COVER PAGE

Clinical Protocol Title:

The utility of in vivo confocal microscopy to assess cellular response and efficacy of long-term topical steroid treatment in patients with dry eye disease

Version number and date: Version 2 – 01/13/2017

Phase of clinical investigation: Phase IV

Name of all sites (if applicable): MEEI and Tufts Medical Center

IND/IDE number: N/A

Investigational drug(s) or device(s):
Loteprednol etabonate 0.5% ophthalmic suspension (Lotemax, Bausch & Lomb, Inc);

Soothe Tired Eyes Lubricant Eye Drop (Glycerin 1%, Bausch & Lomb Inc.)

Regulatory Sponsor:

Pedram Hamrah, MD

Funding Sponsor: GlaxoSmithKline plc

Study Monitor

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Acronym List:

AE	Adverse Event
ANOVA	Analysis of Variance
BCVA	Best Corrected Visual Acuity
BID	Twice Daily
CRF	Case Report Form
DED	Dry Eye Disease
FDA	Food and Drug Administration
IOP	Intraocular Pressure
IRB	Institutional Review Board
IVCM	In-Vivo Confocal Microscopy
LMR	Longitudinal Medical Record
MEEI	Massachusetts Eye and Ear Infirmary
HSC	Human Studies Committee
NEI	National Eye Institute
OSDI	Ocular Surface Disease Index
PI	Principal Investigator
SAE	Serious Adverse Event
SANDE	Symptom Assessment In Dry Eye
TBUT	Tear break-up time

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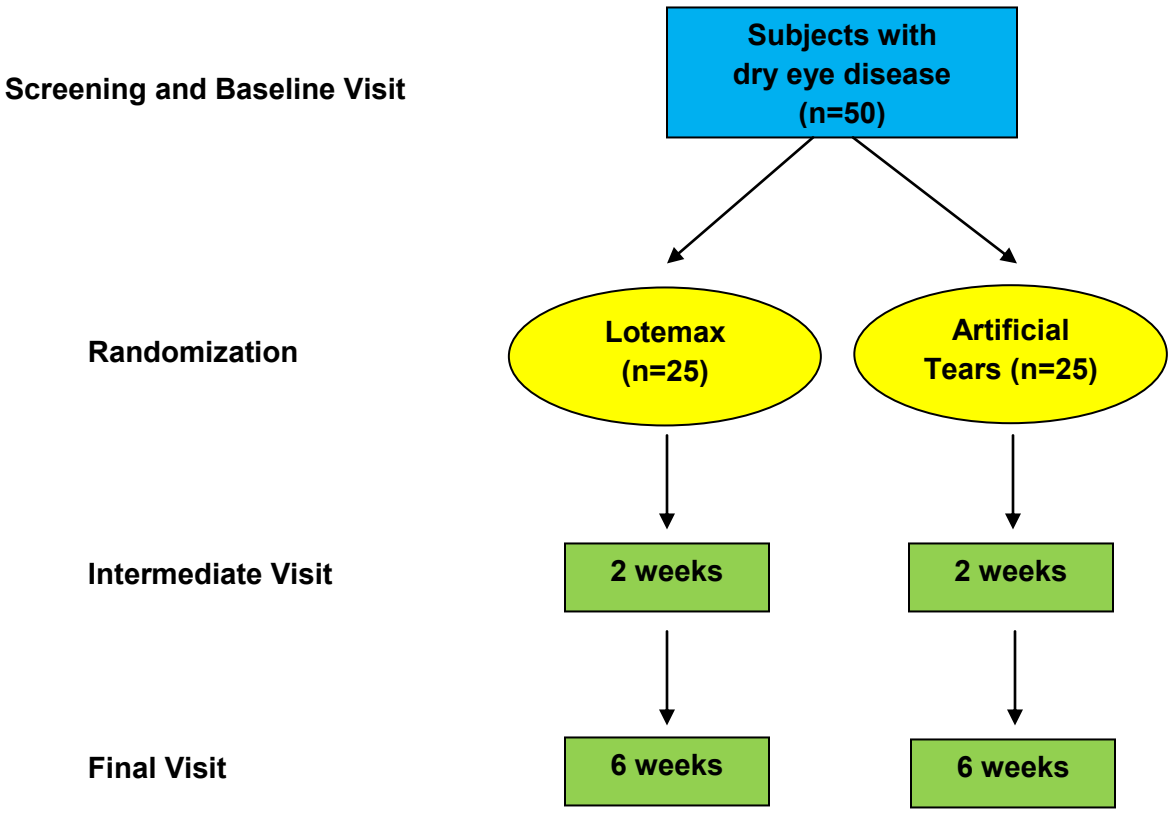
STUDY DESIGN SCHEMATIC



1. CLINICAL PROTOCOL

1.1 Background

Recent studies have shown that inflammation plays an important role in the pathogenesis of Dry Eye Disease (DED). Ocular surface inflammation often exacerbates the subject's signs and symptoms, and results in an increase in the immune cells and other inflammatory mediators located in the ocular surface. Dry eye disease is one of the most commonly encountered ophthalmic disorders. It is a multifactorial disease of the ocular surface and tear film, characterized by symptoms of eye irritation, tear instability and vision impairment. Despite being very common, standardized therapy is not available.

Due to the underlying inflammation associated with DED, anti-inflammatory steroid medications are used for the treatment of DED. "Soft steroids" are often preferred because their chance of increasing intraocular pressure (IOP) is lower than other steroids. One of the "soft steroids" that is commonly used for the treatment of inflammation associated with ocular surface disease is loteprednol etabonate 0.5% ophthalmic suspension (Lotemax, Bausch & Lomb, Inc).

Current steroid therapy in DED is comprised of pulse therapy (to avoid adverse events associated with long-term steroid use) of usually 2 weeks duration, administered BID. This timeframe is often too short to meaningfully resolve the inflammation associated with DED. More recently steroid treatment of at least 6 weeks with tapered dosing has been advocated.

1.2 Rationale

Lotemax, an FDA-approved medication for ocular inflammatory disease, is commonly used to treat inflammation associated with DED with a regimen of twice daily for 2

weeks. However, because DED is a chronic disease, this short duration of steroid therapy may not be enough to meaningfully resolve the inflammation associated with DED. Thus, corneal specialists, including here at MEEI, have begun using Lotemax for at least 6 weeks with tapered dosing. This study has been designed to evaluate the effects of this tapering regimen on inflammation associated with DED.

Clinical signs and symptoms are used to evaluate the efficacy of a treatment for DED, including Schirmer's test, tear break-up test, corneal fluorescein staining, and conjunctival lissamine green staining. However, none of these tests evaluate the underlying inflammatory and immune response changes in DED. Therefore, to determine the efficacy of any treatment for DED, it is ideal to evaluate these underlying changes in addition to the clinical parameters.

In vivo confocal microscopy (IVCM) is a novel imaging technology that allows the visualization and quantification of corneal structures at the cellular level. IVCM has recently been used to evaluate the corneal changes in DED, such as hyperfluorescent superficial epithelial cells, immune dendritic cells, and sub-basal nerves.

Therefore, in this randomized clinical trial, IVCM images will be used to determine the changes in corneal immune cells and nerves during a 6-week taper regimen of Lotemax versus Soothe Tired Eyes Lubricant Eye Drop (Glycerin 1.0%, Bausch & Lomb Inc.) (an artificial tear) for treatment of inflammation associated with DED.

2. STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the efficacy of Lotemax as compared to Artificial Tear over a period of 6 weeks, using clinical and in vivo confocal microscopic findings.

2.2 Secondary Objective(s)

N/A

3. STUDY DESIGN

3.1 Study Design Description

This is a single site, prospective, randomized, controlled, double-masked clinical trial including 50 subjects with DED. The participants will be randomized to take either Lotemax or Soothe Tired Eyes Lubricant Eye Drops for 6 weeks. Both Lotemax and the artificial tear will be used 4 times a day for 2 weeks, 2 times a day for 2 weeks, and once a day for 2 weeks. After screening/baseline examination and enrolling into the study, the participants will have follow-up visits at 2 weeks as well as 6 weeks.

3.2 Allocation to Treatment

The subjects will be randomized in two groups, one group will receive Lotemax and the other group will receive Soothe Tired Eye Lubricant Eye Drops.

3.2.1 Randomization Procedures

Once the investigator determines that the subject has met all of the inclusion and exclusion criteria, the subject will be randomized to take either Lotemax or Soothe Tired Eyes Lubricant Eye Drops (artificial tears). The randomization will be in a 1:1 ratio using a permuted-block design (which will be obtained using a computer-based random code generator) into the two groups.

3.2.2 Masking Procedures

The study drugs will be labeled by the Massachusetts Eye & Ear pharmacy with instructions for storage, the name of the study, an expiration date, and a notice that

the bottle may contain Lotemax. The pharmacy will maintain a master list of the participants' name, unique identifier, and the coded treatment assignment. The randomization scheme and treatment assignment will be held in the pharmacy to ensure that the investigator and study team remain masked. Participants, investigators, image analysts, and the sponsor (GSK) will be masked to the treatment group.

3.2.3 Breaking the Mask

A subject's medication may be unmasked at any point for a medical emergency or to supplement a serious adverse event (SAE) report. The subjects will be unmasked at the end of the study in order to provide optimal ongoing care.

4. SUBJECT SELECTION

4.1 Subject Inclusion Criteria

- Age 18-89 years.
- Willing and able to provide written informed consent.
- Willing and able to comply with study assessments for the full duration of the study.
- Diagnosis of dry eye disease based on the followings:
    - Symptoms of dry eye disease such as foreign body sensation, burning, stinging, light sensitivity for at least 6 months.
    - Two or more of the following objective signs:
        - Schirmer test with anesthesia <10 mm at 5 minutes [mean Schirmer between eyes.

  - Tear break-up time (TBUT) of <10 seconds.
  - Corneal fluorescein staining of 4 (NEI grading scheme, 0-15) in at least one eye
  - Lissamine green staining of the nasal and temporal conjunctiva (NEI grading scheme, 0-18) in at least one eye
- Corneal dendritiform cell count by confocal microscopy of >=75/mm^2 (13 immune cells per image)
- In good stable overall health.

4.2 Subject Exclusion Criteria

- Central corneal subbasal dendritic cell count by *in vivo* confocal microscopy of <75/mm^2 in both eyes
- Active ocular allergies
- Active allergies to steroids, aminoglycosides, or benzalkonium chloride (BAK)
- History of contact lens wear within 3 months before enrollment.
- Intraocular surgery or ocular laser surgery within 3 months before enrollment.
- History of ocular infection within 3 months before enrollment.
- History of topical or systemic steroid treatment within 1 month before enrollment. In case of topical steroid use, a wash-out period of 1 month is required.
- History of increased intraocular pressure after using topical steroids (steroid responsive)

- History of systemic immunosuppressive treatment within 1 month before enrollment.
- History of any change in the frequency of topical cyclosporine or oral tetracycline compounds (tetracycline, doxycycline, and minocycline) within 1 month before enrollment.
- Any condition (including language barrier) that precludes subject's ability to comply with study requirements including completion of study.

5. STUDY DRUG(S)/DEVICE(S)

5.1 Study Drug/Device Information

The participants will receive either Lotemax or Soothe Tired Eye Drop.

Lotemax

Lotemax (loteprednol etabonate) 0.5% is a prescription-only, preserved ophthalmic suspension supplied by Bausch & Lomb, Inc. Lotemax (loteprednol etabonate) 0.5% has been approved by the FDA for treatment of ocular inflammation with a maximum dosing frequency of 24 drops per eye per day. It is a C-20 ester-based corticosteroid, with a potent anti-inflammatory efficacy, but decreased impact on intraocular pressure (IOP) compared to other corticosteroids, which may increase IOP. The medication will be applied topically to both eyes for 6 weeks with the following regimen: four times a day for 2 weeks, twice daily for 2 weeks, and once daily for 2 weeks.

Soothe Tired Eyes Lubricant Eye Drop

Soothe Tired Eyes Lubricant Eye Drop (Bausch & Lomb Inc.) is a preserved artificial tear whichis used to relieve the dryness of the eye and to prevent further irritation. Its active ingredient is glycerin 1%. The artificial tear will be applied topically to both eyes for 6 weeks with the following regimen: four times a day for 2 weeks, twice daily for 2 weeks, and once daily for 2 weeks.

In the case of increased intraocular pressure due to Lotemax, the medication will be tapered rapidly and stopped within 1 week. In addition, for cases where subjects withdraw from the study, the medication will be tapered and stopped within 1 week.

5.2 Study Drug/Device Compliance/Adherence

Directed questioning using the (Ocular Tolerability and Compliance Questionnaire – Appendix I) will be administerd by the investigator or research staff. Results on the participants' compliance and adherence to the study protocol and study drug will be recorded on the paper case report form (CRF) at the second and third visits (2 and 6 weeks after enrollment).

Subjects will not be withdrawn for lack of compliance with the study drug regimen.

During the study, all concomitant medication treatment regimens, ocular hygiene treatments (i.e., lid scrubs and warm compresses), or insertion of punctal plugs will be kept constant as permitted by accepted medical practice. If one of the aforementioned treatment regimens needs to be modified, the participant may be withdrawn based on the investigator's discretion.

Additionally, if there is an administration of a prohibited medication (including any other steroid) or procedure (any ocular surgery), the participant may be withdrawn based on the investigator's discretion.

Participants must be compliant with IVCM imaging on at least 2 of their 3 visits or they will be withdrawn. Subjects who are withdrawn may be replaced by new enrolled subjects to maintain the sample size in each group.

5.3 Study Drug Supplies

Both Lotemax and Soothe Tired Eyes Lubricant Eye Drop are commercially available from Bausch & Lomb Inc as eye drops.

- **Preparing and Dispensing**
  To mask the identity of the medication, the Massachusetts Eye and Ear Pharmacy will prepare, label and distribute both Lotemax and Soothe Tired Eyes Lubricant Eye Drop to the study participants.

- **Administration**
  Regardless of study drug assignment, each participant will self-administer one drop of the study medication to both eyes with the following regimen: 4 times a day for 2 weeks, 2 times a day for 2 weeks, and once daily for 2 weeks. The participants are being advised not to allow the dropper tip to touch any surface, as this may contaminate the medication.

  Participants will be instructed on how to instill an eye drop at the baseline visit and will be asked to demonstrate application of an eye drop in front of research personnel to confirm that the appropriate technique is being used.

5.4 Study Drug/Device Storage and Accountability

The subjects will be instructed to store the medication at room temperature (15-25ºC / 59-77ºF) and to shake it vigorously before using it. If a subject contracts infectious conjunctivitis, they will be instructed to discard the remainder of the study drug. The subject will be scheduled for a clinic visit where an investigator will assess the severity of the infectious conjunctivitis as well as the subject's eligibility. If the investigator determines that the subject is still eligible, a new study drug will be sent from the host site pharmacy, Massachusetts Eye and Ear Infirmary, and given to the subject during their visit.

The participants will not be asked to return the unused drug following the completion of the study. Additionally, subjects who are withdrawn from the study will be asked

to discard the rest of their unused study drug. Therefore, the study drug will not be accounted for following dispensing and will not be destroyed by the MEEI pharmacy.

5.5 Other Medications

- **Administration**

  The use of any concurrent, prescription, or over-the-counter medication, is to be recorded along with the reason the medication was taken. During the study, all concomitant medication treatment regimens, ocular hygiene treatments (i.e., lid scrubs and warm compresses), or insertion of punctal plugs will be kept as constant as permitted by accepted medical practice.

  Using artificial tears (preserved and non-preserved) are permissible at the screening visits and during the study. However, concomitant use of artificial tears will be monitored. At each study visit, subjects will be queried about the average number of times they used artificial tears each day during the past week and the number of days during the past week when they did not use any artificial tears.

  Systemic and topical ophthalmic medications, that the subjects are already taking as part of their current treatment for dry eye disease will be maintained, without a change in frequency.

  When necessary, treatment and administration of any other therapy, besides their current and study's regimen, will be done as the safety of the study participant is the primary consideration. The administration of a prohibited medication or procedure will be considered a protocol violation and the subject may be discontinued from the study. Prohibited medications include other ophthalmic steroids. Prohibited procedures include any ocular surgery.

- **Rescue Medication or Therapy**
  When necessary, administration of any other therapy besides the study medication and their current medications will occur for the safety of the subject, which is the primary concern. If treatment with artificial tears is inadequate, or if the subject develops a severe form of ocular surface disease and dry eye, the subject may be unmasked by the investigator. To ensure the subject's safety and proper treatment, unmasking of the study treatment assignment will allow the investigator to institute appropriate follow-up care. The subject will be kept in the study so that we can do an "intention to treat" analysis.

6. BIOSPECIMEN COLLECTION (IF APPLICABLE)

6.1 Specimen preparation, handling, and shipping: N/A

6.2 Instruction for specimen preparation, handling and storage: N/A

6.3 Specimen shipment: N/A

6.4 Future use of stored specimens: N/A

7. STUDY PROCEDURES

7.1 Screening Procedures

Prospective participants, as defined by the inclusion/exclusion criteria, will be considered for entry into this study. Concurrent enrollment of subjects who are participants in other studies will be allowed based on the investigator's discretion.

During the consent process, study design and treatment regimen will be discussed with each subject. Written informed consent will be obtained before any study specific procedures are performed. However, standard of care clinical information (such as: medical history, TBUT, staining, and Schirmer's test) obtained during a subject's clinical visit within the previous 48 hours can be used during the screening visit to determine eligibility and as their baseline assessments. Additionally, standard of care IVCM images taken within a week of, or during, the subject's screening visit may be used to determine eligibility and as a baseline assessment.
The following evaluations and procedures will be performed for all subjects during the screening and baseline period:

- Written informed consent
- Record of current ocular and systemic medications
- Record of significant medical/surgical history in the past 5 years
- Record of demographic data, including date of birth, sex, and race/ethnicity
- Tear Break-up Time (TBUT)
- Eye examination (best corrected visual acuity, slit lamp biomicroscopy, applanation tonometry, funduscopy)
- Corneal fluorescein staining
- Conjunctival lissamine green staining

- Schirmer's test with anesthesia
- Central corneal in vivo confocal microscopy (IVCM)

Researchers will document type and frequency of artificial tear, ocular ointment, and other dry eye therapies use by the subject.

Subjects will start the study medication the first day after their screening visit, while continuing the use of their current medications.

Tear Break-Up Time (TBUT): The standard TBUT measurement will be performed by dropping of a fluorescein drop to the inferior tarsal conjunctiva. After several blinks, the tear film will be examined using a broad beam of the slit lamp with a blue filter. The time lapse between the last blink and the appearance of the first randomly distributed dark discontinuity in the fluorescein stained tear film will be measured three times and the mean value of the measurements will be calculated. The tear break-up time will be evaluated prior to the instillation of any eye drops and before the eyelids are manipulated in any way. Break-up times less than 10 seconds are considered abnormal. A positive change from baseline indicates improvement.

Corneal Fluorescein Staining (Appendix IV): Corneal fluorescein staining will be performed after 3 minutes of application of a fluorescein drop. The entire cornea will be then examined using slit-lamp evaluation with cobalt blue illumination. Staining will be graded using the NEI grading scheme, with a total range of 0-15.

Schirmer's Test with Anesthesia: The Schirmer's test will be performed 3 minutes after a drop of topical anesthesia with 0.5% proparacaine (Alcon Inc., Ft Worth TX) is

applied to the eye. The Schirmer test is performed by placing a narrow filter-paper strip (5 x 35 mm strip of Whatman #41 filter paper) in the inferior cul-de-sac. This test is to be conducted in a dimly lit room. The subject should gently close their eyes until five minutes have elapsed and the strips are removed. Since the tear front will continue advancing a few millimeters after it has been removed from the eyes, it is important to mark the tear front with a ball-point pen at precisely five minutes. Aqueous tear production will be measured by the length in millimeters that the strip wets during 5 minutes.

Conjunctival Lissamine Green Staining (Appendix IV): Grading of conjunctival lissamine green staining will be performed after 2 minutes of application of a lissamine green drop. The nasal and temporal conjunctiva will be examined using slit-lamp evaluation with white light. Staining will be graded using the NEI grading scheme, with a total range of 0-18.

***In Vivo* Confocal Microscopy (IVCM):**

In vivo confocal microscopy (IVCM) is a new imaging method which allows visualization of the corneal structures at the cellular level. With a magnification of 800 times, it makes it possible to detect and quantify changes in the epithelial layers and sub-basal nerve plexus. With this technology, size and reflectivity of superficial epithelial cells, as well as the number of immune dendritic cells (DC) can be readily evaluated.

This is a contact imaging procedure and therefore a drop of topical anesthesia will be used for both eyes. A topical gel is applied to the imaging cap as well as the subject's eye to provide a coupling medium between these two surfaces. Three to four locations in the central part of the cornea will be imaged at the depth of the superficial epithelium and sub-basal layer which is 50 to 80 microns deep. A sequence of 100 images will be obtained.

7.2 Enrollment/Baseline Procedures

The baseline visit may occur immediately after the screening assessments. Following the baseline assessments, subjects will be randomized into a treatment group. If an individual is deemed eligible to participate, the following baseline procedures will be performed:

- Symptom Assessment iN Dry Eye (SANDE) questionnaire:

  SANDE Questionnaire (Appendix III): The Symptom Assessment in Dry Eye (SANDE) questionnaire utilizes a 100-mm horizontal visual analog scale (a 100-mm line) to quantify subject symptoms of ocular dryness and/or irritation. In the SANDE symptom questionnaire-I, subjects will be asked to put a mark on two given lines to depict the extent of their dry eye symptoms separately in terms of frequency and severity. At the screening visit and all follow-up visits, the SANDE symptom questionnaire-I will be administered. At each follow-up visit, the SANDE symptom questionnaire-II will be also administered. To indicate whether there are any differences in symptoms from the previous visit, subjects will compare the frequency and severity of their current level of symptoms with the level at their previous visit by placing a mark to the left of central anchor (if less than previous visit) or to the right of central anchor (if more than the previous visit), according to how much of a change they perceive. The location of the mark made by the subject for each question of the SANDE-I will be measured in mm from the left-hand side of the 100 mm line, and the number will be recorded in mm. For the second version of the questionnaire SANDE-II, the distance between the mark made by the subject and the central anchor will be measured and it will be recorded as a negative value if to the left of the central anchor and as a positive value if placed to the right of the central anchor. Then a global score will be calculated by multiplying the frequency score by the severity score and taking the square root of the result to transform back to the original centesimal scale.

- Ocular Surface Disease Index (OSDI) questionnaire:

  OSDI Questionnaire (Appendix II): 12-item questionnaire designed to provide a rapid assessment of the symptoms of ocular irritation consistent with dry eye disease and their impact on vision-related functioning. The 12 items of the OSDI questionnaire are graded on a scale of 0 to 4, where 0 indicates none of the time; 1, some of the time; 2, half of the time; 3, most of the time; and 4, all of the time. The total OSDI score is then calculated on the basis of the following formula: OSDI=[(sum of scores for all questions answered) x 100]/[(total number of questions answered) x 4].

7.3 Study Drug or Device Procedures

At visits 1 and 3, the following procedures will be done for study specific purposes in addition to standard of care procedures:

- Schirmer's Test with anesthesia (may be standard of care)
- Ocular Surface Disease Index questionnaire
- Symptom Assessment iN Dry Eye questionnaire
- Central Cornea In Vivo Confocal Microscopy (may be standard of care)
- Corneal fluorescein staining

At visit 2, the following procedures will be done for study specific purposes:

- Eye examination (best corrected visual acuity, bio-microscopy, applanation tonometry, funduscopy)
- Tear Break-up Time
- Corneal fluorescein staining
- Conjunctival lissamine green staining
- Schirmer's Test with anesthesia
- Ocular Surface Disease Index questionnaire

- Symptom Assessment in Dry Eye (SANDE) questionnaire
- Central Cornea In Vivo Confocal Microscopy

7.4 Standard of Care Procedures

The following procedures are the standard of care:

- Eye examination (best corrected visual acuity, bio-microscopy, applanation tonometry, funduscopy)
- Tear Break-up Time
- Corneal fluorescein staining
- Conjunctival lissamine green staining
- Schirmer's Test with anesthesia (may be study specific)
- Central Cornea In Vivo Confocal Microscopy (may be study specific)

7.5 Follow-up Procedures

After enrollment, the subjects will be scheduled for a second visit (Week 2 ± 3 days) and a third visit (Week 6 ± 1 week).

The following procedures will be performed at all follow-up visits:

- Eye examination (best corrected visual acuity, bio-microscopy, applanation tonometry, funduscopy)
- Tear Break-up Time
- Corneal fluorescein staining
- Conjunctival lissamine green staining
- Schirmer's Test with anesthesia
- Ocular Surface Disease Index questionnaire
- Symptom Assessment in Dry Eye (SANDE) questionnaire

- Central Cornea In Vivo Confocal Microscopy

7.6 Unscheduled Visits

If the subject reports to the Cornea Department for a visit that is not related to the study, only a review of adverse assessments will be required per the study.

7.7 Early Termination

If a subject withdraws or is withdrawn from the study prior to completion of all study visits, there will be no additional procedures performed

7.8 Schedule of Activities (Study Table)

Table1- Schedule of events and procedures

Visit #	1			2	3
Visit Description	Screening	Baseline	Day 1	Week 2 (± 3 days)	Week 6 (± 1 week)
Obtain Informed Consent	X		START OF STUDY MEDICATION		
Inclusion/ exclusion criteria	X				
Medical and Ophthalmic History	X			X	X
Medication History	X				
BCVA	X			X	X
OSDI		X		X	X
SANDE		X		X	X
TBUT	X			X	X
Biomicroscopy	X			X	X
Corneal staining	X			X	X
Schirmer with anesthesia	X			X	X
Conjunctival staining	X			X	X

Intraocular Pressure	X			X	X
Funduscopy	X			X	X
In vivo confocal microscopy	X			X	X
Concommitant medications				X	X
Compliance and adverse events check	X	X		X	X

8. SAFETY AND EFFECTIVENESS ASSESSMENTS

8.1 Safety Assessments

The primary safety variable monitored will be the occurrence of adverse events. The severity of each adverse event observed will be evaluated as serious or not serious and the occurrence will be evaluated as expected or unexpected. The relationship of the event to the study medication will be assessed by the investigator as possibly, probably, or definitely related. Safety variables will be evaluated at all study visits. If adverse events occur, the first concern will be the safety of the study participants. Any serious adverse event occurring during the study period will be immediately reported to MEEI IRB. At each visit throughout the study, the investigator will begin querying for adverse events by asking each subject a general, non-directed question such as “How have you been feeling since the last visit?” or “How have your eyes been since the last visit?”

The investigator will ask questions to subjects at each visit to determine if they have had any changes to the use of concomitant medications since the previous visit. A comprehensive eye examination including best-corrected visual acuity, measuring intraocular pressure, evaluation of the condition of conjunctiva, cornea, anterior chamber, iris/pupil, lens, vitreous, macula and optic nerve will be performed. Any changes in the study eyes from the screening visit will be recorded. If a subject contracts infectious conjunctivitis, they will be instructed to discard the remainder of the study drug and new drug will be sent from the host site pharmacy, Massachusetts Eye and Ear Infirmary.

If treatment with artificial tears is inadequate, or the subject develops severe form of ocular surface disease and dry eye, for the safety and proper treatment of the subject, the investigator can unmask the subject's treatment assignment to determine which

treatment has been assigned and institute appropriate follow-up care. However, the subject will be kept in the study so we can do the "intent-to-treat" analysis.

Withdrawal from the study will happen if any of the following occurs:

- Investigator determination that it is not in the best interest of the subject to continue participation.
- Subject's wish to withdraw for any reason.

After being withdrawn, subjects will be followed according to each case until the adverse event is resolved.

8.2 Effectiveness Assessments

Comparison of the efficacy of the taper Lotemax versus artificial tear will be evaluated using both clinical and IVCM parameters which include the followings:

Ocular Signs

- Corneal epitheliopathy (corneal fluorescein staining using the NEI grading scheme). (Appendix IV)
- Conjunctival epitheliopathy (conjunctival lissamine green staining using NEI grading scheme). (Appendix IV)
- Tear Film Break Up Time (TBUT)
- Schirmer's Test with Anesthesia

Ocular Symptoms

- Ocular Surface Disease Index (OSDI) questionnaire. (Appendix II)
- Symptom Assessment iN Dry Eye (SANDE) questionnaire. (Appendix III)

In Vivo Confocal Microscopy

- Superficial corneal epithelial cells: Density, size, and hypereflectivity
- Corneal subbasal immune dendritiform cells: Density, size, and cell field
- Corneal subbasal nerves: Number and length of the total, main nerves and the branches

9. ADVERSE EVENT RECORDING AND REPORTING

9.1 Recording Requirements

Research subjects will be routinely questioned about adverse events at study visits. All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study drug(s) or device(s) will be recorded in the subjects' case histories (source data, case report form). For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a serious adverse event) and; 2) an assessment of the causal relationship between the adverse event and the study drug(s) or device(s).

Adverse events or abnormal test findings thought to be associated with the study drug(s) or device(s) will be followed until the event (or its sequel) or the abnormal test finding resolves or stabilizes at a level acceptable to the Investigator.

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy
- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study
- The test finding is considered an adverse event by the Investigator. Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.

The Investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the Investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s)", the adverse event will be classified as associated with the use of the study drug(s) for reporting purposes. If the Investigator's final determination of causality is "unknown but not related to the study drug(s)", this determination and the rationale for the determination will be documented in the respective subject's case history (source data or case report form).

9.2 REPORTING PROCEDURES

- **REPORTING OF ADVERSE EVENTS TO FDA**

  If an investigator determines that a serious adverse event (SAE) is related to one of the study medications the SAE will be reported to the FDA.

- **Reporting Adverse Events to Other External Entities**

  The sponsor (GSK) does not require reports on adverse events. Therefore no reports on adverse events will be submitted to external entities.

Reporting Adverse Events to the Human Studies Committee

MEEI HSC policy for "REPORTING ADVERSE EVENTS AND UNANTICIPATED PROBLEMS" will be followed.

9.3 Withdrawal of Subjects due to Adverse Events

Withdrawal from the study will happen if any of the following occurs:

- Investigator determines that is not in the best interest of the subject to continue participation.
- Subject's wish to withdraw for any reason.

After being withdrawn, subjects will be followed according to each case until the adverse event is resolved.

10. STATISTICAL METHODS/DATA ANALYSIS

10.1 Primary endpoint(s) or outcome measure(s)

In Vivo Confocal Microscopy

- Superficial corneal epithelial cells: Density, size, and hypereflectivity
- Corneal subbasal immune dendritiform cells: Density, size, and cell field
- Corneal subbasal nerves: Number and length of the main nerves and the branches

10.2 Secondary endpoints or outcome measure(s)

Ocular Signs

- Corneal epitheliopathy (corneal fluorescein staining using the NEI grading scheme). (Appendix IV)
- Conjunctival epitheliopathy (conjunctival lissamine green staining using NEI grading scheme). (Appendix IV)
- Tear Break Up Time (TBUT)
- Schirmer's Test with Anesthesia
- Intraocular pressure (IOP) by measure of applanation tonometry

Ocular Symptoms

- Ocular Surface Disease Index (OSDI) questionnaire. (Appendix II)
- Symptom Assessment iN Dry Eye (SANDE) questionnaire. (Appendix III)

10.3 Sample Size Determination

To calculate sample size, density of sub-basal dendritic cells in the central cornea was used as an outcome measure. Based on a previous published paper (Villani et al, Innate Immunity. 2013;19(4) 420–427), a difference of 52 cell/mm^2 with a standard deviation of 44 were used to calculate the sample size with the following formula:[1-3]

$$N = \frac{4 \times \sigma^2 \times Z(1-\alpha/2)^2}{d^2}$$

With $\alpha = 0.05$ ($Z_\alpha = 1.96$), $d = 52$, and $p = 44$, a sample size of 22 was calculated for each group (44 for both groups). To compensate for potential loss to follow-up, a total of 50 subjects will be enrolled in this study.

1) Daniel WW. Biostatistics: a foundation for analysis in the health sciences 7th ed. New York, NY: Wiley, 1999; 180-185, 268– 270.
2) Snedecor GW, Cochran WG. Statistical methods 8th ed. Ames, Iowa: Iowa State University Press, 1989; 52, 439.
3) Eng G. Sample size estimation: how many individuals should be studied? Radiology. 2003; 227:309-13.

10.4 Analysis Population

N/A

10.5 Effectiveness Analysis

For efficacy variables and any other variables except for safety, all subjects will be analyzed with the treatment to which they were randomized (the intent-to-treat population). Subjects that are not compliant with the study medication or study procedures may or may not be withdrawn according to the investigators discretion. Subjects that are noncompliant, but remain active in the study, will be followed so that an intent-to-treat analysis can be conducted. For safety variables, subjects will be analyzed with the treatment actually received (the safety population).

The primary analysis for the proposed study will be based on a standard intention-to-treat analysis with each study participant analyzed with respect to the randomized treatment assignment, regardless of eventual compliance. A secondary analysis may include imputation of missing data for select variables. A per-protocol analysis, disqualifying subjects or subject visits, might also be done, but is not planned because observational analyses of actual treatment use could introduce bias if the pattern of use is in some way related to the outcome.

Despite the randomized nature of the treatment assignments, in this relatively small sample of study subjects there may be imbalances with regard to potential confounding variables. Thus, as an initial step in the analysis, we will compare those assigned to Lotemax versus those randomized to the artificial tear with regard to demographic characteristics and potential confounding variables, using the non-parametric Wilcoxon rank sum test for continuous or ordinal variables, and chi-square or Fisher's exact tests for categorical variables.

The changes in primary end-points within each group during the course of treatment will be analyzed using Analysis of Variance (ANOVA) with post hoc analysis of Bonferroni correction. The values between the Lotemax and artificial tear groups will be compared with non-parametric Wilcoxon rank sum test. P values of 0.05 or less will be considered as statistically significant.

10.6 Safety Analysis

The primary safety variable monitored will be the occurrence of adverse events. The severity of each adverse event observed will be evaluated as serious or not serious as defined by the MEEI HSC and the occurrence will be evaluated as expected or unexpected. The relationship of the event to the study medication will be assessed by the investigator as possibly, probably, or definitely related. Safety variables will be evaluated at screening and at all study visits. If adverse events occur, the first concern will be the safety of the study participants. Any serious adverse event occurring

during the study period will be immediately reported to MEEI IRB. Directed questioning (Ocular Tolerability and Compliance Questionnaire – Appendix I) and examination will then be done to assess tolerability. These questions are used to assess common and expected symptoms related to instillation or use of the study eye drops. Subjects will be asked to rate their symptoms from trace to severe.

Additional steps taken to assess safety will be followed as outlined in section 8.1 of this SUPP M form.

10.7 Interim Analysis

N/A

11. DATA AND SAFETY MONITORING

11.1 Data and Safety Monitoring Plan

The PI, research fellows, research coordinators, and technicians involved with this protocol will be required to read the protocol, participate in a group meeting to discuss roles before the protocol is approved, and participate in weekly clinical research meetings. The pharmacy and the clinical fellows on the protocol will be required to read the protocol and separate meetings will be created to discuss roles as they relate to the protocol.

A copy of the approved protocol and study related materials will be placed on a shared network drive accessible to authorized research staff involved in this study and on IRBnet.org.

The primary safety variable monitored will be the occurrence of adverse events. The severity of each adverse event observed will be evaluated as outlined in section 10.6 by the treating investigator. The PI will be made aware of all AEs within one week. The number of AEs related to increased pressure will be monitored and collated throughout the course of the study. Should the investigator need to be un-masked to treatment assignment to determine if there is a relationship with one of the study medications, treatment information will be provided by the MEEI Pharmacy.

The ocular surface exam and IVCM imaging will only be performed by a research technician, fellow, or investigator to ensure adherence to the required tests and accurate assessments.

The study coordinator will be responsible for monitoring each visit to ensure that the procedures are performed as outlined in the protocol and documented appropriately.

The images acquired will be checked for accuracy by the research fellows after acquisition and also during image analysis to ensure that the nasal conjunctiva has *been imaged appropriately as laid out in the IRB-approved protocol.*

Raw data collected for this study will be entered into a master database by the study coordinator, research technician, or fellow and uploaded for access to the principal investigator and all authorized research staff members on the U-Drive of password-protected computers located in the Cornea Research Department.

12. DATA HANDLING, RECORD-KEEPING AND MONITORING

12.1 Data Recording, Record-Keeping and Monitoring

A Case Report Form (CRF) will be completed for each subject enrolled into the clinical study. The Investigator will review, approve and sign/date each completed CRF; the Investigator's signature serving as attestation of the responsibility for ensuring that all clinical data entered on the CRF are complete, accurate and authentic.

The LMR will be considered Source Data and will contain the clinical findings and observations, and other information collected during the visit. Subject consents will be maintained within the Department of Ophthalmology Clinical Research Office. Drug dispensing and randomization logs will be maintained within the Clinical Pharmacy, Massachusetts Eye and Ear Pharmacy.

All data will be stored on in the U-drive located on encrypted, password protected computers in the Cornea Research Department. Only the PI and researchers specific to this study who have been granted access to the data by the PI will be able to view the data in the MEEI network protected folder. When data is sent out to be analyzed the data will be de-identified. The data will contain subject identification numbers, which are linked to identifiers on a separately secured spreadsheet. The data will be coded by assigning each participant a subject identification number and removing any identifiable information. The code will be secured by the PI and Study Coordinator in in the U-drive located on encrypted, password protected computers in the Cornea Research Department. The code that links information that can identify the participant to the data collected for this research will be kept separate from their health information, which will be destroyed once this study is complete and the manuscript has been published.

13. STUDY DISCONTINUATION CRITERIA

13.1 Discontinuation of Individual Research Subjects

Please see sections 9.3.

13.2 Sponsor-Investigator Discontinuation of the Clinical Research Study

Potential reasons for discontinuation of this study include if 50% of subjects experience an increase in IOP resulting in a total IOP of greater than 30. Additionally, if one of the study drugs is discontinued by the manufacturer and is no longer commercially available, the study will be discontinued.

Should the clinical research study be discontinued, the MEEI HSC will be notified immediately. Given the frequency of the visits, all enrolled subjects will be notified by phone of discontinuation of the study and ensured that discontinuation will have no effect on standard of care treatment.

14. APPENDICES

Appendix I: Ocular Tolerability and Compliance Questionnaire

Appendix II: Ocular Surface Disease Index (OSDI)

Appendix III: Symptom Assessment in Dry Eye (SANDE) Questionnaire I and II

Appendix IV: Corneal Fluorescein Staining and Conjunctival Lissamine Green Staining

14.1 Schedule of Events

See Section 7.8

14.2 Case Report Form(s)

Appendix I: Ocular Tolerability and Compliance Questionnaire

After you instill the study medication, how intensely have you experienced the following symptoms in each eye?

Itching

Right Eye:	None	Trace	Mild	Moderate	Severe
Left Eye:	None	Trace	Mild	Moderate	Severe

Burning Sensation

Right Eye:	None	Trace	Mild	Moderate	Severe
Left Eye:	None	Trace	Mild	Moderate	Severe

Foreign Body Sensation

Right Eye:	None	Trace	Mild	Moderate	Severe
Left Eye:	None	Trace	Mild	Moderate	Severe

Discharge

Right Eye:	None	Trace	Mild	Moderate	Severe
Left Eye:	None	Trace	Mild	Moderate	Severe

Redness

Right Eye:	None	Trace	Mild	Moderate	Severe
Left Eye:	None	Trace	Mild	Moderate	Severe

Appendix I: Ocular Tolerability and Compliance Questionnaire (Continued)

Have you experienced any other symptoms after instillation of the study drug?

YES NO

If yes, which one(s)_____
_____

In the past week, approximately how many times per day have you used the following?

Artificial Tears _____

Warm Compresses _____

Study Medications _____

Appendix II: Ocular Surface Disease Index (OSDI)

Have you experienced any of the following during the last week:

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

Have problems with your eyes limited you in performing any of the following during the last week:

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

Have your eyes felt uncomfortable in any of the following situations during the last week:

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

Total score for answers 1 to 12: ____

Total number of questions answered: ____

(Do not include questions answered N/A)

OSDI = (sum of scores) x 25/(# of questions answered): ____

Appendix III: Symptom Assessment in Dry Eye (SANDE) Questionnaire-I

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 feels **dry and/or irritated**:

_____

Rarely **All of the Time**

2. Severity of symptoms:

Please place an 'X' on the line to indicate how severe, on average, your eyes feel **dry and/or irritated**:

_____

Very Mild **Very Severe**

Appendix III: Symptom Assessment in Dry Eye (SANDE) Questionnaire-II

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 feels dry and/or irritated **now** *compared to your last visit*:

2. Severity of symptoms:

Please place an 'X' on the line to indicate how severe, on average, your eyes feels dry and/or irritated **now** *compared to your last visit*:

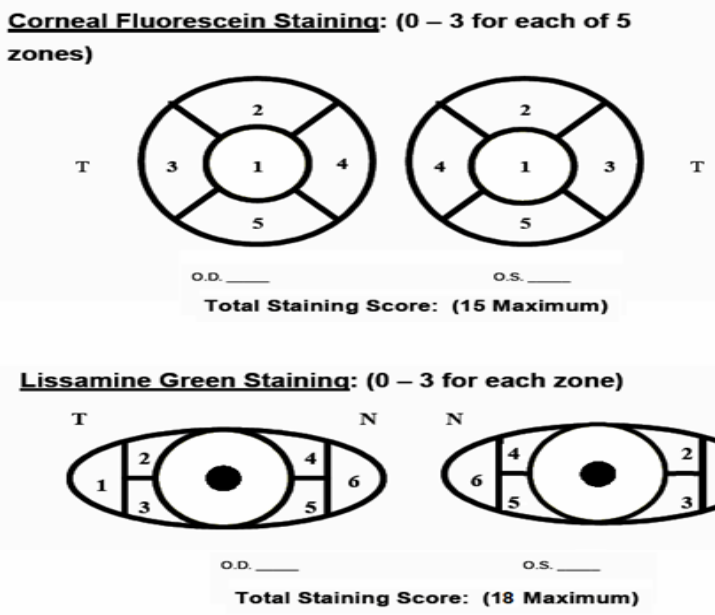
Appendix IV: Corneal Fluorescein Staining and Conjunctival Lissamine Green Staining

Fluorescein instillation:

Fluorescein strip wetted with buffered saline. Drop instilled on inferior palpebral conjunctiva. The participant is asked to blink several times.

Staining:

5 corneal regions and 6 conjunctival regions (shown below) will be graded with a staining scale of 0-3. The "total scores" of the cornea as well as of the conjunctiva will be measured.



Appendix V: Study Medical Calendar

Study Medication Calendar: Please bring calendar back to each study visit							
Week 1 & 2: Instill 1 drop in BOTH eyes 4 times a day							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1							
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Week 2							
Week 3 &4: Instill 1 drop in both eyes 2 times a day							
	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Week 3							
	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Week 4							
Week 5 & 6: Instill 1 drop in both eyes 1 time a day							
	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35
Week 5							
	Day 36	Day 37	Day 38	Day 39	Day 40	Day 41	Day 42
Week 6							