note of any missed doses together with the reason for the omission. Subjects will return four weeks later (Day 28) for further study assessments, and thereafter at Day 56 and Day 84 (primary endpoint assessment visit), and finally at Day 105 after three weeks (or 21 days) of no study drug treatment.

There is no primary efficacy endpoint in this study. Instead several exploratory efficacy endpoints will be assessed.

The primary tolerability endpoint is the change in the test substance tolerance between Day 1 (post-dose) and 12 weeks (84 days).

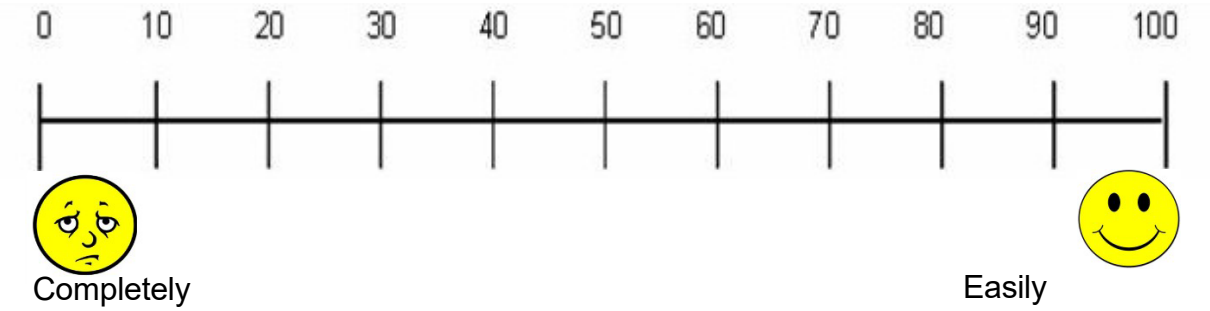
3.1.1 Primary Tolerability Endpoint: Test Substance Tolerance (Visual Analogue Scale)

Subjects will assess their tolerance to the administration of the study drug, utilizing a Visual Analogue Scale (VAS). The VAS is a 100-mm horizontal line with verbal descriptors at either end. The VAS ratings will be completed after administration of the study drug on Day 1 (post-dose), Day 28, Day 56, and Day 84,. Subjects will place a single slash mark across the horizontal line between the end labeled "completely intolerable" (0 mm) and "easily tolerable" (100mm).

The VAS rating is as follows:

Visual Analogue

On the scale of 0 to 100 seen below, please mark where you would rate your tolerability to administration of the study drug.



3.2 Secondary Study Endpoints

There are no secondary endpoints in this study

3.3 Exploratory Study Endpoints

The exploratory study endpoints will include:

1. Symptom Assessment Questionnaire iN Dry Eye (SANDE)
2. Change in corneal and conjunctival staining scores by Lissamine Green dye staining
3. Change in tear secretion as measured by Schirmer I test
4. Change in Keratograph Ocular Redness Score (ORS)
5. Change in Validated Bulbar Redness (VBR) grading scale
6. Clinical Global Impression (CGI) of change in symptoms from baseline (physician's rating)
7. Subject Global Assessment (SGA) of overall change from baseline (subject's rating)

Note: In these exploratory analyses, endpoint values will be compared to baseline values, where "baseline" is defined as the measurement done on Day 1 before initial study drug is administered.

3.3.1 Exploratory Efficacy Endpoint: Symptom Assessment iN Dry Eye (SANDE)

The Symptom Assessment Questionnaire iN Dry Eye (SANDE) is a short questionnaire based on a visual analog scale that quantifies both severity and frequency of dry eye symptoms. The SANDE is comprised of two questions, and each question employs a 100mm horizontal linear visual analog scale. The measurement of symptom frequency ranges from "rarely" to "all of the time," and the symptom severity from "very mild" to "very severe." Subjects will complete this scale on Day 1 prior to first dose (Baseline), Day 28, Day 56, Day 84, and Day 105. At each visit, subjects will be asked to place a mark on the two given lines based on the extent of their symptoms. The locations of the marks made by the subjects on the 100 mm horizontal lines will be measured in mm from left to right and recorded. The overall SANDE score will be calculated by multiplying the frequency score by the severity score and obtaining the square root (Amparo, 2015; Saboo, 2015). The questions and scoring system are shown below:

SANDE Questionnaire

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

1. Frequency of symptoms:
Please place an 'X' on the line to indicate how often, on average, your eyes feel **dry and/or irritated:**

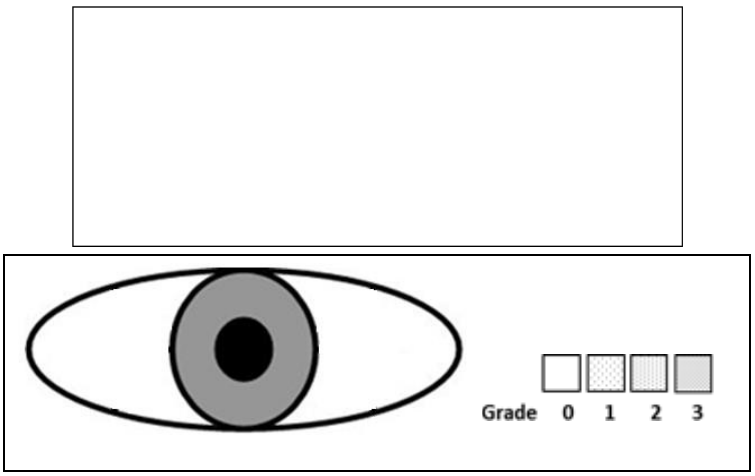
Rarely ——————————————— **All the time**

2. Severity of symptoms:
Please place an 'X' on the line to indicate how severe, on average, you feel your symptoms of **dryness and/or irritation**:

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

3.3.2 Exploratory Efficacy Endpoint: Ocular Surface Lissamine Green Dye Staining Score (via Slit Lamp)

Lissamine Green dye (approximately 5uL of 1% solution) will be applied over the surface of both eyes. Using the slit lamp, Corneal and Conjunctival Staining will be graded using the grading system described below. Corneal staining will be graded in 5 zones. Each zone will be graded from 0 to 3 based on the density of punctate staining (maximum score =15). Similarly, conjunctiva will be graded for each eye from 0 to 3 based on the density of punctate staining in the nasal-bulbar and temporal-bulbar zones staining (maximum score =6). Because Lissamine Green dye can fade variably within 4-5 minutes, results should be recorded within approximately 1 minute of application to the eye surface. The scoring pattern is represented below:



3.3.3 Exploratory Efficacy Endpoint: Schirmer I Test

Schirmer's test uses paper strips inserted into the eye for several minutes to measure the production of tears. After instilling one drop of a topical anesthetic (e.g., proparacaine) to each eye, wait

approximately 30 seconds, then blot the inferior cul-de-sac with a tissue. Place a small strip of filter paper inside the lower eyelid (inferior fornix). The eyes are closed for 5 minutes. The paper is then removed and the amount of moisture is measured. The study site will capture the actual measurement in millimeters (mm) and the Sponsor will convert to categories based on the number of mm of wetting of the paper after 5 minutes, as follows:

1. Normal is ≥15 mm.
2. Mild dry eye is 11-14 mm
3. Moderate dry eye is 6-10mm
4. Severe dry eye is ≤5 mm

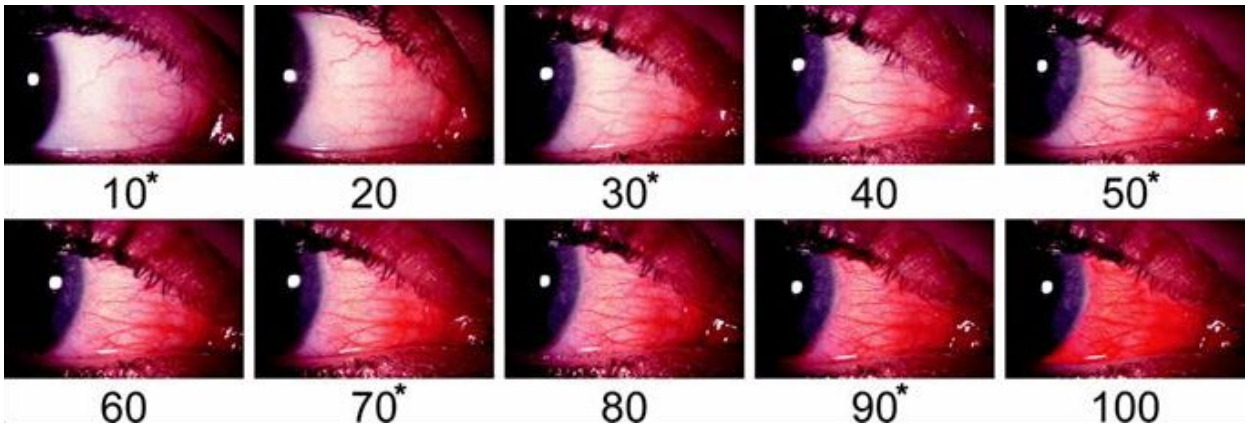
3.3.4 Exploratory Efficacy Endpoint: Ocular Redness Score (ORS) (via Keratograph)

Keratograph is FDA approved and is used in clinical care of patients in the US. The Oculus Keratograph 5M performs a non-invasive tear film analysis. It uses a Placido bowl with a camera aperture that has a fixation mark in the center. The device provides consistent illumination, allowing scanning of the exposed bulbar conjunctiva to take place. The keratograph then analyzes the scanned area. This system generates an ocular redness score (ORS) automatically, which is based on the area percentage ratio between the vessels and the rest of the analyzed area. For instance, if the ratio is 16%, then the score is 1.6. The maximum ratio, according to the manufacturer, is 40%; therefore, the ocular redness scores that the machine generates range between 0.0 and 4.0.
Note: The Keratograph 5M will also be used for measurement of average NITBUT, although this is not considered an Exploratory Efficacy Endpoint in this study.

3.3.5 Exploratory Efficacy Endpoint: Validated Bulbar Redness (VBR) Grading Scale (via Slit Lamp)

Ocular surface redness (nasal or temporal) will be assessed using the Validated Bulbar Redness grading scale (VBR) (Schulze, 2007). The VBR consists of a set of ten images illustrating different degrees of ocular surface redness (OR), ranging from normal to severe, and each image is assigned a value in an order of ascending severity. Colored copies of these images will be made and put up in all the examination rooms. Subjects will be examined by a slit-lamp at 10X magnification using direct diffuse illumination (slit fully opened, angled at 30°- 50° approximately; at half illumination intensity with rheostat set to maximum voltage) and the bulbar conjunctival injection of the subject's eye (nasal and temporal) will be compared to the reference images and graded accordingly. To maintain uniformity, all subjects will be graded by a single physician (Principal Investigator) at each study site under constant illumination conditions. The subjects will be asked to look at nasal or temporal fixation marks while the physician will examine the temporal or nasal bulbar conjunctivae, respectively.

Photographic anchors and their respective grades for ocular surface redness are shown below:



3.3.6 Exploratory Efficacy Endpoint: Clinical Global Impression (CGI)

At each visit after Day 1, the Principal Investigator will use his/her clinical judgement of all signs and symptoms to provide a global assessment of each subject's change in signs and symptoms from Day 1.

The CGI is as follows (Miller, 2010): Question (to physician): *In general, compared with the subject's symptoms and signs on Day 1, how would you characterize his/her overall signs and symptoms now?*

The responses will be categorized on a seven-point scale as follows:

- Marked worsening
- Moderate worsening
- Minimal worsening
- Unchanged
- Minimal improvement
- Moderate improvement
- Marked improvement

3.3.7 Exploratory Efficacy Endpoint: Subject Global Assessment (SGA)

At each visit after Day 1, subjects will be asked to assess their overall change from Day 1. The SGA is as follows (Miller, 2010):

Question 1 (to subject): *Compared to when you first received the study medication (Day 1 visit), how are your eye symptoms now?*

The responses will be categorized on a five-point scale as follows:

- Much worse
- Worse
- About the same
- Improved
- Much improved

3.4 Safety Endpoints

Safety assessments include Vital Signs, adverse events, and findings from ophthalmic examinations. All ocular and non-ocular adverse events will be assessed for severity and relationship to the investigational product.

In addition to the primary tolerability endpoint (Section 3.1.1), the following safety endpoints will be assessed:

- The proportion of subjects at Day 84 who successfully complete (i.e., tolerate) a full twelve weeks (i.e., 84 days) of therapy with topical administration two times per day (BID).
- All reported adverse events, classified by frequency, severity, and relatedness, from baseline (Day 1) through the last study visit (Day 105).
- Clinically significant changes in vital signs or ophthalmic examination from baseline.

3.4.1 Vital Signs

Vital signs will be obtained and recorded at the Day 1 Visit, prior to the first administration of the study drug, and on Day 84 and Day 105. The following vital signs will be measured: 1) blood pressure measurements (mm Hg) will be taken while the subject is relaxed in a sitting position for at least 3 minutes with the arm at heart level. 2) Heart rate will be recorded as beats per minute (bpm). 3) Body temperature (forehead) will be recorded in degrees Celsius (^{0}C).

Clinically significant changes (worsening) from baseline (Day 1) will be recorded on the adverse event forms.

3.4.2 Ophthalmic Examination

At all visits, the Investigator will conduct a complete undilated examination of the eyes using a binocular slit lamp. The Investigator will examine the tear film, eye lids, lashes, bulbar and palpebral conjunctiva, upper and lower lid puncta, cornea, anterior chamber, iris, lens, and anterior vitreous. Conjunctival hyperemia (ocular surface redness) will be graded at each visit using the VBR grading system as explained in Section 3.3.5. Also, measurement of visual acuity (via Snellen, with eyeglasses if applicable), and intraocular pressure (via Goldmann tonometry) will be performed. Clinically significant changes (worsening) from the baseline examination (Day 1) will be recorded on the adverse event forms.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Subjects will be eligible for the study if **all** the following criteria are met:

1. Aged 18 years or older.
2. Sign and date informed consent form approved by the IRB
3. History of Dry Eye Disease
4. Objective evidence of DED in at least one eye by having 2 or more of the following 4 signs in the same eye at Screening and Baseline (Day 1) visits (same qualifying signs must be present at both visits):

    i. Conjunctival staining at $\geq$ 1 (out of a possible score of 6 per eye)
    ii. Corneal staining at $\geq$2 (out of a possible score of 15 per eye)
    iii. Noninvasive Tear Break-Up Time (NITBUT) at $\leq$ 7 seconds

iv. Schirmer test at <10mm in 5 minutes

Note: For those subjects undergoing a combined Screening/Baseline visit, these criteria need only be met during that one visit.

5. Symptomatic evidence of DED by having a global symptom score (SANDE) ≥25 mm at both Screening and Baseline (Day 1) visits
6. Intraocular pressure (IOP) ≥ 5 mmHg and ≤22 mmHg in each eye
7. Women who satisfy one of the following:
    a. Are of child-bearing potential (WOCP) who are not pregnant or lactating and who are either abstinent or sexually active on an acceptable method of birth control for at least 4 weeks prior to Visit 1 and throughout the study (i.e., until Day 105), OR
    b. Are post-menopausal or have undergone a sterilization procedure

4.2 Exclusion Criteria

Subjects will not be eligible for the study if **any** of the following criteria are met prior to initial dosing:

1. Allergic to brimonidine, loteprednol or any similar products, or excipients of brimonidine or loteprednol, including benzalkonium chloride (BAK)
2. Use of contact lenses within 2 weeks prior to Screening visit or during study
3. Currently receiving brimonidine or other treatment for glaucoma or ocular hypertension or history of glaucoma surgery.
4. Receiving or have received any experimental or investigational drug or device within 30 days prior to Screening visit
5. Intraocular pressure <5 mmHg or >22 mmHg in either eye
6. Active ocular infection or history of ocular herpetic keratitis
7. History of neurotrophic keratitis or ocular neuropathic pain
8. Any history of eyelid surgery or intraocular/ocular surgery within the past 3 months
9. Punctal occlusion within 3 months prior to Screening visit or during study
10. Corneal epithelial defect larger than 1 mm^2 in either eye
11. Have active drug/alcohol dependence or abuse history
12. Are neonates, pregnant/lactating women, children, institutionalized individuals, or others who may be considered vulnerable populations
13. Received corticosteroid-containing eye drops within the past 7 days, or a change in systemic corticosteroids/immunosuppressives within the past 30 days
14. A change in cyclosporine ophthalmic emulsion 0.05% (Restasis®) or lifitegrast ophthalmic solution 5% (Xiidra™) within 30 days prior to Screening visit
15. In the opinion of Investigator or Study Coordinator, be unwilling or unable to comply with study protocol or unable to successfully instill eye drops
16. Disease, condition, or disorder that in the judgement of Investigator could confound study assessments or limit compliance to study protocol

Subjects will be permitted to continue all their over-the-counter dry eye treatments, including the use of artificial tears, eyelid massage, or warm compresses, if they commit to the same brand/regimen throughout the study. In addition, they can continue their use of cyclosporine ophthalmic emulsion 0.05% (Restasis®) or lifitegrast ophthalmic solution 5% (Xiidra™) or systemic corticosteroids/immunosuppressives as long as the dosing regimen has not changed within the 30 days prior to Screening. None of the dry eye treatments, whether over-the-counter or prescription (e.g.,

Restasis® or Xiidra™), should be used within 2 hours of instilling study medication or within 2 hours prior to a study visit.

4.3 Subject Recruitment and Screening

Potential subjects will be recruited from the clinical practice of the Investigator at the time of their routine eye examination visit. Subjects will include patients who have been diagnosed with DED in the Investigator's eye clinic. All subjects will be screened, recruited, and will attend all study related visits only at their Investigator's clinic.

Patients with diagnosed DED and annoying or activity limiting visual symptoms will be approached by a member of the research staff to determine if the subject might be interested in participating in a research study. If the patient is interested, the research staff member will describe the study. If the patient is willing to enter the study, the study will be discussed and the patient will be asked to sign the Informed Consent form. Screening procedures include documentation of DED as well as other assessments as detailed in Section 6.3.1. Eligible subjects will be enrolled in the study.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects have the right to withdraw from the study at any time, for any reason, without jeopardizing their medical care. Where possible, subjects will be followed for safety and encouraged to return for follow-up visits for any unresolved safety events.

The IRB and Investigator also have the right to withdraw subjects from the study for the following reasons: when continuation may jeopardize the health of the subject, protocol violations, adverse events or concurrent conditions that affect the patient's well-being, and administrative or other reasons.

4.4.2 Data Collection and Follow-Up for Withdrawn Subjects

If a subject withdraws from the study prior to 12 weeks (84 days), the subject will be asked to complete the procedures outlined in the Day 84 visit as soon as possible. Subjects who voluntarily withdraw from the study between Day 84 and Day 105 will be asked to complete procedures outlined in the Day 105 visit as soon as possible. Subjects who are withdrawn due to adverse events will be followed at least until resolution or stabilization of the adverse event.

If the subject remains in the study for safety evaluation, follow-up visits will be scheduled according to the schedule of visits and procedures.

5 Study Drug

5.1 Description

The assigned study treatment will be provided to subjects in two bottles. In the brimonidine + loteprednol combination arm, brimonidine 0.2% will be supplied as an ophthalmic solution in Bottle #1 for administration as a single eye drop, followed by a loteprednol ophthalmic suspension (0.2%) application from Bottle #2 approximately 5-10 minutes later in each eye two times a day (BID) for twelve weeks (84 days). In the brimonidine monotherapy arm, each subject will receive the study drug as a single eye drop from Bottle #1(brimonidine) followed by an application from Bottle #2 (placebo) approximately 5-10 minutes later in each eye two times a day (BID) for twelve weeks (84 days).

Similarly, the control group will receive placebo (sodium carboxymethylcellulose, 0.25%) as a single eye drop from Bottle #1 followed by another application from Bottle #2 approximately 5-10 minutes later in each eye two times a day (BID) for twelve weeks (84 days). Except for the first dose during the clinic visit on Day 1, subjects will self-administer the study eye drops at home. Study drug will be blinded to both patient and Investigator. To maintain the blind, commercial dropper vials of approximately the same size (minimum 10mL) will be used for all study medication, and the original labels will be covered, obscured, or removed. There will be a uniform-appearing study-specific label for all study medication.

In summary, regardless of study arm, subjects will administer a single drop from Bottle #1 in each eye and wait 5-10 minutes before administering a single drop from Bottle #2 in each eye. This regimen will be conducted two times each day (BID) for twelve weeks (84 days).

Subjects will not be charged for the study drug in any way (neither the cost of the medication nor its dispensing cost).

5.2 Treatment Regimen

After Day 1, the subject will be instructed to instill the first dose of the study medication in the morning at approximately 8 a.m., and the next dose at approximately a 12-hour interval. Therefore, doses will be scheduled at approximately 8 a.m. and 8 p.m. During the treatment period, on days in which the patient will be in the clinic for evaluation (Days 1, 28, 56, and 84), patients should still receive both doses of study medication, on or close to their usual schedule, approximately 12 hours apart. However, no eye treatment (whether study medication or other eye drops/products) should be used within 2 hours prior to the clinic evaluation

5.3 Method for Assigning Subjects to Treatment Groups

This placebo-controlled, double-blind study will have three treatment groups utilizing a 1:1:1 randomization. One group will receive the study drug combination (brimonidine 0.2% then loteprednol ophthalmic suspension 0.2% separated by 5-10 minutes), one group will receive study drug monotherapy (brimonidine 0.2% then placebo separated by 5-10 minutes), and the third group will receive placebo (sodium carboxymethylcellulose, 0.25% x 2 applications separated by 5-10 minutes). Randomization/treatment assignment will be stratified by site and performed by an unblinded study team member.

To maintain confidentiality, the subject's name will not be recorded on any study document other than the informed consent form. A subject identification number will be created and linked to the drug vial number in a way that maintains the blind. Commercial dropper vials of approximately the same size (minimum 10mL) will be used for all study medication, and the original labels will be covered, obscured, or removed. There will be a uniform-appearing study-specific label for all study medication.

5.4 Preparation and Administration of Study Drug

The study drug will be procured, packaged, labeled, and coded by an outside vendor. One concentration of brimonidine tartrate ophthalmic solution will be used (0.2%), one concentration of loteprednol etabonate ophthalmic suspension will be used (0.2%), and one concentration of sodium carboxymethylcellulose, eye drops will be used (0.25%). All three agents will contain benzalkonium chloride. The eye droppers will be used by subjects as multiple-dose applications. The agents are regularly stored at room temperature (15°-25° C/ 59-77°F).

Instructions for Drug Administration (for clinic staff on Day 1):

1. Wash your hands thoroughly with soap and water
2. Check the dropper tip to make sure that it is not chipped or cracked.
3. Avoid touching the dropper tip against the subject's eye or anything else – eye drops and droppers must be kept clean
4. Have the subject tilt their head back and pull down the lower lid of his/her eye with your index finger to form a pocket
5. Hold the dropper (tip down) with the other hand, as close to the eye as possible without touching it
6. While looking up, gently squeeze the dropper so that a single drop falls into the pocket made by the lower eyelid. Remove your index finger from the lower eyelid
7. Ask the subject to close the eye for 2 to 3 minutes and tip his/her head down as though looking at the floor. Ask them to try not to blink or squeeze their eyelids
8. Place a finger on the tear duct and apply gentle pressure

Subjects should repeat the above procedures for the other eye in the clinic to demonstrate to the Investigator or designee that they are able to perform the drug administration satisfactorily. Subjects will be instructed to perform these steps on each subsequent administration of the study medication. Instructions for use will be provided to the subjects with the study drug.

5.5 Subject Compliance Monitoring

Subjects will receive their first dose of study drug on study Day 1 in the clinic, consistent with the treatment arm to which they were randomized. All subsequent doses will be self-administered.
Subjects will be given diaries to record each dose. In addition, they will be asked to make a note of any missed doses together with the reason for the omission. Subjects will be asked to bring their diaries, along with any used and unused dropper vials, with them to the Day 28, 56, and Day 84 visits to aid in determining study drug compliance. Enough study drug to last until the next visit will be given to the subject at each visit, starting with Day 1 (Baseline visit). No study drug will be given at the Screening visit (if separate from the Day 1 visit), Day 84, and Day 105 visits.

5.6 Prior and Concomitant Therapy

All eye medication (prescription and over-the-counter), steroids, antibiotics, blood pressure medication, anti-inflammatory medication, or immunosuppressive medication, whether topical or systemic, should be recorded if taken within 30 days prior to Day 1, whether there is continued use or not. All concomitant medications must be identified in the subject's medical record, including all eye treatments administered for DED. These medications will be recorded in the case report form (CRF).

For each medication taken, the following information will be collected:

1. Medication trade name
2. Eye that was treated, if applicable
3. Indication for which the medication was given
4. Date started
5. Date stopped
6. Dose, frequency and route of administration of the medication

Subjects will be permitted to continue all their over-the-counter dry eye treatments, including the use of artificial tears, eyelid massage, or warm compresses, if they commit to the same brand/regimen throughout the study. In addition, they can continue their use of cyclosporine ophthalmic emulsion 0.05% (Restasis®) or lifitegrast ophthalmic solution 5% (Xiidra™) or systemic

corticosteroids/immunosuppressives as long as the dosing regimen has not changed within the 30 days prior to Screening. None of the dry eye treatments, whether over-the-counter or prescription (e.g., Restasis® or Xiidra™), should be used within 2 hours of instilling study medication or within 2 hours prior to a study visit.

The use of any investigational agent during the 30 days prior to enrollment is prohibited.

5.7 Rescue Plan

Subjects will be monitored by the Principal Investigator and/or Sub-Investigator or designee at each study visit (approximately every 4 weeks). Subjects will also be encouraged to contact the research team in case they experience any ocular discomfort during the study. Any worsening of DED or any adverse event (AE) will be recorded, and in the case of AEs, followed to resolution. Based on the individual subject's clinical condition, one or more of the following therapeutic decisions may be implemented:

(1) Increasing the frequency of artificial tears or other over-the-counter dry eye treatments, and/or

(2) Discontinuing the study drug.

The decision will be made by the Principal Investigator based on his/her clinical judgment and the individual subject's clinical condition. The subject shall receive the treatment required for his/ her eye condition as per established clinical guidelines. Such treatment, and reason for administration, will be recorded in the case report form. In this situation, the subject will be followed closely (at least weekly) until complete resolution of symptoms and return to the subject's previous baseline.

5.8 Packaging

The study drug will be procured, packaged, labeled, and coded by an outside vendor. To maintain the blind, commercial dropper vials of approximately the same size (minimum 10mL) will be used for all study medication, and the original labels will be covered, obscured, or removed. There will be a uniform-appearing study-specific label for all study medication. The two bottles of study drug for the assigned arm will be packaged together in a plastic bag with a label that will be customized with the subject's identifiers. The bag label will also include the study protocol number, guidance for product storage, and a statement that the drug is "New Drug – Limited by Federal Law to Investigational Use Only". Instructions on medication self-administration will be included in each medication bag distributed to the subject. The dispensing of medication to subjects will be done at each study site. The eye dropper bottles will be used by subjects as multiple-dose applications.

The first dose will be administered to the subject by the researcher at the first treatment visit (Day 1).

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Each Investigator will receive a standard shipment of study materials and will order additional study drug as required. The study drug will be stored in a controlled environment and dispensed to subjects as needed.

5.9.2 Storage

Study drug will be stored at each site at room temperature (15°-25° C/ 59-77°F). The study drug will be directly dispensed to the subject from the site on each treatment visit.

5.9.3 Dispensing of Study Drug

At the first treatment visit (Day 1), the first study medication dose will be administered to subjects in the clinic and the eyedropper bottle containing sufficient drug to last for 4 weeks will be given to them to take home. The subjects will be asked to return the used eyedropper bottle at the follow-up visits. The designated study team member will then retrieve the previously dispensed eyedropper bottle and a fresh 4-week supply will be dispensed by the site.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug ordered/received, drug consumed, and drug remaining. Any discrepancies will be investigated, resolved, and documented. The used drug eyedroppers will be disposed by the study staff according to the site's standard protocols.

6 Study Procedures

6.1 Assignment of Subject Identification

Subjects meeting the enrollment criteria (see Sections 4.1 and 4.2) will be eligible for the study. To maintain confidentiality, the subject's name will not be recorded on any study document other than the informed consent. At the time of Screening, each subject will be assigned a five-digit site-specific subject number, and for subjects who qualify for the study, a unique 6-digit randomization number will be assigned. All data submitted to the sponsor will be de-identified.

6.2 Screen Failure

A record of screen failures and the reasons for non-eligibility will be maintained.

6.3 Study Assessments

The following detailed procedures are performed at the designated clinic visit. All results will be documented on the subject's medical/research charts, source documents, and CRFs as required. All ophthalmic procedures will be performed on both eyes. In general, assessments may be performed by the Principal Investigator, a Sub-investigator, a research coordinator, nursing staff, or other members of the research team. However, all tear film and ocular surface parameters are to be measured by the same investigator within each study site.

6.3.1 Day -7 to 0 (Screening Visit)

The Screening Visit and Day 1 Visit may be combined into a single visit. In this case, do not duplicate any of the Screening/Day 1 procedures. Subjects taking steroid-containing eye drops at the Screening Visit must discontinue those drops and wait a full 7 days before attending the Day 1 Visit.

After obtaining Informed Consent, the following assessments will be performed within 7 days prior to the subject receiving the first dose of study medication:

- Demographic information, including: birth date, gender, race and ethnic origin
- Medical History, including a review of the following systems: cardiovascular, dermatologic, gastrointestinal, genitourinary, musculoskeletal, neurologic and respiratory. Any history of cerebral or coronary insufficiency, postural hypotension or Raynaud's disease will be noted. An allergic history (including medications and food) will also be obtained. A history of prior and

concomitant medication use (including prescription, OTC, and herbal products) during the past 30 days will also be completed

- Ocular History, including: date when the subject began treatment for DED, as well as medications and procedures for DED.
- SANDE Questionnaire (Section 3.3.1) to quantify ocular symptoms due to Dry Eye Disease
- Keratograph Noninvasive Tear Breakup Time (NIBUT). The Keratograph 5M will automatically record NITBUT values based on manufacturer's guidelines
- Ophthalmic Examination (slit lamp examination, Lissamine Green staining, and a Schirmer I test). Eyelid margins and meibomian gland openings will be evaluated during a slit lamp examination by the physician, and changes such as lid margin vascularization (including telangiectasia) will be recorded
- IOP (Goldmann tonometry) assessment

6.3.2 Day 1 *(Baseline, Randomization, and First Treatment Visit)*

Note: Day 1, by definition, is when the first dose of study drug is administered. Endpoint assessments done at subsequent visits will be compared to baseline assessments done at the Day 1 visit. For this reason, the Day 1 Visit is also known as the Baseline visit.

-Prior to first dose of study drug (Baseline):

- SANDE Questionnaire
- Vital Signs (blood pressure taken while subject is relaxed in a sitting position for at least 3 minutes, pulse, and temperature)
- Visual Acuity (Snellen chart)
- Keratograph Ocular Redness Score (ORS)
- Keratograph Noninvasive Tear Breakup Time (NIBUT)
- Baseline Ophthalmic Examination (slit lamp examination, VBR scale and Lissamine Green staining)
- Schirmer I test
- IOP (Goldmann tonometry) assessment
- Record changes in concomitant medication
- Adverse events since screening visit.

-Randomization and administration of first dose of study drug by clinic staff

-After first dose of study drug:

- Subjects will be trained on how to self-administer the study eye drops and be given a sufficient supply to last for 4 weeks
- Subjects will receive a study diary on which to record the day/time of the first dose and each subsequent dose
- Post-dose evaluation for any adverse effects
- Tolerability Visual Analog Scale (VAS)

6.3.3 *Day 28 (± 7 days)*

Treatment Visit

- SANDE Questionnaire

- Review the subject's diary
- Visual Acuity (Snellen chart)
- Keratograph Ocular Redness Score (ORS)
- Ophthalmic Examination (slit lamp examination, VBR scale)
- IOP (Goldmann tonometry) assessment
- Clinical Global Impression, Subject Global Assessment
- Subjects will be given study medication refill
- Tolerability VAS
- Record changes in concomitant medication and adverse events

6.3.4 Day 56 (± 7 days)

Treatment Visit

- SANDE Questionnaire
- Review the subject's diary
- Visual Acuity (Snellen chart)
- Keratograph Ocular Redness Score (ORS)
- Ophthalmic Examination (slit lamp examination, VBR score)
- Clinical Global Impression, Subject Global Assessment
- Subjects will be given study medication refill
- Tolerability VAS
- Record changes in concomitant medication and adverse events

6.3.5 Day 84 (± 7 days)

Primary Endpoint Assessment Visit

- SANDE Questionnaire
- Review the subject's diary
- Vital Signs
- Visual Acuity (Snellen chart)
- Keratograph Ocular Redness Score (ORS)
- Keratograph Noninvasive Tear Breakup Time (NIBUT)
- Ophthalmic Examination (slit lamp examination, VBR scale, Lissamine Green staining)
- Schirmer I test
- IOP (Goldmann tonometry) assessment
- Clinical Global Impression, Subject Global Assessment
- Tolerability VAS
- Record changes in concomitant medication and adverse events

6.3.6 Day 105 (± 7 days)

End-of-Study Visit

- SANDE Questionnaire
- Vital Signs
- Visual Acuity (Snellen chart)
- Keratograph Ocular Redness Score (ORS)
- Ophthalmic Examination (slit lamp examination, VBR scale)
- IOP (Goldmann tonometry) assessment
- Clinical Global Impression, Subject Global Assessment
- Record changes in concomitant medication and adverse events

7 Statistical Plan

7.1 Sample Size Determination

Thirty (30) subjects per treatment group and a common standard deviation of 20 mm yields precision (half-width) of the point estimate of pairwise difference (active – placebo) in tolerability of +/- 10.3 mm for a two-sided 95% confidence interval (CI) and +/- 8.6 mm for a two-sided 90% CI. Therefore, if the observed mean difference in tolerability is 5.0 mm, the true mean difference in tolerability will be within (-5.3, 15.3) with 95% confidence and (-3.6, 13.6) with 90% confidence.

Additionally, with a sample size of 30 subjects within a treatment group, adverse events that are not observed will be concluded to occur at a true rate of <10% with 95% confidence within that treatment group. For example, if headache is not seen within the combination treatment group, then with 95% confidence, headache would be concluded to occur at a true rate of <10%.

The sample size was not calculated to have pre-determined power for efficacy in this proof-of-concept study.

7.2 Statistical Methods

7.2.1 Unit of Analysis

The unit of analysis for efficacy will be the study eye. Each subject will have a single eye identified as the study eye as follows: (i) if only 1 eye meets inclusion criteria, this eye will be the study eye and the other eye will be considered the non-qualified fellow eye; (ii) if both eyes meet inclusion criteria, the eye with the higher Ocular Redness Score (ORS) will be the study eye and the other eye will be considered the qualified fellow eye; (iii) if both eyes have the same Ocular Redness Score (ORS), then the eye with the lower Schirmer I score will be the study eye and the other eye will be considered the qualified fellow eye; (iv) if both eyes have same Schirmer 1 score, the right eye will be the study eye and the other eye will be considered the qualified fellow eye. Safety and Efficacy analyses will be primarily performed on the study eyes and secondarily on the qualified fellow eyes (for efficacy) and all fellow eyes (for safety).

7.2.2 Analysis Populations

Randomized Population: The randomized population will include all subjects who were randomized to treatment. Baseline variables and demographic characteristics will be summarized for this population.

Safety Population: The safety population will include all randomized subjects who have received at least one dose of study medication. This population will be used to summarize safety variables and will summarize subjects as treated.

Intent-to-Treat Population (ITT): The ITT population will include all randomized subjects who have received at least one dose of study medication. This population will be the primary population for efficacy analyses and will be used to summarize all efficacy variables and will summarize subjects as randomized.

Per-protocol population (PP): The PP population is a subset of the ITT population, which will include those subjects who do not have major protocol violations likely to seriously affect the primary outcome of the study as judged by a masked evaluation prior to the unmasking of the study treatment. This population will be the secondary population for efficacy analyses and will be used to summarize a subset of efficacy variables. If the PP and ITT populations are exactly the same, then additional efficacy

analyses on the PP population will not be performed. The PP population will summarize subjects as treated.

7.2.3 General Considerations

All continuous study assessments will be summarized by treatment and visit (as applicable) and change from baseline to each post-baseline visit using descriptive statistics (n, mean, median, standard deviation, minimum, and maximum). All categorical study assessments will be summarized by treatment and visit (as applicable) using frequency counts and percentages.

Baseline is defined as the last measurement prior to the first dose of study medication.

Missing values of less than 25% will be imputed by using multiple imputations; otherwise will not be included in the analysis. Descriptive statistics will be utilized before the modeling to describe the sample. Assumptions behind each statistical method will be examined. Two-sided *P*-value less than 0.05 would be considered as statistical significance. All the analyses will be conducted by using SAS 9.2 (SAS Inst., Cary, NC).

7.2.4 Safety Analysis

Tolerability will be summarized by treatment group at each visit using continuous summary statistics; p-values from pairwise two-sample t-tests and 90% and 95% CIs will be presented to test the difference in mean tolerability among the three treatment groups.

Verbatim descriptions of AEs will be mapped to MedDRA thesaurus terms. Treatment emergent AEs (TEAEs), those that occur after the first dose of study medication, will be summarized by treatment group using frequency and percent for each system organ class (SOC) and preferred term (PT) within each SOC. Summaries will be presented at the subject level for both ocular and non-ocular TEAEs. Summaries will also be presented for serious TEAEs, TEAEs related to Investigational Product, TEAEs by severity, and TEAEs leading to discontinuation.

Slit lamp biomicroscopy measures will be summarized by treatment group at each visit using discrete summary statistics.

Visual acuity data will be summarized by treatment group at each visit and for change from baseline to each visit using discrete summaries including change from baseline in the number of lines and the proportion of subjects with a worsening of ≥ 3 lines from baseline.

Intraocular pressure will be summarized by treatment group at each visit and for change from baseline to each visit using both continuous and discrete summaries.

Vital signs will be summarized by treatment group at each visit and for change from baseline to each visit using continuous summary statistics.

7.2.5 Efficacy Analysis

The efficacy parameters, SANDE, CGI, SGA, Lissamine Green corneal and conjunctival staining, Schirmer's Test I, Keratograph Ocular Redness Score (ORS), and Validated Bulbar Redness (VBR) scores will be summarized by treatment group at each visit and for change from baseline to each visit using continuous and discrete summary statistics as appropriate and using pairwise two-sample t-tests or Pearson's chi-squared test (or Fisher's exact test if expected cell count is <5) and corresponding asymptotic 90% and 95% CI around the difference in means or proportions.

Lissamine Green corneal and conjunctival staining will be summarized by region and overall.

7.2.6 Missing Data

Efficacy analyses will be performed using the ITT (primary) and PP (secondarily) populations and will summarize both observed data only and with missing data imputed using: multiple imputation techniques, trimmed mean analyses, and last observation carried forward (for endpoints as detailed in the formal statistical analysis plan; additionally, the statistical analysis plan will detail which imputation or observed data only will be considered primary and which imputations or observed data only will be considered secondary).

7.2.7 Multiplicity Considerations

As this is a proof of concept study for which there is no pre-determined power for a specific endpoint measure, there will be no multiplicity adjustments to account for the testing of the multiple endpoints and for the testing of both the combination arm and monotherapy arm to placebo.

8 Safety and Adverse Events

8.1 Definition of Adverse Events and Serious Adverse Events (SAEs)

The following are specific definitions of terms guided by the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and the U.S. Code of Federal Regulations that apply to this section:

Adverse Event: Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can be any unfavorable sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether considered related or not related to the investigational product.

Subjects will be reminded to inform the study staff of any adverse effects that they have experienced or are experiencing after the first administration of study drug. All reports of adverse events during the study will be recorded on an Adverse Event Case Report Form (CRF). The subject should not be prompted about any adverse events that may occur during this trial.

The study staff should instruct the subject to notify the research facility should any adverse event occur within 7 days of study completion. Subjects who withdraw due to an adverse event may be replaced.

Serious Adverse Event (SAE): An Adverse Event should be considered a Serious Adverse Event (SAE) if any of the following criteria are met:

- results in death
- is life-threatening*
- requires inpatient hospitalization or prolongs existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/ birth defect
- requires medical or surgical intervention to prevent any of the occurrences noted above

*__Life-Threatening:__ Any adverse drug experience in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

If a subject experiences an SAE, the study staff should notify the Sponsor **within 24 hours** of the staff becoming aware of the event.

Unexpected Adverse Event: Any adverse event, the specificity or severity of which is not consistent with the current Package Insert.

8.2 Classification of Adverse Events by Severity

All toxicities/adverse events will be graded according to the following definitions to code the intensity of the event.

Mild: Usually transient, requiring no special treatment, and does not interfere with the subject's daily activities.

Moderate: Traditionally introduces a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually relieved by simple therapeutic measures.

Severe: Causes an interruption of the subject's usual daily activity and traditionally required systemic drug therapy or other treatment.

Note: If the intensity of an adverse event changes, the event will be reentered as a separate event.

There is a distinction between the severity and the seriousness of an adverse event. Severity is a measurement of intensity; thus, a severe reaction is not necessarily a serious adverse event. For example, a headache may be severe in intensity, but would not be serious unless it met one of the criteria for serious adverse events listed previously.

8.3 Classification of Adverse Events by Relationship to Study Treatment

The relationship or association of the study medication to an adverse event, as causing or contributing to the adverse event, will be characterized as defined below:

Probably/Definitely Related: Either a direct association can be demonstrated or the adverse event follows a reasonable temporal sequence from administration of the drug (including the course after withdrawal of the drug) and the possibilities of factors other than the drug, such as underlying disease, concomitant drugs or concurrent treatment, can be excluded.

Possibly Related: The adverse event follows a reasonable temporal sequence from administration of the drug (including the course after withdrawal of the drug) and the possibility that drug involvement cannot be excluded, e.g. existence of similar reports attributable to the suspected drug, its analog or its pharmacological effect. However, other factors such as underlying disease, concomitant drugs or concurrent treatment are presumable.

Not Related: The adverse event has no temporal sequence from administration of the drug, or it can be explained by other factors, including underlying disease, concomitant drugs or concurrent treatment.

8.4 Action(s) Taken

The investigator will select one or more of the following choices in the CRF for each adverse event:

Action taken with the study drug:

- Dose not changed
- Drug Interrupted
- Drug Discontinued
- Unknown
- Not Applicable

Action taken for subject:

- None
- Subject Withdrawn
- Administered Therapy
- Hospitalized Subject
- Other

8.5 Outcome

The outcome for each adverse event documented will also be recorded in the CRF. The selection of options include the following:

- Recovered/Resolved
- Not Recovered/Not Resolved
- Recovered/Resolved with Sequelae
- Fatal
- Unknown

8.6 Adverse Event Reporting

All subjects who have been exposed to study drug will be evaluated for adverse events. Adverse events will be recorded starting after the first dose of study drug and continuing until the end of the study. All adverse events will be evaluated beginning with onset, and evaluation will continue until resolution is noted, or until the Investigator determines that the subject's condition is stable, whichever is earlier. The Investigator will take all appropriate and necessary therapeutic measures required for resolution of the adverse event. Any medication necessary for the treatment of an adverse event must be recorded on the Concomitant Medication case report form. If more than one distinct adverse event occurs, each event should be recorded separately. Procedures such as surgery should not be recorded as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of adverse event as described previously.

8.7 Serious Adverse Event Reporting

All SAEs (as defined in Section 8.1) that occur during the study will be reported to the Sponsor within 24 hours of the study staff becoming aware of the event. SAEs will be recorded starting after first dose of study drug and continuing until the end of the study. A copy of the report will be sent to the FDA.

In cases where an event is both Serious and Unexpected, the study Sponsor is responsible for submitting an IND Safety Report to the FDA and all participating investigators. Any adverse events or IND Safety reports that require a change to this protocol or the associated Informed Consent document will be reported to the IRB within 5 days.

8.8 Medical Emergencies

In medical emergencies, the Investigator should use medical judgment and remove the subject from immediate hazard. The IRB should be notified as to the type of emergency and the course of action taken. In case of an Emergency, a code list will be accessible to one member of the study team for the purpose of emergency unblinding, if required.

8.9 Study Oversight

The Study PI has primary oversight responsibility for this study. The Principal Investigator and his/her research team are responsible for identifying adverse events. Safety monitoring will include careful assessment and appropriate reporting of adverse events. Subjects will be reminded to inform the study staff of any adverse effects that they have experienced or are experiencing after the first administration of study drug. All reports of adverse events during the study will be recorded on an Adverse Event Case Report Form (CRF). In addition, subjects will be asked to make a note of any missed doses together with the reason for the omission. A member of the research staff will review diary entries with the subject at each study visit. Subjects will be asked to bring back the leftover drug at each study visit. The amount of drug remaining in the used vial will also give an estimate of the compliance.

It is the responsibility of each Investigator and the study Sponsor, Ocugen Inc. to conduct this study in compliance with all aspects of the protocol, IRB requirements, Declaration of Helsinki, all applicable Federal Regulations (21 CFR Parts 812, 50, 54 and 56) and general Good Clinical Practice (GCP). In the event of a protocol deviation, the Sponsor should be notified, as should the IRB if the deviation meets their stated requirements for deviation reporting. As necessary, corrective action should be taken to ensure the subject's safety and to maintain the integrity of the clinical investigation.

9 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

If a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10 Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medical-technical departments involved in the clinical trial.

10.1 Records Retention

It is the investigator's responsibility to retain study essential documents for the full duration of the study. This includes, but is not limited to, the following: completed Informed Consent forms, Case Report Forms, AE/SAE report forms, all study correspondence and associated documentation. Per regulations, these records should be maintained for a period of two years following a marketing approval for the study drug or two years following shipment and delivery of the drug for investigational use is discontinued. In the event the site is not able to store records for the full 2-year duration, the study Sponsor should be informed so that alternative retention and storage plans can be confirmed.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator. The study may not commence until IRB approval is granted.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject (or legally authorized representative) and the investigator-designated research professional obtaining the consent.

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