Clinical Trial Protocol: AXR201701

NCT03598699

Protocol Title:	A Multicenter, Vehicle-controlled, Randomized Study to Evaluate the Safety, Tolerability, Systemic Pharmacokinetics, and Pharmacodynamics of AXR-159 Ophthalmic Solution 3 mg/mL, 30 mg/mL, and 50 mg/mL in Patients with Dry Eye Disease (DED)
Protocol Number:	AXR201701
Study Phase:	2
Investigational Product Name:	AXR-159 (α4β1 and α4β7 Antagonist)
IND/IDE/PMA Number:	IND123799
Indication:	Dry Eye Disease
Investigators:	Multi-center
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Original Protocol:	February 17, 2018
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SYNOPSIS

Protocol Title:	A Multicenter, Vehicle-controlled, Randomized Study to Evaluate the Safety, Tolerability, Systemic Pharmacokinetics, and Pharmacodynamics of AXR-159 Ophthalmic Solution 3 mg/mL, 30 mg/mL, and 50 mg/mL in Patients with Dry Eye Disease (DED)
Protocol Number:	AXR201701
Investigational Product:	Stage 1: AXR-159 Ophthalmic Solution (30 mg/mL or 50 mg/mL) Stage 2: AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) Controls: AXR-159 Ophthalmic Solution Vehicle (Stages 1 and 2)
Study Phase:	2
Primary Objective(s):	Stage 1: To evaluate the safety, tolerability, and exploratory pharmacodynamics of up to 2 different concentrations of AXR-159 Ophthalmic Solution (30 mg/mL or 50 mg/mL) dosed three-times daily (TID) or twice-daily (BID) for up to 12 weeks compared to its vehicle in patients with Dry Eye Disease (DED). Stage 2: To evaluate the safety, tolerability, and pharmacodynamics of up to 3 different concentrations of AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) dosed three-times daily (TID) or twice-daily (BID) for up to 12 weeks compared to its vehicle in patients with Dry Eye Disease (DED).

Secondary Endpoints(s):	Stage 1 and 2: • Schirmer's test w/o anesthesia • Tear film break-up time (TBUT) • Conjunctival redness score • Sodium fluorescein corneal staining • Lissamine green conjunctival staining • Ocular Discomfort Score • Patient symptoms (visual analog scale [VAS]) • OSDI (subscales and items) • Symptom Assessment iN Dry Eye (SANDE) • Tear collection (for exploratory analyses) (selected sites)
Overall Study Design:	
Structure:	Multi-center, double-masked, vehicle-controlled, randomized, parallel group study
Duration:	An individual subject's participation is estimated to be approximately 14 weeks in stage 1 and 14 weeks in stage 2.
Controls:	AXR-159 Ophthalmic Solution Vehicle (Stages 1 and 2)
Dosage/Dose Regimen/ Instillation/Application/Use:	Stage 1: Study patients will receive a 2-week run-in on AXR-159 Ophthalmic Solution Vehicle to be administered either TID (Stage 1, Cohort 1; Stage 1, Cohort 2) or BID (Stage 1, Cohort 3). The assigned randomized study medication will be instilled into the eye TID for 3 months in Stage 1, Cohort 1; or Stage 1, Cohort 2 and BID in Cohort 3. Stage 2: Study patients will receive a 2-week run-in on AXR-159 Ophthalmic Solution Vehicle to be administered either TID or BID corresponding to the frequency of treatment dosing to be assigned post-randomization. The assigned randomized study medication will be instilled into the eye either TID or BID for 3 months in Stage 2 depending upon the groups selected for evaluation by the DRC.

Summary of Visit Schedule:	5 visits over the course of approximately 14 weeks • Visit 1 = Screening (Day -14 ± 2) • Visit 2 = Baseline & Randomization, Day 1 • Visit 3 = 2-Week Follow-Up (Day 15 ± 2) • Visit 4 = 1.5 Month Follow-up (Day 43 ± 2) • Visit 5 = 3 Month Follow-up (Day 85 ± 2)
Measures Taken to Reduce Bias:	This is a randomized treatment assignment, double-masked study
Study Population Characteristics:	
Number of Subjects:	Stage 1: The total number of randomized patients for Stage 1 of the study will be approximately 100 at up to 2 sites. Approximately 50% of patients should have a baseline eye dryness score of ≥ 60 and approximately 50% of patients should have a baseline inferior corneal staining score (ICCS) > 1.5 according to the Ora Calibra® staining scale. Based upon data from Xiidra®, OPUS 2 a screen failure rate of ~ 50% is expected. Thus, ~200 patients will need to be screened to achieve ~100 patients randomized to treatment. Stage 2: The total number of randomized patients for Stage 2 of the study will be approximately 330 (see Figure 6.1-1, Stage 2) at up to 7 sites. Approximately 50% of patients should have a baseline eye dryness score of ≥ 60 and approximately 50% of patients should have a baseline inferior corneal staining score (ICCS) > 1.5. Based upon data from Xiidra®, OPUS 2 a screen failure rate of ~ 50% is expected. Thus, ~660 patients will need to be screened to achieve ~330 patients randomized to treatment. Note: patients can be reallocated from discontinued groups to increase the power in groups that are continued at the discretion of the DRC.

Condition/Disease:	Dry Eye Disease (DED)
Inclusion Criteria:	1. Male or female, 18 years of age or older at screening visit 2. Provide written informed consent 3. Be willing and able to follow instructions and participate in all study assessments and visits 4. Best-corrected visual acuity (BCVA) of 20/100 or better (Snellen equivalent), using the logarithm of the minimum angle of resolution (LogMAR) in each eye at Visits 1 and 2 5. Must have patient reported history of dry eye for at least 6 months prior to Visit 1 6. Use of non-prescription artificial tears for the symptoms of DED within the 30 days preceding the screening visit and willing to suspend use of artificial tears for the study duration 7. Corneal fluorescein staining score ≥ 2 according to the Ora Calibra® scale in at least one region in at least one eye at the screening Visit 1 (Pre-CAE®) and Visit 2 8. Conjunctival redness score ≥ 1 according to the Ora Calibra® Conjunctival Redness for Dry Eye Scale in at least one eye at Visit 1 (Pre-CAE®) and Visit 2 9. Eye dryness score ≥ 40 (0-100 VAS scale in both eyes) at Visit 1 (Pre-CAE®) and Visit 2 10. Total Ocular Surface Disease Index (OSDI) score > 18 at Visit 1 (Pre-CAE®) and Visit 2 11. In the same eye at Visits 1 and 2, subjects must have: a. Inferior corneal fluorescein staining score ≥ 0.5 according to the Ora Calibra® Corneal and Conjunctival Staining Scale for Grading of Fluorescein Staining, Pre-CAE® at Visit 1 b. Schirmer's Tear Test without anesthesia ≥ 1 and ≤ 10 mm

Exclusion Criteria:	1. Uncontrolled ocular disease (except for dry eye disease/keratoconjunctivitis sicca) or uncontrolled systemic disease 2. Patient has glaucoma, ocular hypertension, on IOP-lowering medications or have previously undergone any glaucoma laser or surgical procedure. 3. Corneal abnormality or disorder that impacts normal spreading of the tear film (keratoconus, pterygia, scarring) or compromised corneal integrity 4. BCVA worse than 20/100 in either eye 5. Current use of punctal plugs, anticipated insertion during the study, or a history of punctal cautery in either eye at any time prior to the screening visit or anticipate such a procedure during the study 6. Keratoconjunctivitis sicca secondary to destruction of conjunctival goblet cells as occurs with vitamin A deficiency or scarring, such as that with cicatricial pemphigoid, alkali burns, Stevens-Johnson syndrome, trachoma, or irradiation 7. Active ocular infection or inflammation other than that caused by DED (including allergic, vernal, or giant papillary conjunctivitis and severe eyelid inflammation) 8. Patients with clinically significant inflammation of the lid margin such as anterior blepharitis or ocular rosacea 9. Recent (within the past 3 months) ocular surgery, trauma or herpes 10. Use of contact lenses in either eye within one month prior to the screening visit or anticipated use during the study 11. Use of any type of scleral lenses or sealed compartment ocular frames within 2 months of the screening visit, or planned use during the study

	12. Use prohibited medication within the prescribed washout window or anticipated use during the study: a. Topical cyclosporine (e.g., Restasis) or integrins (i.e., Xiidra): 12 weeks prior to Visit 1 b. Corticosteroids or mast cell stabilizers: 2 weeks prior to Visit 1 c. Antihistamines (including ocular): 72 hours prior to Visit 1 d. Oral aspirin or aspirin containing products only if a stable dosing regimen has been established for at least 30 days before Visit 1 e. Medications known to impact ocular dryness must be on a stable dosing regimen for at least 30 days before Visit 1 f. All other ophthalmic preparations including artificial tears: 72 hours prior to Visit 1 13. Eyelid abnormalities that affect lid function or impact lid closure 14. Systemic disease condition that causes dry eye 15. Diagnosis of hepatitis C infection, human immunodeficiency virus (HIV) infection, sarcoidosis, amyloidosis, active tuberculosis, or graft versus host disease 16. History of anterior segment surgery or trauma that could affect corneal sensitivity (e.g., cataract surgery, refractive surgery or any surgery involving a limbal or corneal incision) in either eye within the 12 months prior to Visit 1 17. Planned anterior segment surgery (e.g., cataract surgery or any surgery involving a limbal or corneal incision) in either eye during the study

	18. Known allergy or sensitivity to fluorescein, lissamine green, or the study medication or its components 19. Patient is unlikely to follow study instructions or to complete all required study visits or has a condition or situation that in the investigator's opinion, may put the patient at significant risk, may confound the study results, or may interfere significantly with the patient's participation in the study 20. Patient is an employee at the investigational site or is related to any member of the study staff 21. Patient cannot tolerate multiple blood draws 22. Pregnant, nursing, or females of childbearing potential and not utilizing adequate birth control measures 23. Participation in another ophthalmic clinical trial involving a therapeutic drug or device within the past 30 days 24. Previous exposure to AXR-159 Ophthalmic Solution or Ophthalmic Solution Vehicle.
Study Formulations and Formulation Numbers:	Stage 1: • 30 mg/mL AXR-159 Ophthalmic Solution • 50 mg/mL AXR-159 Ophthalmic Solution • AXR-159 Ophthalmic Solution Vehicle Stage 2: • 3 mg/mL AXR-159 Ophthalmic Solution • 30 mg/mL AXR-159 Ophthalmic Solution • 50 mg/mL AXR-159 Ophthalmic Solution • AXR-159 Ophthalmic Solution Vehicle

Evaluation Criteria:	
Efficacy Measures and Endpoints:	Primary Efficacy (pre-CAE) • Change from Baseline to Month 3 in inferior corneal staining score (Ora Calibra® scale) in the study eye • Change from Baseline to Month 3 in total corneal staining score (Oxford and Ora Calibra® scales) in the study eye • Change from Baseline to Month 3 in eye dryness measured using a visual analogue scale (VAS) • Change from Baseline to Month 3 in Total Ocular Surface Disease Index (OSDI) score Secondary Efficacy Measures • Schirmer's test w/o anesthesia • Tear film break-up time (TBUT) • Conjunctival redness score • Sodium fluorescein corneal staining • Lissamine green conjunctival staining • Ocular Discomfort Score • Patient symptoms (visual analog scale [VAS]) • OSDI (subscales and items) • Symptom Assessment iN Dry Eye (SANDE) • Proportion of patients with a TBUT ≥ 10 seconds at each visit • Proportion of patients with a Schirmer's test ≥ 10 mm at each visit • Proportion of patients with a Total OSDI < 18 at each visit • Proportion of patients with an Ocular Discomfort Score = 0 at each visit • Tear collection (for exploratory analyses) (selected sites)

Safety Measures:	• Adverse events • Vital signs • Study medication tolerability as measured by the Ocular Comfort Questionnaire • Urine pregnancy test • Best-corrected visual acuity (BCVA; Logarithmic visual acuity chart) • Biomicroscopy • Ophthalmoscopy • Intraocular pressure (IOP) • Lab analysis of blood and urine (including hematology, chemistry, and urinalysis)
Other:	Pharmacokinetics (Stage 1 Only) • Systemic pharmacokinetics

General Statistical Methods and Types of Analyses

The safety population will include all randomized subjects who have received at least one dose of the investigational product. The safety population will be analyzed for all safety assessments. Subjects in the Safety population will be analyzed as treated. The modified intent-to-treat (mITT) population will be comprised of all randomized subjects that have baseline and at least 1 post-baseline assessment for 1 or more of the efficacy measurements. All patients in the mITT population will be analyzed by the treatment as randomized with last observation carried forward (LOCF) for missing data. This population will be used for the primary and the secondary efficacy analyses.

The modified intent-to-treat 2 (mITT2) population will be comprised of patients who are included in the mITT and have baseline inferior corneal staining score (Ora Calibra® scale) in the study eye > 1.5. The mITT2 population will be analyzed as randomized.

In general, continuous data will be summarized with descriptive statistics (number of subjects, mean, standard deviation, median, minimum, and maximum) and will be analyzed using analysis of variance (ANOVA), analysis of covariance (ANCOVA) techniques, two-sample t-tests, or Wilcoxon rank sum tests for between-group comparisons, and paired t-tests or sign-rank tests for within-group analyses. Categorical variables will be summarized by number of subjects (n), frequency counts, and percentages, and they will be analyzed using Pearson's chi-square test or Fisher's exact test (if the expected cell count is less than 5 in 25% or more of the cells). Ordinal variables will be analyzed using the Cochran-Mantel-Haenszel (CMH) or the Wilcoxon rank-sum test for between-treatment comparisons and the sign-rank test for within-treatment comparisons.

The primary efficacy variables are:

- Change from Baseline to Month 3 in inferior corneal staining score (Ora Calibra® scale) in the study eye
- Change from Baseline to Month 3 in total corneal staining score (Oxford and Ora Calibra® scales) in the study eye
- Change from Baseline to Month 3 in eye dryness measured using a visual analogue scale (VAS)

And

- Change from Baseline to Month 3 in Total Ocular Surface Disease Index (OSDI) score.

A patient will be considered a responder if a cure is reached for TFBUT (i.e., ≥ 10 sec), a Schirmer's test (i,e., ≥ 10 mm), Total OSDI (< 18), or Ocular Discomfort Score (Score = 0) at a post-randomization visit. The visit for the primary variable is month 3 and the primary analysis population is mITT with last observation carried forward (LOCF). Statistical tests will be performed for each AXR-159 Ophthalmic Solution group versus vehicle group. Pairwise comparisons of the proportion of responders will be performed using the CMH method stratifying by baseline eye dryness score and baseline inferior corneal staining score. There will be no alpha adjustment for the multiple tests for the pairwise comparisons or multiple endpoints.

An interim analysis will be performed when the last enrolled patient in Stage 1 completes Month 3 of the Expansion Cohort. There is no plan to stop the study for efficacy on the basis of the interim analysis. However, termination of individual dose groups due to safety, tolerability and/or futility may occur. Statistical significance will not be declared at the time of the interim analysis.

A final database lock will occur at the completion of Stage 2 and the safety, tolerability, and efficacy data from both Stage 1 and 2 will be analyzed separately and in a combined fashion to fully leverage the advantage of this two-stage study design. At the final analysis, statistical significance will be declared for two-sided p-values < 0.05.

Safety: Safety measures will be analyzed using the safety population. Medical Dictionary for Regulatory Activities (MedDRA) nomenclature will be used to code adverse events. Incidence rates of each treatment-emergent adverse event will be summarized by primary system organ class and preferred term. Summary tables will be generated for all treatment-emergent adverse events regardless of causality as well as for those considered to be treatment-related.

Sample Size Calculation: The sample size is determined empirically. Xiidra achieved a 0.25 unit difference between lifitegrast ophthalmic solution and vehicle in mean change from baseline to day 84 in inferior cornel staining and a standard deviation of approximately 0.75 in Phase 2. Sample sizes for this study are based on detecting a 0.35 unit difference between the active and vehicle groups, assuming a standard deviation of 0.75. In Stage 1 the number of expected enrolled subjects varies between 43 and 53 subjects in the active group and between 37 and 47 subjects in the vehicle group. In Stage 2 the number of expected enrolled subjects is 60 in the active group and 30 to 60 subjects in the vehicle group. Thus, the combined sample provides between 103 and 113 subjects in the active group and between 67 and 77 subjects in the matching vehicle group or between 97 and 107 subjects in the combined vehicle group. Under these assumptions and allowing a dropout rate of 10%, sample sizes of 93 in the active treatment group and 60 in the vehicle group will yield 80% power to show a significant difference at the $\alpha = 0.05$ level using a two-sample t-test.

Summary of Known and Potential Risks and Benefits to Human Subjects

AXR-159X has previously been well tolerated clinically when administered via oral inhalation or intranasal routes to healthy volunteers, asthma and allergic rhinitis patients. This is the first time that a competitive antagonist of the α4 subunit, α4β1 and α4β7 integrins, (AXR-159 Ophthalmic Solution) is being administered as an eye drop in humans. The eye drop formulation was very well tolerated in preclinical species (i.e., rabbits, dogs) when administered at 50 mg/mL up to 12 times per day for up to 3 months. Eye drop administration can be expected to produce negligible systemic levels of drug.

Most reported adverse events from AXR-159 were considered mild to moderate in intensity and there have been no serious adverse events associated with the administration of AXR-159. Findings from pre-clinical studies support the administration of AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) BID or TID in patients with DED. While it is hoped that topical ocular dosing with AXR-159 Ophthalmic Solution will impart some degree of relief from dry eye signs or symptoms including redness, itching, foreign body sensation or blurred vision while on study, this is a first in patient study and these benefits cannot be predicted from preclinical models of DED with certainty.

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2 LIST OF ABBREVIATIONS

AE	Adverse Event
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
AXR-159	GW559090
AXR-159A	GW559090A, potassium salt of GW559090
AXR-159X	GW559090X, free acid of GW559090
BCVA	Best-Corrected Visual Acuity
BID	Twice Daily
CAE	Controlled Adverse Environment
CFR	Code of Federal Regulations
C_{max}	Maximum Concentration
CMH	Cochran-Mantel-Haenszel
CRF	Case Report Form
DED	Dry Eye Disease
DHHS	Department of Health and Human Services
DRC	Data Review Committee
ESOE	Early Signal of Efficacy
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICCS	Inferior Corneal Staining Score
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
IOP	Intraocular Pressure
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intent To Treat
LASIK	Laser In Situ Keratomileusis
LOCF	Last Observation Carried Forward
logMAR	Logarithm of the Minimum Angle of Resolution
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures

NCS	Not Clinically Significant
OD	Right Eye
OS	Left Eye
OSDI	Ocular Surface Disease Index
OU	Both Eyes
PK	Pharmacokinetics
PI	Principal Investigator
PP	Per Protocol
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDC	Statistics and Data Corporation
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TID	Three Times Daily
TF	Tear Film
TBUT/TFBUT	Tear Film Break-up Time
US	United States
VA	Visual Acuity
VAS	Visual Analog Scale

3 INTRODUCTION

Dry eye disease (DED) is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neuronal sensory abnormalities play etiological roles (Craig et al, 2017). DEWS II hypothesizes that dry eye disease (DED) is "…initiated by desiccating stress and perpetuated by a Vicious Circle of ocular surface inflammation (Bron et al, 2017)." Thus, no matter what initiates a patient's dry eye disease (e.g., allergic eye disease, topical preservative toxicity, or xerophthalmia) all patients eventually enter a chain of inflammatory events that perpetuate the disease.

Experimentally, the expression of IL-1, TNF-α, and IL-6 by ocular surface epithelia are critical to the ocular surface dry eye inflammatory response. A key step in the amplification process for inflammation is the generation of signals that recruit inflammatory cells to the site of inflammation: 1) chemokines and 2) integrin adhesion molecules. Chemokines produced at the ocular surface (e.g., CCL3, CCL4, CCL5, CXCL9, CXCL10, and CX3CL1) can bind macrophages, dendritic cells, neutrophils, and activated T cells. The other key step in homing inflammatory cells to the ocular surface is the expression of endothelial integrin adhesion molecules (e.g., ICAM-1, VCAM-1). ICAM-1 and VCAM-1 are adhesion molecules that bind to inflammatory cells expressing the ligand, integrated leukocyte function antigen 1 (LFA-1; integrin αLβ2) and very late antigen 4 (VLA-4; integrin α4β1), respectively. Integrins facilitate cell-cell and cell-extracellular matrix (ECM) adhesion and include the β1, β2, β4, immunoglobulin, and selectin super families. Known targets for treating ocular inflammation include αLβ2 and α4β1 (Bron et al, 2017).

Lifitegrast ophthalmic solution (Xiidra®) 5% was approved by the US FDA for the treatment of DED in 2016. Lifitegrast is an antagonist of LFA-1 (also known as CD11a/CD18 or αLβ2). Lifitegrast demonstrated a statistically significant treatment response in the objective endpoint, change from baseline to Day 84 in inferior corneal staining score in the Phase 2 and OPUS 1 studies and a statically significant treatment response in the subjective endpoint, change from baseline to Day 84 in eye dryness score (VAS) in the OPUS 2 and 3 studies (Semba & Gadek, 2016).

Natalizumab, an antibody directed against the α4- integrin subunit, has been shown to profoundly inhibit inflammation and improve clinical outcomes in both multiple sclerosis and Crohn's disease. Topical, ocular application of BIO-8809 (Anti-VLA-4 small molecule) decreased corneal fluorescein staining, conjunctival T-cell infiltrates, and TNFα expression in cornea and conjunctiva in a murine dry eye model (Ecoiffier et al, 2008).

AXR-159 (GW559090) is potent and selective antagonist for the α4 subunit, α4β1 and α4β7 integrins, which has been formulated as an eye drop and has been shown to be safe and well tolerated in the eye with no target organ toxicity identified at doses up to 30 mg/kg/day in animal models. It lacks significant systemic exposure following topical ocular administration due to low systemic exposure and its high clearance rate from the circulation.

In preclinical murine models of DED, AXR-159A improved corneal staining with similar efficacy and onset of action, as the topical steroid dexamethasone. Topical steroids are an unapproved, but effective treatment for DED and often serve as positive controls in preclinical models of DED. Topical ophthalmic corticosteroids are commonly used for the treatment of dry eye disease (DED) (Holland et al, 2007; DEWS, 2007; Avunduk et al, 2003; Pflugfelder et al, 1999; Pflugfelder et al, 2004; Yang et al, 2006). Topical AXR-159A also significantly reduced corneal uptake of Oregon Green Dextran (OGD) compared to vehicle-treated disease controls, murine model of dry eye, in a dose-dependent manner (1, 3, 10, and 30 mg/mL) with 30 mg/mL showing the greatest reduction in OGD staining. When administered topically, corneal expression of IL-1α, matrix metalloproteinase (MMP)–9, chemokine ligand 9 (CXCL9), and TGF-β1 were reduced in AXR-159A -treated eyes. Topical treatment with AXR-159A decreased dendritic cell activation in lymph nodes. The effects on corneal staining and cellular composition in draining cervical lymph nodes (CLN) were not reproduced by systemic administration of AXR-159A, suggestive of a local role for integrin antagonism in the treatment of dry eye (Krauss et al, 2015).

The presence of CD4+ T cells at the ocular surface in DED and the successful treatment of ocular surface inflammation with cyclosporine (i.e., Restasis® and Ikervis®) suggest that adaptive immunity is important in the pathophysiology of DED. Initiation of an adaptive immune response requires that antigens at the site of inflammation are presented by professional antigen presenting cells that migrate to regional lymphoid tissue to activate and expand antigen-specific T cells. The ability of AXR-159A to decreased dendritic cell activation in lymph nodes suggests it has the ability to block adaptive immunity associated with DED.

Given the above clinical experience in man with an antibody directed against the α4-integrin subunit and preclinical work with novel α4 integrin antagonists, i.e., AXR-159A and BIO-8809, in pre-clinical models of DED, AxeroVision Inc. believes that AXR-159 has potential as a novel agent for the treatment of dry eye disease.

4 STUDY OBJECTIVES

TABLE 4–1 TABLE OF OBJECTIVES, ENDPOINT, AND ENDPOINT JUSTIFICATION

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Stage 1: To evaluate the safety, tolerability, and exploratory pharmacodynamics of up to 2 different concentrations of AXR-159 Ophthalmic Solution (30 mg/mL or 50 mg/mL) dosed three-times daily (TID) or twice-daily (BID) for up to 12 weeks compared to its vehicle in patients with Dry Eye Disease (DED).	Adverse Events: • Incidence rates of each treatment-emergent adverse event summarized by primary system organ class and preferred term. • Tables for all treatment-emergent adverse events regardless of causality. • Tables for all treatment-emergent adverse events considered to be treatment-related. • Shift tables for safety variables (e.g., IOP, biomicroscopy, and ophthalmoscopy). Signal of Efficacy: • Change from Baseline in inferior corneal staining score in the study eye • Change from Baseline in total corneal staining score in the study eye • Change from Baseline in eye dryness measured using a visual analogue scale (VAS) • Change from Baseline in Total Ocular Surface Disease Index (OSDI) score	The endpoints for safety and tolerability are all commonly used in drug trials. The efficacy endpoints match those captured in the Xiidra clinical development program and are listed on the U.S. Food & Drug Administration webpage (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208073Orig1s000TOC.cfm).
Stage 2: To evaluate the safety, tolerability, and pharmacodynamics of up to 3 different concentrations of	*See Above for Stage 1*	*See Above for Stage 1*

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) dosed three-times daily (TID) or twice-daily (BID) for up to 12 weeks compared to its vehicle in patients with Dry Eye Disease (DED).		
Secondary		
See Above for Stages 1 & 2	• Schirmer's test w/o anesthesia • Tear film break-up time (TBUT) • Conjunctival redness score • Sodium fluorescein corneal staining • Lissamine green conjunctival staining • Ocular Discomfort Score • Patient symptoms (visual analog scale [VAS]) • OSDI (subscales and items) • Symptom Assessment iN Dry Eye (SANDE) • Proportion of patients with a TBUT ≥ 10 seconds at each visit • Proportion of patients with a Schirmer's test ≥ 10 mm at each visit • Proportion of patients with a Total OSDI < 18 at each visit • Proportion of patients with an Ocular Discomfort Score = 0 at each visit • Tear collection (for exploratory analyses) (selected sites)	*See Above for Stages 1 & 2*

5 CLINICAL HYPOTHESES

Stage 1:

AXR-159 Ophthalmic Solution (50 mg/mL or 30 mg/mL) has an acceptable safety and tolerability profile following TID or BID administration for the study treatment duration.

AXR-159 Ophthalmic Solution (50 mg/mL or 30 mg/mL) demonstrates an Early Signal of Efficacy (ESOE) for either a sign (e.g., Inferior Corneal Staining) or Symptom (e.g., eye dryness measured by VAS or Total OSDI) of DED.

Stage 2:

At least 1 concentration of AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) has an acceptable safety and tolerability profile following TID or BID administration for the study treatment duration.

At least 1 concentration of AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) is more effective than vehicle for treating DED as measured by either a sign (e.g., Inferior Corneal Staining) or Symptom (e.g., eye dryness measured by VAS or Total OSDI) of DED.

6 OVERALL STUDY DESIGN

6.1 Overall Design

This is a multicenter, double-masked, vehicle-controlled, randomized, parallel group study carried out in 2 stages (Stage 1: AXR-159 Ophthalmic Solution (30 mg/mL or 50 mg/mL) dosed three-times daily (TID) or twice-daily (BID); Stage 2: AXR-159 Ophthalmic Solution (3 mg/mL, 30 mg/mL or 50 mg/mL) dosed three-times daily (TID) or twice-daily (BID).

For both Stages 1 and 2, patients with signs and symptoms of DED will be randomly assigned in a 1:1 (Stage 1) or up to a 2:2:2:2:1:1:1 (Stage 2) ratio to receive either a single concentration of AXR-159 Ophthalmic Solution or AXR-159 Ophthalmic Solution Vehicle. A screening visit will be followed by a baseline period where subjects will dose with AXR-159 Ophthalmic Solution Vehicle for 14 days. At the end of the baseline period patients who still exhibit signs and symptoms of DED will be enrolled into a 3-month treatment period (See Appendix 1 for study visit structure across Stages 1 and 2 and Figure 6.1-1 for the study flow diagram). If only a single dosing regimen is selected for Stage 2 a 1:1:1:1 regime may be selected: AXR-159 Ophthalmic Solutions and AXR-159 Ophthalmic Solution Vehicle.

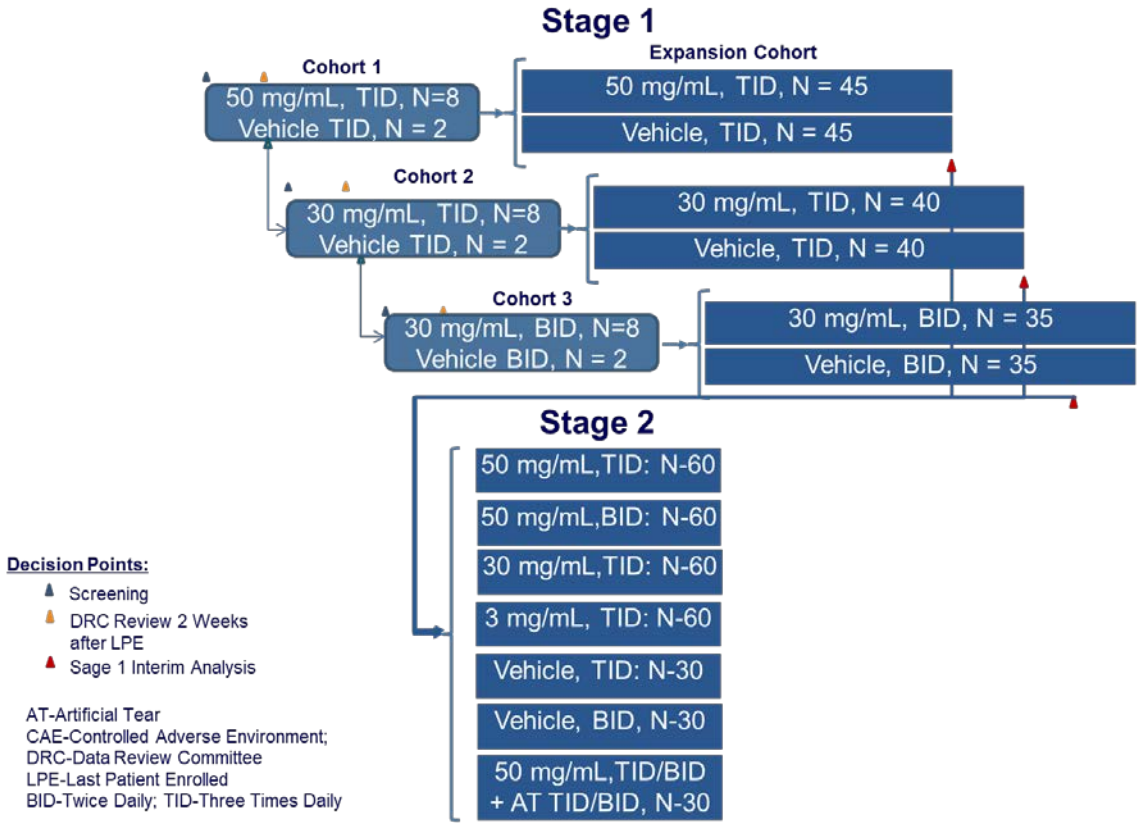
When the 10th patient enrolled in Stage 1, Cohort 1 (8 patients on AXR-159 Ophthalmic Solution 50 mg/mL TID and 2 patients on vehicle TID) completes the Week 2 visit, a Data Review Committee (DRC) will evaluate all available safety data. If AXR-159 Ophthalmic Solution 50 mg/mL TID is safe and well tolerated the DRC will recommend

expansion of the AXR-159 Ophthalmic Solution 50 mg/mL and vehicle TID groups (N = 45 per group) and to halt the initiation of Stage 1, Cohorts 2 and 3. If AXR-159 Ophthalmic Solution 50 mg/mL TID is NOT safe and well tolerated the DRC will recommend Stage 1, Cohort 2 (8 patients on AXR-159 Ophthalmic Solution 30 mg/mL TID and 2 patients on vehicle TID) and to halt the Cohort 1 Expansion Cohort. Patients already enrolled in Stage 1, Cohort 1 will continue at the investigator's discretion. Any patients that discontinue during the study will be followed through the resolution of their adverse event(s) or end of study. When the 10th patient enrolled in Stage 1, Cohort 2 completes the Week 2 visit, a DRC will evaluate all available safety data. If AXR-159 Ophthalmic Solution 30 mg/mL TID is safe and well tolerated the DRC will recommend expansion of the AXR-159 Ophthalmic Solution 30 mg/mL and vehicle TID Expansion Cohort (N = 40 per group) and to halt Cohort 3. If AXR-159 Ophthalmic Solution 30 mg/mL TID is NOT safe and well tolerated the DRC will recommend Stage 1, Cohort 3 (8 patients on AXR-159 Ophthalmic Solution 30 mg/mL BID and 2 patients on vehicle BID) and to halt the Cohort 2 Expansion Cohort. Patients already enrolled in Stage 1, Cohort 2 will continue at the investigator's discretion. When the 10th patient enrolled in Stage 1, Cohort 3 completes the Week 2 visit, a DRC will evaluate all available safety data. If AXR-159 Ophthalmic Solution 30 mg/mL BID is safe and well tolerated the DRC will recommend expansion of the AXR-159 Ophthalmic Solution 30 mg/mL and vehicle BID Expansion Cohort (N = 35 per group). If AXR-159 Ophthalmic Solution 30 mg/mL BID is NOT safe and well tolerated the DRC will recommend stopping the study. Thus, in stage 1 a successive cohort will only be started if the previous cohort is felt to be either unsafe or poorly tolerated by the DRC.

When the last patient enrolled in Stage 1 completes the Month 3 visit in the Expansion Cohort, a formal Interim Analysis will be completed and the DRC will evaluate all available safety and efficacy data from Stage 1 before recommending initiation of Stage 2. Stage 2 is a parallel group design and the DRC may recommend up to a maximum of 7 groups as depicted in Figure 6.1-1. Groups that are either unsafe, poorly tolerated, or likely futile may be discontinued by the DRC. The DRC may reallocate patients to the surviving AXR-159 Ophthalmic Solution dose groups.

A final database lock will occur at the completion of Stage 2 and the safety, tolerability, and efficacy data from both Stage 1 and 2 will be analyzed separately and in a combined fashion to fully leverage the advantage of this two-stage study design.

FIGURE 6.1-1 STUDY DESIGN



6.2 Scientific Rationale for Study Design

Prior studies in dry eye have been marked by poor reproducibility. This is due partly to the heterogeneity of the population, inadequate assessment methods, and poor correlation between signs and symptoms.

Xiidra (lifitegrast ophthalmic solution) which was approved by the US FDA on July 11th, 2016 addressed these challenges through a variety of design considerations which are incorporated into the current study design:

1. The registration studies used a vehicle which was not palliative
2. Subjects showing active signs and symptoms of DED after a 2 week run-in on the vehicle either switched to lifitegrast ophthalmic solution 5% or stayed on vehicle
3. The studies were enriched for both inferior corneal staining and/or eye dryness

Thus, clinical study AXR201701 incorporates the learnings from the recently successful clinical program for Xiidra.

6.3 Data Review Committee (DRC)

A DRC consisting of physicians and trained study personnel, in addition to ad hoc internal/external experts, will assess study treatment effects. The composition and activities of the committee are described in the DRC charter. The DRC will review available data to determine the appropriateness of continuing dosing and enrollment in Stage 1. Based on review of data from all executed Stage 1 cohorts, the DRC will recommend whether to proceed to Stage 2 and the dose levels to be employed. The DRC may recommend at any time during Stage 1 to de-escalate, delay, or to stop patient recruitment. The number of treatment groups, doses, and number of subjects per-group in Stage 2 will be based on recommendations by the DRC following review of available Stage 1 data. Details of the planned analyses will be provided in the DRC analysis plan. The DRC may also request ad-hoc review of masked or unmasked data at any time throughout the course of the study to evaluate new findings or recommend changes to study progression.

6.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Appendix 1.

7 STUDY POPULATION

7.1 Number of Subjects (approximate)

Stage 1:

The total number of randomized patients for Stage 1 of the study will be approximately 100 at up to 2 sites. Approximately 50% of patients should have a baseline eye dryness score of ≥ 60 and approximately 50% of patients should have a baseline inferior corneal staining score (ICCS) > 1.5 according to the Ora Calibra® staining scale. Based upon data from Xiidra®, OPUS 2 a screen failure rate of ~ 50% is expected. Thus, ~200 patients will need to be screened to achieve ~100 patients randomized to treatment.

Stage 2:

The total number of randomized patients for Stage 2 of the study will be approximately 330 (see Figure 6.1-1, Stage 2) at up to 7 sites. Approximately 50% of patients should have a baseline eye dryness score of ≥ 60 and approximately 50% of patients should have a baseline inferior corneal staining score (ICCS) > 1.5 according to the Ora Calibra® staining scale. Based upon data from Xiidra®, OPUS 2 a screen failure rate of ~ 50% is expected. Thus, ~660 patients will need to be screened to achieve ~330 patients randomized to treatment.

Note: patients can be reallocated from discontinued groups to increase the power in groups that are continued at the discretion of the DRC.

7.2 Study Population Characteristics

The study will consist of patients with DED, who at the screening visit (day -14) are administering a conventional artificial tear for the symptoms of DED within the 30 days preceding the screening visit and are willing to suspend use of artificial tears for the study duration.

Patients with systemic autoimmune disorders (including, but not limited to, primary and secondary Sjögren's Syndrome, systemic lupus erythematosus, rheumatoid arthritis, chronic osteoarthritis, eczema, ankylosing spondylitis, scleroderma, and atopic dermatitis) may be included in the study.

7.3 Inclusion Criteria

1. Male or female, 18 years of age or older at screening visit
2. Provide written informed consent
3. Be willing and able to follow instructions and participate in all study assessments and visits
4. Best-corrected visual acuity (BCVA) of 20/100 or better (Snellen equivalent), using the logarithm of the minimum angle of resolution (LogMAR) in each eye at Visits 1 and 2
5. Must have patient reported history of dry eye for at least 6 months prior to Visit 1
6. Use of non-prescription artificial tears for the symptoms of DED within the 30 days preceding the screening visit and willing to suspend use of artificial tears for the study duration
7. Corneal fluorescein staining score ≥ 2 according to the Ora Calibra® scale in at least one region in at least one eye at the screening Visit 1 (Pre-CAE®) and Visit 2
8. Conjunctival redness score ≥ 1 according to the Ora Calibra® Conjunctival Redness for Dry Eye Scale in at least one eye at Visit 1 (Pre-CAE®) and Visit 2
9. Eye dryness score ≥ 40 (0-100 VAS scale in both eyes) at Visit 1 (Pre-CAE®) and Visit 2
10. Total Ocular Surface Disease Index (OSDI) score > 18 at Visit 1 (Pre-CAE®) and Visit 2
11. In the same eye at Visits 1 and 2, subjects must have:
    a. Inferior corneal fluorescein staining score ≥ 0.5 according to the Ora Calibra® Corneal and Conjunctival Staining Scale for Grading of Fluorescein Staining, Pre-CAE® at Visit 1
    b. Schirmer's Tear Test without anesthesia ≥ 1 and ≤ 10 mm

7.4 Exclusion Criteria

1. Uncontrolled ocular disease (except for dry eye disease/keratoconjunctivitis sicca) or uncontrolled systemic disease
2. Patient has glaucoma, ocular hypertension, on IOP-lowering medications or have previously undergone any glaucoma laser or surgical procedure

3. Corneal abnormality or disorder that impacts normal spreading of the tear film (keratoconus, pterygia, scarring) or compromised corneal integrity
4. BCVA worse than 20/100 in either eye
5. Current use of punctal plugs, anticipated insertion during the study, or a history of punctal cautery in either eye at any time prior to the screening visit or anticipate such a procedure during the study
6. Keratoconjunctivitis sicca secondary to destruction of conjunctival goblet cells as occurs with vitamin A deficiency or scarring, such as that with cicatricial pemphigoid, alkali burns, Stevens-Johnson syndrome, trachoma, or irradiation
7. Active ocular infection or inflammation other than that caused by DED (including allergic, vernal, or giant papillary conjunctivitis and severe eyelid inflammation)
8. Patients with clinically significant inflammation of the lid margin such as anterior blepharitis or ocular rosacea
9. Recent (within the past 3 months) ocular surgery, trauma or herpes
10. Use of contact lenses in either eye within one month prior to the screening visit or anticipated use during the study
11. Use of any type of scleral lenses or sealed compartment ocular frames within 2 months of the screening visit, or planned use during the study
12. Use prohibited medication within the prescribed washout window or anticipated use during the study:
    a. Topical cyclosporine (e.g., Restasis) or integrins (i.e., Xiidra): 12 weeks prior to Visit 1
    b. Corticosteroids or mast cell stabilizers: 2 weeks prior to Visit 1
    c. Antihistamines (including ocular): 72 hours prior to Visit 1
    d. Oral aspirin or aspirin containing products only if a stable dosing regimen has been established for at least 30 days before Visit 1
    e. Medications known to impact ocular dryness must be on a stable dosing regimen for at least 30 days before Visit 1
    f. All other ophthalmic preparations including artificial tears: 72 hours prior to Visit 1
13. Eyelid abnormalities that affect lid function or impact lid closure
14. Systemic disease condition that causes dry eye
15. Diagnosis of hepatitis C infection, human immunodeficiency virus (HIV) infection, sarcoidosis, amyloidosis, active tuberculosis, or graft versus host disease
16. History of anterior segment surgery or trauma that could affect corneal sensitivity (e.g., cataract surgery, refractive surgery or any surgery involving a limbal or corneal incision) in either eye within the 12 months prior to Visit 1
17. Planned anterior segment surgery (e.g., cataract surgery or any surgery involving a limbal or corneal incision) in either eye during the study

18. Known allergy or sensitivity to fluorescein, lissamine green, or the study medication or its components
19. Patient is unlikely to follow study instructions or to complete all required study visits or has a condition or situation that in the investigator's opinion, may put the patient at significant risk, may confound the study results, or may interfere significantly with the patient's participation in the study
20. Patient is an employee at the investigational site or is related to any member of the study staff
21. Patient cannot tolerate multiple blood draws
22. Pregnant, nursing, or females of childbearing potential and not utilizing adequate birth control measures
23. Participation in another ophthalmic clinical trial involving a therapeutic drug or device within the past 30 days
24. Previous exposure to AXR-159 Ophthalmic Solution or Ophthalmic Solution Vehicle.

7.5 Withdrawal Criteria (if applicable)

Patients can voluntarily withdraw from the study at any time. The investigator and AxeroVision Inc. can withdraw a patient from the study at any time for any reason. Additionally, patients can be discontinued from the study by an investigator if any of the following criteria are met:

- Patient develops (or had an exacerbation of) any medical condition that, in the opinion of the investigator, would put the patient at an unacceptable medical risk or compromised the patient’s ability to participate in the study
- Patient is unwilling or unable to continue to comply with study procedures
- Patient becomes pregnant

The study can be stopped at the study site(s) at any time by the site investigator(s). AxeroVision Inc. can stop the study (and/or the study site[s]) with appropriate notification.

If a patient discontinued participation in the study early, every attempt will be made to complete the exit procedures. Notification of early patient discontinuation from the study and the reason for discontinuation should be made to AxeroVision Inc. and clearly documented on the appropriate eCRF.

8 STUDY PARAMETERS

8.1 Efficacy Measures and Endpoints

8.1.1 Primary Efficacy Measure(s)

Primary efficacy measures for Stage 1 and Stage 2 include total sodium fluorescein corneal staining (Oxford and Ora Calibra® scales), sodium fluorescein corneal staining in the inferior cornea (Ora scale), patient symptoms of dryness (visual analog scale [VAS]), and total OSDI. For specific timings of efficacy measures see Appendix 1.

- Change from Baseline to Month 3 in inferior corneal staining score (Ora Calibra® scale) in the study eye
- Change from Baseline to Month 3 in total corneal staining score (Oxford and Ora Calibra® scales) in the study eye
- Change from Baseline to Month 3 in eye dryness measured using a visual analogue scale (VAS)
- Change from Baseline to Month 3 in Total Ocular Surface Disease Index (OSDI) score

8.1.2 Secondary Efficacy Measure(s)

Secondary efficacy measures for Stage 1 and Stage 2 include Schirmer's test without anesthesia, TBUT, conjunctival redness score, sodium fluorescein corneal staining (Oxford and Ora Calibra® scales), lissamine green conjunctival staining (Oxford and Ora Calibra® scales), ocular discomfort score, patient symptoms (VAS), OSDI (total and subscales), Symptom Assessment iN Dry Eye (SANDE), and tear sampling for mechanism of action biomarkers. Refer to Appendix 1 for specific timings of exploratory efficacy measures.

- Schirmer's test w/o anesthesia
- Tear film break-up time (TBUT)
- Conjunctival redness score
- Sodium fluorescein corneal staining
- Lissamine green conjunctival staining
- Ocular Discomfort Score
- Patient symptoms (visual analog scale [VAS])
- OSDI (subscales and items)
- Symptom Assessment iN Dry Eye (SANDE)
- Tear collection (for exploratory analyses) (selected sites)

8.2 Safety Measures

For stages 1 and 2, the following safety measures will be examined:

- Adverse events (ocular and non-ocular)
- Ora Calibra® Drop Comfort Assessment
- Best-corrected visual acuity (BCVA)
- Slit-lamp biomicroscopy
- Intraocular pressure (IOP)
- Ophthalmoscopy
- Ora Calibra® Conjunctival Redness for Dry Eye Scale
- Vital signs
- Laboratory tests (chemistry, hematology, urinalysis)
- Urine pregnancy test

Laboratory test results will be forwarded from the central laboratory to the study site and to AxeroVision Inc. or its designee. The laboratory results from screening will be reviewed prior to randomization. Laboratory tests at screening may be repeated once at the discretion of the investigator and pending discussion with AxeroVision Inc., to confirm exclusionary status. Study treatment will be performed only if the investigator deems the laboratory results to be acceptable. The most current specimen results will be reviewed prior to randomization. The investigator will review all laboratory results for the clinical significance of any abnormalities. Evaluation and management of abnormal laboratory results should be conducted according to local site practice. Clinically significant abnormalities are to be recorded on an adverse event electronic case report form (eCRF) page.

8.3 Other Measures

Stage 1:

Systemic pharmacokinetic samples will be collected in a subset of patients. For determination of drug concentration in the circulation, blood samples will be collected at baseline (prior to treatment) and month 1.5. (see Appendix 1).

- Systemic pharmacokinetics

9 STUDY MATERIALS

9.1 Study Treatment(s)

9.1.1 Study Treatment(s)/ Formulation(s)/ Medical Device Composition or Design

All concentrations of AXR-159 Ophthalmic Solution are preservative-free and contain the drug product, AXR-159 (GW559090) (3 mg/mL, 30 mg/mL or 50 mg/mL), and suitable excipients. Excipients in the formulation include phosphate buffer and sodium chloride. The formulations will be supplied in identical unit dose containers (see Table 9.1.1-1).

TABLE 9.1.1–1 INVESTIGATIONAL PRODUCT AND PACKAGING CHARACTERISTICS

	Investigational Product	
Product name:	AXR-159	Vehicle (Placebo)
Formulation description:	AXR-159 solutions contain 3 mg/mL, 30 mg/mL or 50 mg/mL AXR-159 as parent.	Placebo solution matches 30 mg/mL or 50 mg/mL AXR-159 as parent solutions.
Dosage form:	Ophthalmic Sterile Solution	Ophthalmic Sterile Solution
Unit dose strength(s)/Dosage level(s):	3 mg/mL 30 mg/mL 50 mg/mL	Placebo - phosphate buffer + sodium chloride
Route of Administration	Topical	Topical
Dosing instructions :	Single use vial - one drop in the same selected eye three times a day (or twice a day). Each container will be used to dose both eyes and should then be discarded. Thus, a patient dosing TID will use 3 units in one day and a patient dosing BID will use 2 units in one day.	Single use vial - one drop in the same selected eye three times a day (or twice a day). Each container will be used to dose both eyes and should then be discarded. Thus, a patient dosing TID will use 3 units in one day and a patient dosing BID will use 2 units in one day.
Physical description:	A clear, colourless solution packaged in a single use container.	A clear, colourless solution packaged in a single use container.
Device:	Single use blow fill seal vial, tear-off cap	Single use blow fill seal vial, tear-off cap
Method for individualizing dosage:	Five single use containers are placed in an individual foil wrap and appropriately labelled.	Five single use containers are placed in an individual foil wrap and appropriately labelled.

9.1.2 Dosing and Administration

The study medication will be self-administer by the patient (or administered by site staff or a caregiver) at the study site after all procedures have been completed for the run-in and post-randomization visits. At these visits, each patient will be asked to stay at the site for 30 minutes after study drug administration.

Patients will be instructed to twist-off the tips of the unit of use containers. They should not pull them off. Patients (or site staff or a caregiver) will then make a pouch with the lower lid and instil the study medication.

Each patient (or caregiver) must instil 1 drop of the vehicle either tree times daily (TID) (once in the morning, once around mid-day, and once in the evening) or twice daily (BID) (once in the morning and once in the evening) to both eyes during the 2 week run-in period. During the double-masked treatment period, each patient (or caregiver) must instil 1 drop of the study medication either tree times daily (TID) (once in the morning, once around mid-day, and once in the evening) or twice daily (BID) (once in the morning and once in the evening) to both eyes for 3 months. For both the dosing regimens the morning and evening study medication doses should be instilled about 12 hours apart. For the TID dosing regimen the mid-day medication doses should be instilled about 6 hours after the morning dose.

One unit dose vial of the masked study medication is to be used for only 1 application to both eyes. The patient should be instructed to place the used vial in the used vial bag after use and should return used and unused vials at the next study visit.

9.2 Other Study Supplies

Urine pregnancy tests, Schirmer's test strips, sodium fluorescein, lissamine green, Fluress, tear collection supplies, phlebotomy supplies, urinalysis supplies.

10 STUDY METHODS AND PROCEDURES

10.1 Subject Entry Procedures

10.1.1 Overview

Subjects as defined by the criteria in sections 7.2, 7.3, and 7.4 will be considered for entry into this study.

10.1.2 Informed Consent

Prior to a subject's participation in the trial (i.e., prior to changes in a subject's medical treatment and/or prior to study related procedures), the study will be discussed with each subject, and subjects wishing to participate must give written informed consent using an informed consent form (ICF). The informed consent form must be the most recent version that has received approval/favorable review by a properly constituted Institutional Review Board (IRB)

10.1.3 Washout Intervals

Prohibited medications, treatments, and activities are outlined in the Exclusion Criteria (Section 7.4).

10.1.4 Procedures for Final Study Entry

Subjects must meet all inclusion and none of the exclusion criteria.

10.1.5 Methods for Assignment to Treatment Groups:

Prior to initiation of study run-in (at Visit 1), each subject who qualifies for entry will be assigned a screening number. All screening numbers will be assigned in strict numerical sequence at a site and no numbers will be skipped or omitted. If all inclusion and exclusion criteria are met at Visits 1 and 2, each qualifying subject will then be assigned a randomization number at the end of Visit 2.

A randomization schedule will be provided to each investigational site. The randomization schedule will use block randomization stratified by site, such that there will be an approximate equal number of subjects assigned to each of the two treatment arms at each site for the Stage 1, Expansion Cohort. For Stage 1, Cohorts 1-3 patients with signs and symptoms of DED will be randomly assigned in a 4:1 ratio to receive either a single concentration of AXR-159 Ophthalmic Solution or AXR-159 Ophthalmic Solution Vehicle. The site staff will dispense to the patient the study kit labelled with the corresponding randomization number. The randomization number will be recorded on the patient's source document and eCRF. New kits will be dispensed at Visits 2, 3, and 4 based on the subject's randomization. The Sponsor, Investigators, and study staff will be masked during the randomization process and throughout the study.

During the run-in period, the patients will all be supplied with AXR-159 Ophthalmic Solution Vehicle. Patients will be informed that they will receive only vehicle during the 2-week run-in period. Following the run-in period the patient, site personnel, the sponsor and patients will be masked to the treatment assignment for the 3 month treatment period. All study medication will be provided in identical unit of dose vials and cartons to maintain masking of the study.

For both Stages 1 and 2, patients with signs and symptoms of DED will be randomly assigned in a 4:1 (Stage 1, Cohorts 1-3) ratio, a 1:1 (Stage 1, Expansion Cohort) ratio, or to a 2:2:2:2:1:1:1 (Stage 2) ratio to receive either a single concentration of AXR-159 Ophthalmic Solution or AXR-159 Ophthalmic Solution Vehicle.

For the expansion cohort in Stage 1 and Stage 2, subjects will be randomized by strata as follows:

- Site
- Baseline eye dryness score ≥ 60, < 60
- Baseline inferior corneal staining > 1.5, ≤ 1.5

Subjects will not be stratified in Cohorts 1, 2 and 3 in Stage 1.

10.2 Concurrent Therapies

The use of any concurrent medication, prescription or over-the-counter, is to be recorded on the subject's source document and corresponding case report form (CRF) along with the reason the medication was taken.

Concurrent enrollment in another investigational drug or medical device study is not permitted.

10.2.1 Prohibited Medications/Treatments

Prohibited medications, treatments, and activities are outlined in the Exclusion Criteria (Section 7.4).

10.2.2 Rescue Medications

In the event that rescue medication is required for worsening signs or symptoms of dry eye disease during the course of the study, patients will be provided an appropriate rescue regimen by the investigator/treating clinician, and will be exited from the study.

10.2.3 Special Diet or Activities

No special diets or activities are required for this study.

10.3 Examination Procedures

10.3.1 Procedures to be Performed at Each Study Visit with Regard to Study Objective(s)

10.3.1.1 Visit 1: Screening (Day -14 ± 2)

Pre–CAE®

- Written informed consent form
- Demographic information
- Medical, ophthalmic, and dry eye disease history
- Concomitant medications and procedures
- Inclusion and exclusion criteria
- Urine pregnancy test (for females of childbearing potential)
- Patient symptoms using the Visual Analog Scale
- Ocular Discomfort Score
- OSDI®

- SANDE
- Best-corrected visual acuity
- Vital signs
- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- Oculus Keratograph 5® procedures: TBUT and tear meniscus height (selected sites)
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales
- Lissamine green conjunctival staining, using the Oxford scale
- 15+ minute wait from end of Lissamine staining to start of Schirmer's Test
- Schirmer's without anesthesia
- 90-minute CAE® exposure;
    - Ora Calibra® Ocular Discomfort Scale upon entering the CAE® and every 5 minutes thereafter;

Post–CAE®

- Inclusion and exclusion criteria
- Patient symptoms using the Visual Analog Scale
- Ocular Discomfort Score
- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales

- Lissamine green conjunctival staining, using the Oxford scale
- Intraocular pressure (IOP)
- Ophthalmoscopy (the dilating drug should not be given until the time of ophthalmoscopy)
- Blood sample collection for Hematology and Chemistry
- Urine sample collection for Urinalysis
- Dispense AXR-159 Ophthalmic Solution Vehicle
- Adverse event query

10.3.1.2 Visit 2: Baseline & Randomization (Day 1)

- Study drug collection
- Medical/medication history update
- Inclusion and exclusion criteria
- Patient symptoms using the Visual Analog Scale
- Ocular Discomfort Score
- OSDI
- SANDE
- BCVA with manifest refraction
- Vital signs
- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- Tear Collection (selected sites)

- Oculus Keratograph 5® procedures: TBUT and tear meniscus height (selected sites)
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales
- Lissamine green conjunctival staining, using the Oxford scale
- 15+ minute wait from end of Lissamine staining to start of Schirmer's Test
- Schirmer without anesthesia
- Intraocular pressure (IOP)
- Randomization
- Blood sample collection for systemic pharmacokinetics (in selected Stage 1 patients)
- Administer AXR-159 Ophthalmic Solution or vehicle in both eyes (must NOT be done before blood and tear sample collection)
- Ora Calibra® Drop Comfort Assessment
- Collect and dispense study drug
- Adverse event query

10.3.1.3 Visit 3: 2-Week Follow-up (Day 15 ± 2 Days)

- Study drug collection
- Medical/medication history update
- Patient symptoms using the visual analog scale
- Ocular Discomfort Score
- OSDI
- SANDE

- BCVA with manifest refraction
- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- Oculus Keratograph 5® procedures: TBUT and tear meniscus height (selected sites)
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales
- Lissamine green conjunctival staining, using the Oxford scale
- 15+ minute wait from end of Lissamine staining to start of Schirmer's Test
- Schirmer without anesthesia
- Intraocular pressure (IOP)
- Dispense study drug
- Administer AXR-159 Ophthalmic Solution or vehicle in both eyes)
- Adverse event query

10.3.1.4 Visit 4: Month 1.5 Follow-up (Day 43 ± 2 Days)

- Study drug collection
- Medical/medication history update
- Patient symptoms using the visual analog scale
- Ocular Discomfort Score
- OSDI
- SANDE
- BCVA with manifest refraction

- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- Oculus Keratograph 5® procedures: TBUT and tear meniscus height (selected sites)
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales
- Lissamine green conjunctival staining, using the Oxford scale
- 15+ minute wait from end of Lissamine staining to start of Schirmer's Test
- Schirmer without anesthesia
- Intraocular pressure (IOP)
- Dispense study drug
- Blood sample collection for systemic pharmacokinetics (in selected Stage 1 patients)
    - Collected pre-dose, and at 15, 30, 60, 90, 180, 360 minutes post-dose
- Administer AXR-159 Ophthalmic Solution or vehicle in both eyes (must NOT be done before blood and tear sample collection)
- Ora Calibra® Drop Comfort Assessment
- Adverse event query

10.3.1.5 Visit 5: 3 Month Follow-up (Day 85 ± 2 Days)

Pre–CAE®

- Study drug collection
- Medical/medication history update
- Urine pregnancy test (for females of childbearing potential)

- Patient symptoms using the visual analog scale
- Ocular Discomfort Score
- OSDI
- SANDE
- BCVA with manifest refraction
- Vital signs
- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- Tear Collection (selected sites)
- Oculus Keratograph 5® procedures: TBUT and tear meniscus height (selected sites)
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales
- Lissamine green conjunctival staining, using the Oxford scale
- 15+ minute wait from end of Lissamine staining to start of Schirmer's Test
- Schirmer without anesthesia
- 90-minute CAE® exposure;
    - Ora Calibra® Ocular Discomfort Scale upon entering the CAE® and every 5 minutes thereafter;

Post–CAE®

- Patient symptoms using the Visual Analog Scale
- Ocular Discomfort Score

- Bulbar conjunctival hyperemia scores (Ora Calibra® Conjunctival Redness for Dry Eye Scale)
- Slit-lamp biomicroscopy
- TFBUT
- Sodium fluorescein corneal staining, using the Oxford scale and Ora Calibra® scales
- Lissamine green conjunctival staining, using the Oxford scale
- Intraocular pressure (IOP)
- Ophthalmoscopy
- Blood sample collection for Hematology and Chemistry
- Urine sample collection for Urinalysis
- Administer AXR-159 Ophthalmic Solution or vehicle in both eyes (must NOT be done before blood and tear sample collection)
- Ora Calibra® Drop Comfort Assessment
- Adverse event query
- Study Exit

Adverse Events (AEs) (both elicited and observed) will be monitored throughout the study. All AEs (both elicited and observed) will be promptly reviewed by the investigator for accuracy and completeness. All AEs will be documented on the appropriate CRF.

If a female becomes pregnant during the study, the investigator will notify AxeroVision Inc. immediately after the pregnancy is confirmed and the patient will be exited from the study after appropriate safety follow-up. The investigator will (1) notify the patient's physician that the patient was being treated with an investigational drug (AXR-159 Ophthalmic Solution), and (2) follow the progress of the pregnancy. The investigator must document the outcome of the pregnancy and provide a copy of the documentation to AxeroVision Inc.

10.4 Schedule of Visits, Measurements and Dosing

10.4.1 Scheduled Visits

Refer to Appendix 1 for a schedule of visits and measurements.

10.4.2 Unscheduled Visits

These visits may be performed in order to ensure subject safety. All procedures performed at an unscheduled visit will be recorded in the source documents and on the Unscheduled Visit eCRF pages. Any procedure indicated in the eCRF that is not performed should be indicated as "Not done."

Evaluations that may be conducted at an Unscheduled Visit include:

- Vital Signs
- Labs
- Slit-lamp Biomicroscopy
- Visual Acuity
- Intraocular Pressure
- Urine Pregnancy Test
- Ophthalmoscopy
- Assessment of Adverse Events
- Assessment of concomitant medications and/or treatments
- Any other assessments needed in the judgment of the investigator.

10.5 Compliance with Protocol

Whilst subjects are dosed at site, they will receive their study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

Subjects will be instructed on proper instillation and storage of study drug at the end of Visits 1, 2, 3, and 4, and given written instructions. The used and unused study drug vials will be collected at each visit from Visit 2 up to and including Visit 5 to assess dosing and symptom assessment compliance. Dosing compliance will be based off of the used and unused vial count. If the subject is less than 80% or more than 125% compliant with dosing based on the expected number of used vials, then the subject will be deemed non-compliant and a deviation should be recorded.

10.6 Subject Disposition

10.6.1 Completed Subjects

A completed subject is one who has not been discontinued from the study.

10.6.2 Discontinued Subjects

Subjects may be discontinued prior to their completion of the study due to:

- subject request/withdrawal
- AEs
- protocol violations
- administrative reasons (e.g., inability to continue, lost to follow up)
- sponsor termination of study
- other

Note: In addition, any subject may be discontinued for any sound medical reason.

Notification of a subject discontinuation and the reason for discontinuation will be made to Ora and/or sponsor and will be clearly documented on the CRF.

10.7 Study Termination

The study may be stopped at any time by the investigator, the sponsor, and/or Ora with appropriate notification.

10.8 Study Duration

The total duration of study is approximately 3.5 months (from screening to study completion).

10.9 Monitoring and Quality Assurance

During the course of the study a monitor, or designee, will make routine site visits to review protocol compliance, assess Investigational Product (IP)/device accountability, and ensure the study is being conducted according to the pertinent regulatory requirements. The review of the subjects' medical records will be performed in a manner that adequately maintains subject confidentiality. Further details of the study monitoring will be outlined in a monitoring plan.

Regulatory authorities of domestic and foreign agencies, Ora, Inc. quality assurance and or its designees may carry out on-site inspections and/or audits which may include source data checks. Therefore, direct access to the original source data will be required for inspections and/or audits. All inspections and audits will be carried out giving consideration to data protection as well as subject confidentiality to the extent that local, state, and federal laws apply.

11 ADVERSE EVENTS

11.1 Adverse Event

An AE is defined as any untoward medical occurrence associated with the use of an IP in humans, whether or not considered IP-related. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IP, without any judgment about causality. An AE can arise from any use of the IP (e.g., off-label use, use in combination with another drug or medical device) and from any route of administration, formulation, or dose, including an overdose. An AE can arise from any delivery, implantation, or use of a medical device, including medical device failure, subject characteristics that may impact medical device performance (e.g., anatomical limitations), and therapeutic parameters (e.g., energy applied, sizing, dose release, and anatomic fit) associated with medical device use.

All AEs spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures will be recorded in the source document and on the appropriate pages of the CRF. Any clinically relevant deterioration in clinical finding is considered an AE and must be recorded. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

Documentation regarding the AE should be made as to the nature, date of onset, end date, severity, and relationship to IP, action(s) taken, seriousness, and outcome of any sign or symptom observed by the physician or reported by the subject upon indirect questioning.

11.1.1 Severity

Severity of an AE is defined as a qualitative assessment of the degree of intensity of an AE as determined by the investigator or reported to him/her by the subject. The assessment of severity is made irrespective of relationship to IP or seriousness of the event and should be evaluated according to the following scale:

- ***Mild*****:** Event is noticeable to the subject, but is easily tolerated and does not interfere with the subject's daily activities.
- ***Moderate*****:** Event is bothersome, possibly requiring additional therapy, and may interfere with the subject's daily activities.
- ***Severe*****:** Event is intolerable, necessitates additional therapy or alteration of therapy, and interferes with the subject's daily activities.

11.1.2 Relationship to Investigational Product

A determination will be made of the relationship (if any) between an adverse event and the study drug or study procedure, as applicable. A causal relationship is present if a determination is made that there is a reasonable possibility that the adverse event may have been caused by the drug or study procedure.

The relationship of each AE to the IP should be determined by the investigator using these explanations:

- ***Suspected*:** A reasonable possibility exists that the IP caused the AE. A suspected AE can be further defined as:
  - *Definite*: Relationship exists when the AE follows a reasonable sequence from the time of IP administration, follows a known response pattern of the drug class, is confirmed by improvement on stopping the IP and no other reasonable cause exists.
  - *Probable*: Relationship exists when the AE follows a reasonable sequence from the time of IP administration, follows a known response pattern of the drug class, is confirmed by improvement on stopping the IP and the suspect IP is the most likely of all causes.
  - *Possible*: Relationship exists when the AE follows a reasonable sequence from the time of IP administration, but could also have been produced by the subject's clinical state or by other drugs administered to the subject.
- ***Not Suspected:*** A reasonable possibility does not exist that the IP caused the AE.
  - *Not Related:* Concurrent illness, concurrent medication, or other known cause is clearly responsible for the AE, the administration of the IP and the occurrence of the AE are not reasonably related in time, OR exposure to IP has not occurred.

Suspected adverse reaction means any AE for which there is a reasonable possibility that the IP caused the AE. "Reasonable possibility" means there is evidence to suggest a causal relationship between the IP and the AE. Types of evidence that would suggest a causal relationship between the IP and the AE event include: a single occurrence of an event that is uncommon and known to be strongly associated with IP exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome); one or more occurrences of an event that is not commonly associated with IP exposure, but is otherwise uncommon in the population exposed to the IP (e.g., tendon rupture); an aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the IP-treatment group than in a concurrent or historical control group.

11.1.3 Expectedness

The expectedness of an AE should be determined based upon existing safety information about the IP using these explanations:

- ***Unexpected:*** An AE that is not listed in the Investigator's Brochure (IB) or is not listed at the specificity or severity that has been observed.
- ***Expected:*** An AE that is listed in the IB at the specificity and severity that has been observed.
- ***Not applicable:*** An AE unrelated to the IP.

AE events that are mentioned in the IB as occurring with a class of products or as anticipated from the pharmacological/mechanical (or other) properties of the product, but are not specifically mentioned as occurring with the particular product under investigation are to be considered unexpected.

The investigator should initially classify the expectedness of an AE, but the final classification is subject to the Medical Monitor's determination.

11.2 Serious Adverse Events

An AE is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening AE;

Note: An AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization;

Note: The term "inpatient hospitalization" refers to any inpatient admission (even if less than 24 hours). For chronic or long-term inpatients, inpatient admission includes transfer within the hospital to an acute/intensive care inpatient unit. Inpatient hospitalization does not include: emergency room visits; outpatient/same-day/ambulatory procedures; observation/short stay units; rehabilitation facilities; hospice facilities; nursing homes; or clinical research/phase 1 units.

Note: The term "prolongation of existing hospitalization" refers to any extension of an inpatient hospitalization beyond the stay anticipated or required for the reason for the initial admission as determined by the investigator or treating physician.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;

Note: A serious adverse event (SAE) specifically related to visual threat would be interpreted as any potential impairment or damage to the subject's eyes (e.g., hemorrhage, retinal detachment, central corneal ulcer or damage to the optic nerve).

- A congenital anomaly/birth defect.

Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.3 Procedures for Reporting Adverse Events

All AEs and their outcomes must be reported to Ora, the sponsor, and the IRB/IEC as required by the IRB/IEC, federal, state, or local regulations and governing health authorities and recorded on the appropriate CRF.

11.3.1 Reporting a Suspected Unexpected Adverse Reaction

All AEs that are 'suspected' and 'unexpected' are to be reported to Ora, the sponsor and the IRB/IEC as required by the IRB/IEC, federal, state, or local regulations and governing health authorities.

11.3.2 Reporting a Serious Adverse Event

To ensure subject safety, all SAEs, regardless of relationship to the IP, must be immediately reported. All information relevant to the SAE must be recorded on the appropriate CRFs. The investigator is obligated to pursue and obtain information requested by Ora and/or the sponsor in addition to that information reported on the CRF. All subjects experiencing a SAE must be followed up and the outcome reported.

In the event of a SAE, the investigator must notify Ora and the sponsor immediately; obtain and maintain in his/her files all pertinent medical records, information, and medical judgments from colleagues who assisted in the treatment and follow-up of the subject; provide Ora and the sponsor with a complete case history, which includes a statement as to whether the event was or was not suspected to be related to the use of the IP; and inform the IRB of the SAE within their guidelines for reporting SAEs.

Contact information for reporting SAEs and ADEs:

Name:	Brian Orrick
Title:	Clinical Project Manager, Dry Eye
Company:	Ora, Inc.
Office Telephone:	978-685-8900 x9584
Mobile Telephone:	978-409-0138
Office Facsimile:	978-849-7371
Name:	Joseph B. Ciolino, MD
Title:	Medical Monitor
Office Telephone:	617-573-5575
Mobile Phone:	401-935-9662
Office Facsimile:	617-573- 4324

11.4 Procedures for Unmasking (if applicable)

When necessary for the safety and proper treatment of the patient, the investigator can unmask the patient's treatment assignment to determine which treatment has been assigned and institute appropriate follow-up care. When possible, the sponsor

(AxeroVision Inc.) should be notified prior to unmasking study medication. The investigator should inform the sponsor (AxeroVision Inc.) of the unmasking if there is no notification prior to the unmasking.

A report of the results of this study may be published, sent to the appropriate health authorities in any country in which the study drug may ultimately be marketed, and published in part as required by appropriate health authorities (e.g., Clinical Trials posting and disclosure), but the patient's name will not be disclosed in these documents.

Patients will be informed that the study is posted and the results eventually disclosed by appropriate health authorities (e.g., Clinical Trials posting or freedom of information by the FDA).

11.5 Type and Duration of the Follow-up of Subjects after Adverse Events

Any serious adverse event occurring during the study period (beginning with informed consent) and for at least 12 weeks after the last dose of study drug must be immediately reported, but no later than 24 hours after learning of a serious adverse event. Serious adverse events must be reported to AxeroVision Inc. (or Agent of AxeroVision Inc.) as listed on the AxeroVision Inc. Study Contacts Sheet and recorded on the serious adverse event form. All patients with a serious adverse event must be followed up and the outcomes reported. The investigator must supply AxeroVision Inc. and the IRB/IEC with any additional requested information (e.g., autopsy reports and discharge summaries).

In the event of a serious adverse event, the investigator must:

1. Notify AxeroVision Inc. immediately by fax or email using the serious adverse event form (contact details can be found on page 1 of the serious adverse event form); phone numbers and relevant AxeroVision Inc. personnel contacts are also on the front page of the protocol.

2. Obtain and maintain in his/her files all pertinent medical records, information, and medical judgments from colleagues who assisted in the treatment and follow-up of the patient.

3. Provide AxeroVision Inc. with a complete, written description of the adverse event(s) on the serious adverse event form describing the event chronologically, including any treatment given (e.g., medications administered, procedures performed) for the adverse event(s). Summarize relevant clinical information about the event: signs, symptoms, diagnosis, clinical course and relevant clinical laboratory tests, etc. Include any additional or alternative explanation(s) for the causality which includes a statement as to whether the event was or was not related to the use of the investigational drug.

4. Promptly inform the governing IRB/IEC of the serious adverse event as required by the IRB/IEC, local regulations, and the governing health authorities.

12 STATISTICAL HYPOTHESES AND METHODS OF ANALYSES

The statistical analysis plan (SAP) will provide a detailed description of the planned statistical analysis.

12.1 Sample Size Determination

The sample size is determined empirically. Xiidra achieved a 0.25 unit difference between lifitegrast ophthalmic solution and vehicle in mean change from baseline to day 84 in inferior cornel staining and a standard deviation of approximately 0.75 in Phase 2. Sample sizes for this study are based on detecting a 0.35 unit difference between the active and vehicle groups, assuming a standard deviation of 0.75. In Stage 1 the number of expected enrolled subjects varies between 43 and 53 subjects in the active group and between 37 and 47 subjects in the vehicle group. In Stage 2 the number of expected enrolled subjects is 60 in the active group and 30 to 60 subjects in the vehicle group. Thus, the combined sample provides between 103 and 113 subjects in the active group and between 67 and 77 subjects in the matching vehicle group or between 97 and 107 subjects in the combined vehicle group. Under these assumptions and allowing a dropout rate of 10%, sample sizes of 93 in the active treatment group and 60 in the vehicle group will yield 80% power to show a significant difference at the $\alpha = 0.05$ level using a two-sample t-test.

12.2 Analysis Populations

The following analysis populations will be considered:

- Modified Intent-to-Treat Population – The modified intent-to-treat (mITT) population includes all randomized subjects that have baseline and at least 1 post-baseline assessment for 1 or more of the efficacy measurements (see Section 8.1). The primary analysis will be performed on the mITT population with the Last Observation Carried Forward (LOCF) imputation method for missing values. The mITT population may also be analyzed with observed data only (i.e., without LOCF) and using multiple imputation methods to assess sensitivity. Subjects in the mITT population will be analyzed as randomized.
- Modified Intent-to-Treat 2 Population – The modified intent-to-treat 2 (mITT2) population includes all subjects in the mITT population that have baseline inferior corneal staining score in the study eye > 1.5. The mITT2 population will be analyzed using LOCF. The mITT2 population may also be analyzed using observed data only to assess sensitivity. Subjects in the mITT2 population will be analyzed as randomized.
- Per Protocol Population – The per protocol (PP) population includes subjects in the mITT population who do not have significant protocol deviations and who

complete the study. Protocol deviations will be assessed prior to database lock and unmasking. The PP population will be analyzed using observed data only for primary efficacy variables. Subjects in the PP population will be analyzed as treated.

- Safety Population – The safety population includes all randomized subjects who have received at least one dose of the investigational product. The safety population will be analyzed for all safety assessments. Subjects in the Safety population will be analyzed as treated.

The statistical analysis of safety data will be performed for the safety population. The analysis of baseline and efficacy data will be performed for the mITT population. The primary efficacy analysis will also be performed on the mITT2 and PP populations as sensitivity analyses.

12.3 Statistical Hypotheses

The statistical hypotheses are stated in terms of one-sided hypotheses, although statistical testing will be two-sided. All hypotheses are Pre-CAE®.

H_{01}: There is no difference between AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) and vehicle (TID or BID) in the change from baseline to Month 3 in inferior corneal staining score in the study eye.

H_{11}: The change from baseline to Month 3 in inferior corneal staining score in the study eye is less with AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) than with vehicle (TID or BID).

H_{02}: There is no difference between AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) and vehicle (TID or BID) in the change from baseline to Month 3 in total corneal staining score in the study eye.

H_{12}: The change from baseline to Month 3 in total corneal staining score in the study eye is less with AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) than with vehicle (TID or BID).

H_{03}: There is no difference between AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) and vehicle (TID or BID) in the change from baseline to Month 3 in eye dryness.

H_{13}: The change from baseline to Month 3 in eye dryness is less with AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) than with vehicle (TID or BID).

H_{04}: There is no difference between AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) and vehicle (TID or BID) in the change from baseline to Month 3 in total OSDI score.

H_{14}: The change from baseline to Month 3 in total OSDI score is less with AXR-159 Ophthalmic Solution (50 mg/ml, 30 mg/ml or 3 mg/ml, TID or BID) than with vehicle (TID or BID).

In the hypotheses above, dosing (TID or BID) of the vehicle will match the dosing of the active treatment.

12.4 Statistical Analyses

12.4.1 General Approach

In general, continuous data will be summarized with descriptive statistics (number of subjects, mean, standard deviation, median, minimum, and maximum) and will be analyzed using analysis of variance (ANOVA), analysis of covariance (ANCOVA) techniques, two-sample t-tests or Wilcoxon rank sum tests for between-group comparisons, and paired t-tests or sign-rank tests for within-group analyses. Categorical variables will be summarized by number of subjects (n), frequency counts, and percentages, and they will be analyzed using Pearson's chi-square test or Fisher's exact test (if the expected cell count is less than 5 in 25% or more of the cells). Ordinal variables will be analyzed using the Cochran-Mantel-Haenszel (CMH) or the Wilcoxon rank-sum test for between-treatment comparisons and the sign-rank test for within-treatment comparisons.

All summaries will be presented by treatment group and visit. Summaries will be provided for demographics, baseline medical history, concurrent therapies, and subject disposition.

For the purpose of summarization, medical history, concurrent therapies, and adverse events will be coded to MedDRA and WHO Drug dictionaries, as appropriate.

Baseline measures are defined as the last measure prior to the initiation of study treatment, usually at Day 1. If a measure is taken both pre-CAE® and post-CAE®, the baseline will be the last time point matched value prior to the initiation of study treatment. For post-CAE® measures, this will be at Day -14. For changes from pre-CAE® to post-CAE® post first treatment, the change from pre-CAE® to post-CAE® at Day -14 will be considered the baseline value.

Changes from baseline will be calculated as visit – baseline and summarized by treatment and visit as described above. Treatment comparisons between an active treatment and vehicle will be calculated as active – vehicle.

All primary and secondary analyses will be two-sided at a significance level of 0.05. 95% confidence intervals will be provided where appropriate.

12.4.2 Unit of Analysis

Safety endpoints will be analyzed for both eyes. For efficacy endpoints, the unit of analysis will be the study eye, or the "worst eye," as defined by the following:

Study Eye/Worst Eye: Eyes are eligible for analysis if they meet all of the inclusion criteria. In the case that both eyes are eligible for analysis, the study eye will be the eye with worse (higher) inferior corneal staining score (Ora Calibra® scale) on Day 1. If the inferior corneal staining score is the same in both eyes, then the right eye will be selected as the study eye.

12.4.3 Missing Data

The primary efficacy analyses will be performed using Last Observation Carried Forward (LOCF) methodology. For the LOCF analyses of the primary efficacy variables at Month 3, the last value from the previous visits will be carried forward, matching pre-CAE® or post-CAE® time points. A pre-CAE® time point will never be imputed for a post-CAE® value, and vice versa.

An analysis using observed data only will also be performed for the primary efficacy variables. As additional sensitivity analyses, Markov Chain Monte Carlo (MCMC) multiple imputation methodology may be used to impute missing data for the analyses of the primary efficacy variables.

No secondary efficacy endpoints or safety endpoints will be imputed.

12.4.4 Multiplicity Consideration

This is a Phase 2 study to evaluate the safety, tolerability, systemic pharmacokinetics, and pharmacodynamics of AXR-159 Ophthalmic Solution in patients with dry eye disease. There will be no multiplicity adjustments for the multiple treatment groups or multiple primary endpoints.

12.4.5 Primary Efficacy Analyses

The primary efficacy variables (Pre-CAE®) are:

- Change from Baseline to Month 3 in inferior corneal staining score (Ora Calibra® scale) in the study eye
- Change from Baseline to Month 3 in total corneal staining score (Oxford and Ora Calibra® scales) in the study eye
- Change from Baseline to Month 3 in eye dryness measured using a visual analogue scale (VAS)
- Change from Baseline to Month 3 in Total Ocular Surface Disease Index (OSDI) score, and

The primary variables will be summarized as described in Section 12.4.1. The primary efficacy analysis will use the mITT population with LOCF.

Statistical tests will be performed for each AXR-159 Ophthalmic Solution group versus vehicle group at matched dosing frequency (TID or BID). ANCOVA models will be used to compare the change from baseline primary efficacy variables. The ANCOVA models will include terms for baseline value, baseline inferior corneal staining score (ICCS) and baseline eye dryness score as covariates and treatment (AXR-159 or vehicle) as a factor in the model. Pairwise comparisons will be performed for each AXR-159 treatment group versus vehicle using t-tests of the least square means from this model. Two-sided confidence intervals (95%) will be provided for the differences between treatments. As supportive analyses, two-sample t-tests and Wilcoxon rank sum tests will also be conducted.

Details of additional supportive primary efficacy analyses will be provided in the statistical analysis plan.

12.4.6 Secondary Efficacy Analyses

Continuous secondary efficacy variables (see Section 8.1.2) and changes from baseline in these measures will be summarized as described in Section 12.4.1, and analyzed by visit using ANCOVA as described for the primary efficacy analyses.

Binary clinical cure variables representing clinical cure will be defined by visit for TBUT ≥ 10, Schirmer's test ≥ 10 mm, Total OSDI < 18, and Ocular Discomfort Score $= 0$. These binary variables will be summarized as described in Section 12.4.1. Additionally, within each treatment group, shift tables for complete treatment response (yes/no) in the study eye relative to baseline will be presented for each variable.

The Cochran-Mantel-Haenszel test, stratified by baseline inferior corneal staining score (ICCS, > 1.5 and ≤ 1.5) and eye dryness score (≥ 60 and < 60) will be used to compare treatments with respect to the proportion of cured patients. Pairwise comparisons will be performed for each AXR-159 treatment group versus vehicle. Two-sided confidence intervals (95%) will be provided for the differences between treatments. The confidence intervals will be constructed using the normal approximation to the binomial distribution.

The secondary efficacy analysis will use the mITT population with observed data only.

Details of additional secondary efficacy analyses will be provided in the statistical analysis plan.

12.4.7 Safety Variables

Safety measures will be analyzed using the safety population. Medical Dictionary for Regulatory Activities (MedDRA) nomenclature will be used to code adverse events. Incidence rates of each treatment-emergent adverse event will be summarized by primary system organ class and preferred term. Summary tables will be generated for all treatment-emergent adverse events regardless of causality as well as for those considered to be treatment-related.

12.4.8 Planned Interim Analyses

An interim analysis will be performed when the last enrolled patient in Stage 1 completes Month 3 of the Expansion Cohort. There is no plan to stop the study for efficacy on the basis of the interim analysis. However, termination in individual dose groups due to safety, tolerability and/or futility may occur. Statistical significance will not be declared at the time of the interim analysis.

A final database lock will occur at the completion of Stage 2 and the safety, tolerability, and efficacy data from both Stage 1 and 2 will be analyzed separately and in a combined fashion to fully leverage the advantage of this two-stage study design. At the final analysis, statistical significance will be declared for two-sided p-values < 0.05.

Given the exploratory nature of the study no adjustments for multiplicity will be applied across the primary or secondary endpoints.

12.4.9 Sub-Group Analyses

Patients will be stratified by the Pre-CAE® inferior corneal staining score (ICCS) (i.e., ≤ 1.5 or > 1.5) according to the Ora Calibra® staining scale and eye dryness score (i.e., < 60 or ≥ 60) in the study eye.

Subgroup analyses are planned for the 4 groups defined by the 2 stratification factors (Pre-CAE®):

1. inferior corneal staining score (ICCS) ≤ 1.5 according to the Ora Calibra® staining scale and eye dryness score < 60
2. inferior corneal staining score (ICCS) ≤ 1.5 according to the Ora Calibra® staining scale and eye dryness score ≥ 60
3. inferior corneal staining score (ICCS) > 1.5 according to the Ora Calibra® staining scale and eye dryness score < 60
4. inferior corneal staining score (ICCS) > 1.5 according to the Ora Calibra® staining scale and eye dryness score ≥ 60.

12.4.10 Tabulation of Individual Participant Data

Individual participant data will be listed by measure and time point.

12.4.11 Exploratory Analyses

The statistical analysis plan (SAP) will provide a detailed description of the planned exploratory statistical analysis.

12.4.12 Pharmacokinetics Analyses

A model independent approach will be used to calculate the pharmacokinetic parameters of AXR-159 in blood. Pharmacokinetic parameters for blood will only be calculated for samples collected from patients who receive study treatment (Stage 1, the first 40 patients in the expansion cohort). To enable only bioanalysis of samples from patients who receive active AXR-159 treatment, the randomization codes will be made available based

on AxeroVision Inc. internal procedures. Pharmacokinetic parameters including area under the curve from time 0 to t (AUC_{0-t}), AUC from time 0 to infinity ($AUC_{0-\infty}$), C_{max}, time to highest observed concentration (T_{max}), and half-life ($T_{1/2}$) will be calculated when possible.

The concentration of AXR-159 in blood and tear samples will be summarized by dose and timepoint using descriptive statistics, if applicable.

13 COMPLIANCE WITH GOOD CLINICAL PRACTICES, ETHICAL CONSIDERATIONS, AND ADMINISTRATIVE ISSUES

This study will be conducted in compliance with the protocol, current Good Clinical Practices (GCPs), including the International Conference on Harmonisation (ICH) Guidelines, and in general, consistent with the Declaration of Helsinki. In addition, all applicable local, state, and federal requirements relevant to the use of IP in the countries involved will be adhered to.

13.1 Protection of Human Subjects

13.1.1 Subject Informed Consent

Informed consent/assent must take place before any study specific procedures are initiated. Signed and dated written informed consent must be obtained from each subject and/or from the subject's parent or legal guardian prior to enrollment into the study. If the subject is under the legal age of consent, the consent form must be signed by a legal guardian or as required by state and/or local laws and regulations.

All informed consent/assent forms must be approved for use by the sponsor and receive approval/favorable opinion from an IRB/IEC prior to their use. If the consent form requires revision (e.g., due to a protocol amendment or significant new safety information), it is the investigator's responsibility to ensure that the amended informed consent is reviewed and approved by Ora prior to submission to the governing IRB/IEC and that it is read, signed and dated by all subjects subsequently enrolled in the study as well as those currently enrolled in the study.

If informed consent is taken under special circumstances (oral informed consent), then the procedures to be followed must be determined by Ora and/or sponsor and provided in writing by Ora and/or sponsor prior to the consent process.

13.1.2 Institutional Review Board (IRB) Approval

This study is to be conducted in accordance with IRB regulations (U.S. 21 CFR Part 56.103). The investigator must obtain appropriate IRB approval before initiating the study and re-approval at least annually.

Only an IRB/IEC approved version of the informed consent form will be used.

13.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that originated with the Declaration of Helsinki.

13.3 Subject Confidentiality

All personal study subject data collected and processed for the purposes of this study should be maintained by the investigator and his/her staff with adequate precautions as to ensure that the confidentiality of the data is in accordance with local, state, and federal laws and regulations.

Monitors, auditors and other authorized representatives of Ora, the sponsor, the IRB/IEC approving this study, the FDA, the DHHS, other domestic government agencies, and other foreign regulatory agencies will be granted direct access to the subject's original medical and study records for verification of the data and/or clinical trial procedures. Access to this information will be permitted to the aforementioned individuals to the extent permitted by law.

A report of the results of this study may be published or sent to the appropriate health authorities in any country in which the IP may ultimately be marketed, but the subject's identity will not be disclosed in these documents.

13.4 Documentation

Source documents may include a subject's medical records, hospital charts, clinic charts, the investigator's study subject files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and EKGs. The investigator's copy of the CRFs serves as the investigator's record of a subject's study-related data.

13.4.1 Retention of Documentation

All study related correspondence, subject records, consent forms, record of the distribution and use of all IP, and copies of CRFs should be maintained on file for at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region; or until at least two years have elapsed since the formal discontinuation of clinical development of the IP. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian.

13.5 Labeling, Packaging, Storage, Accountability, and Return or Disposal of Investigational Product

13.5.1 Labeling/Packaging

Each vial is embossed with lot-specific information on the vial tab. The vials are produced as a card of five (5) vials with each card being packaged in hermetically sealed aluminum foil pouches containing an identifying label on the pouch exterior. Ten (10) pouched cards are further packaged into a paper board carton comprising a two week supply of drug product. The carton has an identifying label with lot-specific information on its exterior.

13.5.2 Storage of Investigational Product

The IP must be stored at refrigerated temperature (2-8 ºC) in its original packaged kit until dispensed to the patient in a secure area accessible only to the investigator and his/her designees. The IP will be administered only to subjects entered into the clinical study, in accordance with the conditions specified in this protocol.

13.5.3 Accountability of Investigational Product

The IP is to only be prescribed by the principal investigator or his/her named sub-investigator(s), and is to only be used in accordance with this protocol. The IP must only be distributed to subjects properly qualified under this protocol to receive IP.

The investigator must keep an accurate accounting of the IP received from the supplier. This includes the amount of IP dispensed to subjects, amount of IP returned to the investigator by the subjects, and the amount returned or disposed upon the completion of the study. A detailed inventory must be completed for the IP.

13.5.4 Return or Disposal of Investigational Product

All IP will be returned to the sponsor or their designee or destroyed at the study site. The return or disposal of IP will be specified in writing.

13.6 Recording of Data on Source Documents and Case Reports Forms (CRFs)

The investigator is responsible for ensuring that study data is completely and accurately recorded on each subject's CRF, source document, and all study-related material. All study data should also be attributable, legible, contemporaneous, and original. Recorded datum should only be corrected in a manner that does not obliterate, destroy, or render illegible the previous entry (e.g., by drawing a single line through the incorrect entry and writing the revision next to the corrected data). An individual who has corrected a data entry should make clear who made the correction and when, by adding to the correction his/her initials as well as the date of the correction.

Data entry of all enrolled and randomized subjects will use software that conforms to 21 CFR Part 11 requirements, and will be performed only by staff who have been trained on the system and have access to the system. Data will not be entered for screen failure subjects. An audit trail will be maintained within the electronic system to capture all

changes made within the eCRF database. After the end of the study and database lock, compact discs (CDs) containing copies of all applicable subjects' eCRFs will be provided to each Investigator Site to be maintained on file by the Investigator.

13.7 Handling of Biological Specimens

13.7.1 Handling of Biological Specimens

All samples will be returned to AxeroVision Inc. or AxeroVision Inc. designee at the completion of the study. Axer0Vision Inc. shall have full ownership rights to any biological specimens/samples derived from the study.

13.7.2 Blood and Urine Samples for Safety Analysis

Samples of blood (non-fasting) will be evaluated for blood chemistry, and hematology [including complete blood count with differential and HbA1c] at a centralized clinical laboratory (ie, ICON Central Laboratory Services, Whitesboro, New York, USA) with certification from a recognized accreditation agency. Details of sample collection are found in the procedure manual.

13.7.3 Blood Samples for Pharmacokinetic Analysis

Blood samples obtained at the study sites will be analyzed for AXR-159 concentrations by an AxeroVision Inc. qualified laboratory (i.e., Intertek Pharmaceutical Services, El Dorado Hills, California, USA) using a validated method. This laboratory has been qualified by AxeroVision Inc.'s Bioanalytical Sciences department to conduct analysis of clinical samples. Details of sample collection and handling are found in the procedure manual.

13.7.4 Tear Samples

Details of sample collection and handling are found in the procedure manual.

All samples will be stored at the clinical site or at ICON until shipment to the bioanalytical laboratory.

13.7.5 Key Roles and Study Governance

The name and contact information of the Principal Investigator and the DRC Chair follow:

Principal Investigator	**DRC Chair**
Gail L. Torkildsen, M.D.	Achim Krauss, Ph.D.
Andover Eye Associates	AxeroVision Inc.
138 Haverhill St, Suite 104 Andover, MA 01810	5857 Owens Ave., Suite 300 Carlsbad, CA 92008
978-475-0705	484-238-6255
mdlasik@comcast.net	Achim@axerovision.com

13.7.6 Safety Oversight

Safety oversight will be under the direction of a DRC composed of individuals with the appropriate expertise (see Section 6.3). Members of the DRC should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DRC will meet at least monthly to assess safety and efficacy data on each arm of the study. The DRC will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DRC. At this time, each data element that the DRC needs to assess will be clearly defined. The DRC will provide its input to AxeroVision Inc., the IRB and regulatory authorities as necessary.

13.7.7 Clinical Monitoring

A representative of Ora, Inc. will monitor the study on a periodic basis. The determination of the extent and nature of monitoring will be based on considerations such as the objective, purpose, design, complexity, blinding, size, and endpoints of the study.

Authorized representatives of AxeroVision Inc., Ora, Inc., or regulatory authority representatives will conduct onsite visits to review, audit, and copy study-related documents. These representatives will meet with the investigator(s) and appropriate staff at mutually convenient times to discuss study-related data and questions.

13.7.8 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.8 Publications

AxeroVision Inc. as the sponsor, has proprietary interest in this study. Authorship and manuscript composition will reflect joint cooperation between multiple investigators and sites and AxeroVision Inc. personnel. Authorship will be established prior to the writing of the manuscript. As this study involves multiple centers, no individual publications will be allowed prior to completion of the final report of the multicenter study except as agreed with AxeroVision Inc.

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15 APPENDICES

APPENDIX 1: SCHEDULE OF VISITS AND MEASUREMENTS

Procedure	Visit 1 Day -14±2		Visit 2 Day 1	Visit 3 Day 15± 2	Visit 4 Day 43± 2	Visit 5 Day 85 ± 2	
	Pre CAE®	Post CAE®	Non CAE®	Non CAE®	Non CAE®	Pre CAE®	Post CAE®
Informed Consent / HIPAA	X						
Medical / Medication History and Demographics	X						
Medical / Medication Update			X	X	X	X	
Vehicle Run-in Collection			X				
Study Drug Collection			X	X	X	X	
Review of Qualification Criteria	X	X	X				
Adverse Event Query		X	X	X	X	X	X
Pregnancy Test	X[a]					X[a]	
Visual Analog Scale	X	X	X	X	X	X	X
Ora Calibra® Ocular Discomfort Scale	X	X	X	X	X	X	X
SANDE Questionnaire	X		X	X	X	X	
OSDI© Questionnaire	X		X	X	X	X	
Visual Acuity (ETDRS)	X		X	X	X	X	
Vital Signs	X		X			X	
Bulbar Conjunctival Hyperemia	X	X	X	X	X	X	X
Slit-lamp Biomicroscopy	X	X	X	X	X	X	X
Tear Collection (Selected Sites)			X			X	
Oculus Keratograph 5®	X[b]		X[b]	X[b]	X[b]	X[b]	
TFBUT	X	X	X	X	X	X	X
Fluorescein Staining (Ora & Oxford Scales)	X	X	X	X	X	X	X
Lissamine Green Staining (Oxford Scale)	X	X	X	X	X	X	X
15 Minute Wait Period	X		X	X	X	X	
Schirmer's Test	X		X	X	X	X	
CAE® Exposure	X					X	
Discomfort Grading during CAE® Exposure	X					X	
Intraocular Pressure		X	X	X	X		X
Ophthalmoscopy Exam		X[c]					X[c]
CBC and Differential Blood Draw		X					X
Pharmacokinetic Blood Draw			X[d]		X[d]		
Randomization			X				
Vehicle Run-In Dispensation		X					
Vehicle Run-In Instillation		X					
Study Drug Dispensation			X	X	X		
Study Drug Instillation			X	X	X		X

Procedure	Visit 1 Day -14±2		Visit 2 Day 1	Visit 3 Day 15± 2	Visit 4 Day 43± 2	Visit 5 Day 85 ± 2	
	Pre CAE®	Post CAE®	Non CAE®	Non CAE®	Non CAE®	Pre CAE®	Post CAE®
Ora Calibra® Drop Comfort Assessment			X		X		X
Exit Subject from Study							X

[a] To women of child-bearing potential, as defined.
[b] TFBUT and Tear meniscus height only; only at selected sites

[c] Ophthalmoscopy examination will be dilated at randomization and undilated for Visit 5 unless dilation is necessary
[d] Blood samples for pharmacokinetic analysis will only be collected for Stage 1, the first 40 patients in the expansion cohort as follows: Visit 2: pre-dose, Visit 4: pre-dose, 15 min, 30 min, 60 min, 90 min, and 3 hr, & 6 hr post dose.

APPENDIX 2: EXAMINATION PROCEDURES, TESTS, EQUIPMENT, AND TECHNIQUES

Visual Acuity Procedures (ETDRS Chart)

LogMAR visual acuity (VA) must be assessed using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart. The procedure used will be consistent with the recommendations provided for using the ETDRS eye chart. VA should be evaluated at the beginning of each visit in the study (i.e., prior to slit-lamp examination). VA testing should be done with most recent correction.

Equipment

The VA chart to be used is the ETDRS chart. If smaller reproduction (18" by 18", e.g., from Prevent Blindness) wall charts are used, the subject viewing distance should be exactly 10 feet (or as specified by the manufacturer). In ALL cases, for purposes of standardizing the testing conditions during the study, all sites must use only the 'R' charts, and the right eye should be tested first. For reflectance (wall) charts, the chart should be placed frontally and well-illuminated.

Measurement Technique

The chart should be at a comfortable viewing angle. The right eye should be tested first. The subject should attempt to read each letter, line-by-line, left to right, beginning with line 1 at the top of the chart. The subject should be told that the chart has letters only, no numbers. If the subject reads a number, he or she should be reminded that the chart contains no numbers, and the examiner should then request a letter in lieu of the number. The subject should be asked to read slowly, so as to achieve the best identification of each letter. He/she is not to proceed to the next letter until he/she has given a definite response.

If the subject changes a response (e.g., that was a "C" not an "O") before he has read aloud the next letter, then the change must be accepted. If the subject changes a response having read the next letter, then the change is not to be accepted. The examiner should never point to the chart or to specific letters on the chart during the test.

A maximum effort should be made to identify each letter on the chart. When the subject says he or she cannot read a letter, he or she should be encouraged to guess. If the subject identifies a letter as 1 of 2 letters, he or she should be asked to choose 1 letter and, if necessary, to guess. When it becomes evident that no further meaningful readings can be made, despite encouragement to read or guess, the examiner should stop the test for that eye. However, all letters on the last line should be attempted as letter difficulties vary and the last may be the only one read correctly. The number of letters missed or read incorrectly should be noted.

LogMAR Visual Acuity Calculations

The last line in which a letter is read correctly will be taken as the base logMAR reading. To this value will be added the number "N x 0.02" where 'N' represents the total number of letters missed up to and included in the last line read. This total sum represents the logMAR VA for that eye.

For example: Subject correctly reads 4 of 5 letters on the 0.2 line, and 2 of 5 letters on the 0.1 line.

Base logMAR	= 0.1
N (total number of letters incorrect on line 0.2 as well as 0.1)	= 4
N x T (T=0.02)	= 0.08
Base logMAR + (N x T)	= 0.1 + 0.08
logMAR VA	= 0.18

Repeat the procedure for the left eye.

In order to provide standardized and well-controlled assessments of VA during the study, all VA assessments at a single site must be consistently done using the same lighting conditions and same correction if possible during the entire study. If the same correction cannot be used (i.e., a subject forgets his glasses), the reason for the change in correction should be documented.

Slit Lamp Biomicroscopy Procedures

Slit lamp biomicroscopic observations will be graded as Normal or Abnormal. Abnormal findings will be categorized as clinically significant (findings that may interfere with study parameters or otherwise confound the data as determined by the investigator) or not clinically significant (NCS). The following will be examined:

- Cornea
- Conjunctiva
- Anterior Chamber
- Iris
- Lens
- Eyelid

External magnification and biomicroscopy will be performed using a slit-lamp. Magnification will be consistent with standard clinical practice. The subject will be seated.

Fundus Exams

Dilated fundus exams will be performed at Visit 1 using indirect ophthalmoscopy. At visit 5, undilated fundus exams will be performed unless dilation is necessary. The investigator will make observations of the vitreous, retina, macula, choroid and optic nerve.

Observations will be graded as Normal or Abnormal. Abnormal findings that are clinically significant (as determined by the investigator that may interfere with study parameters or otherwise confound the data) and those that are not clinically significant will be described. An indirect Fundoscopy examination should be performed if retinal disease is detected.

- Vitreous: Examination should emphasize the visual axis.
- Retina, Macula, Choroid: Include an observation of the retina and its blood vessels. Eyes should be excluded from the study if active inflammation is present.
- Optic Nerve: Significant damage or cupping to the optic nerve should be noted.

It is recommended that tropicamide 1% ophthalmic solution be used to dilate subjects.

Vital Signs

Each subject will have vital signs assessments (resting blood pressure and pulse) conducted at Visits 1, 2, and 5. Vital signs are to be conducted by a qualified staff member who may be any of the following: a board-certified investigator or sub-investigator, nurse practitioner, registered nurse, or physician assistant.

Systolic/Diastolic Blood Pressure (mmHg)

Systolic and diastolic blood pressure should be measured in the same arm each time using a sphygmomanometer with the subjects who have been in a resting state (seated upright) at least 5 minutes. Blood pressure will be recorded in mmHg.

Pulse (bpm)

Pulse will be measured with the subjects who have been in a resting state (seated) for at least 5 minutes. Pulse will be counted for 30 seconds and multiplied by 2, and recorded in beats per minute (bpm).

Intraocular Pressure

Intraocular pressure (IOP) will be measured in each eye by contact tonometry by the examiner and the results will be recorded in mmHg. A single measurement is made to obtain a determination of IOP. The same tonometer employing the investigator's standard technique will be used throughout the study. In addition, all reasonable efforts will be made to have the same examiner obtain all IOP measurements for a given subject.

Ora proprietary scales – Not for distribution without permission

Ora Calibra® Conjunctival Redness Scale for Dry Eye

This procedure will be performed according to Ora, Inc. SOPs and/or guidance documents.

None	0 = Normal, without vasodilation
Trace	1 = Trace ciliary or conjunctival vasodilation
Mild	2 = Broad ciliary vasodilation;
Moderate	3 = Broad ciliary and slight, horizontal conjunctival vasodilation
Severe	4 = Broad ciliary and prominent, horizontal conjunctival vasodilation
Half (0.5) unit increments are allowed.	

Ora Calibra® Ocular Discomfort Scale for Dry Eye

This procedure will be performed according to Ora, Inc. SOPs and/or guidance documents.

Ocular discomfort scores will be subjectively graded by the subjects according to the following scale, rating each eye separately.

0	**No discomfort**
1	**Intermittent awareness**
2	**Constant awareness**
3	**Intermittent discomfort**
4	**Constant discomfort**

Ora Calibra® Ocular Discomfort & 4-Symptom Questionnaire for Dry Eye

This procedure will be performed according to Ora, Inc. SOPs and/or guidance documents.

Subjects will rate the severity of each of the following symptoms, with regard to how both their eyes feel, in general – overall ocular discomfort, burning, dryness, grittiness and stinging according to the following 6-point (0 to 5) scale where 0 = none and 5 = worst.

0	1	2	3	4	5
(None)					(Worst)

Ocular Surface and Disease Index (OSDI) © for Dry Eye

Ocular Surface Disease Index© (OSDI©)[2]

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

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

Subtotal score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?.	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?.	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9 (B)

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?.	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?. . .	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

Add subtotals A, B, and C to obtain D
(D = sum of scores for all questions answered) (D)

Total number of questions answered
(do not include questions answered N/A) (E)

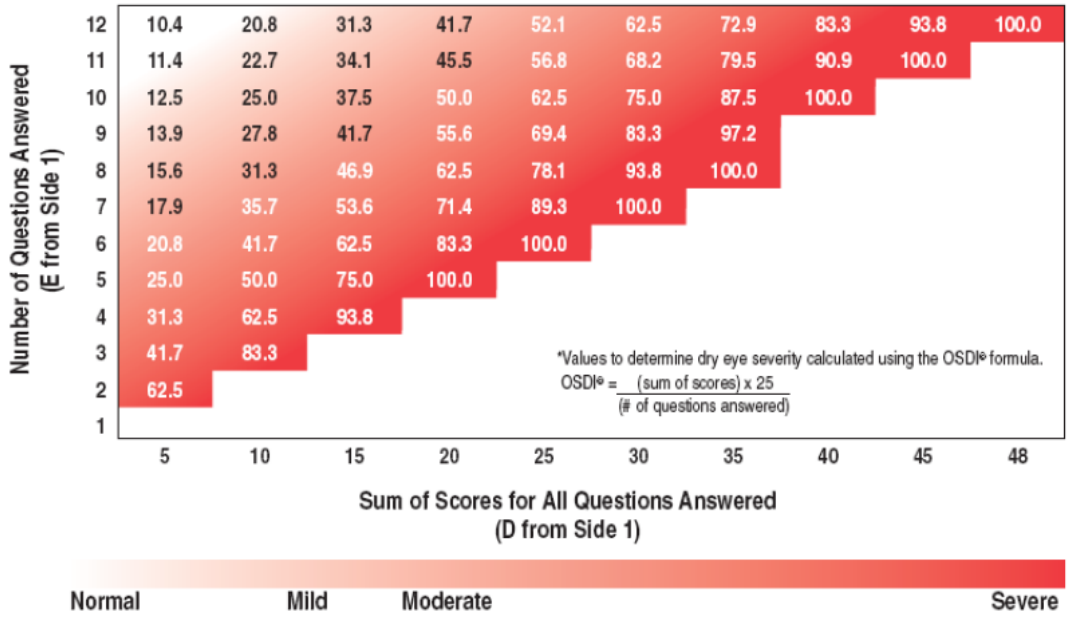
Please turn over the questionnaire to calculate the patient's final OSDI® score.

Evaluating the OSDI® Score[1]

The OSDI® is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI® is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.

Assessing Your Patient's Dry Eye Disease[1, 2]

Use your answers D and E from side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below.* Find where your patient's score would fall. Match the corresponding shade of red to the key below to determine whether your patient's score indicates normal, mild, moderate, or severe dry eye disease.



1. Data on file, Allergan, Inc.
2. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615-621

Tear Film Break-Up Time (TFBUT) ©

The examiner will instill 5 µL of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. To thoroughly mix the fluorescein with the tear film, the subject will be instructed to blink several times. In order to achieve maximum fluorescence, the examiner should wait approximately 30 seconds after instillation before evaluating TFBUT.

With the aid of a slit-lamp, the examiner will monitor the integrity of the tear film, noting the time it takes to form micelles from the time that the eye is opened. TFBUT will be measured in seconds using a stopwatch and a digital image recording system for the right eye followed by the left eye. A Wratten #12 yellow filter will be used to enhance the ability to grade TFBUT.

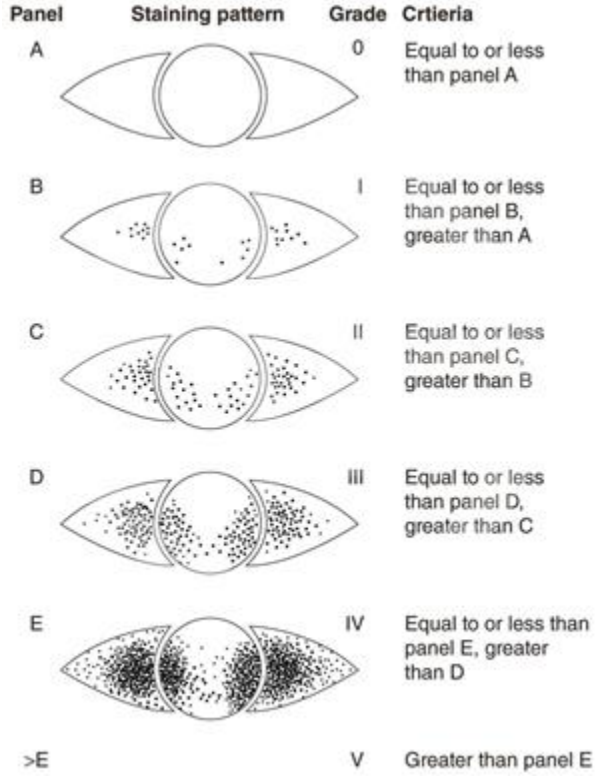
For each eye, 2 measurements will be taken and averaged unless the 2 measurements are > 2 seconds apart and are each < 10 seconds, in which case, a third measurement would be taken and the 2 closest of the 3 would be averaged.

Fluorescein Staining

The examiner will instill 5 µL of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. In order to achieve maximum fluorescence, the examiner should wait approximately 3-5 minutes after instillation before evaluating fluorescein staining. A Wratten #12 yellow filter will be used to enhance the ability to grade fluorescein staining. The staining will be graded with the Oxford Scale and Ora Calibra® Scale.

Oxford Grading Scale

Fluorescein staining is represented by punctate dots on a series of panels (A-E). Staining ranges from 0-5 for each panel and 0-10 for the total exposed inter-palpebral conjunctiva. Inferior, superior, and Central regions will be graded separately. The dots are ordered on a log scale. Conjunctiva: temporal zone grading performed when the subject looks nasally; grading nasally by looking temporally.


Panel
Staining pattern
Grade
Crtieria
A
0
Equal to or less than panel A
B
I
Equal to or less than panel B, greater than A
C
II
Equal to or less than panel C, greater than B
D
III
Equal to or less than panel D, greater than C
E
IV
Equal to or less than panel E, greater than D
>E
V
Greater than panel E

Ora proprietary scale – Not for distribution without permission

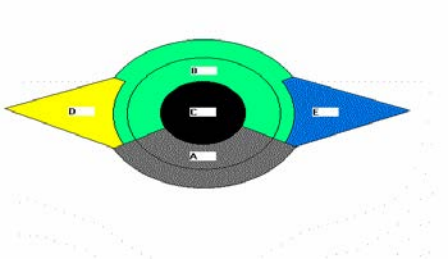
Ora Calibra® Corneal and Conjunctival Staining Scale for Grading of Fluorescein Staining

This procedure will be performed according to Ora, Inc. SOPs and/or guidance documents.

The following scale will be used to grade staining of the ocular surface (areas A, B, C, D, and E). Half (0.5) grade increments may be used.

None	0 = no staining
Trace	1 = occasional
Mild	2 = countable
Moderate	3 = uncountable, but not confluent
Severe	4 = confluent

Staining areas:



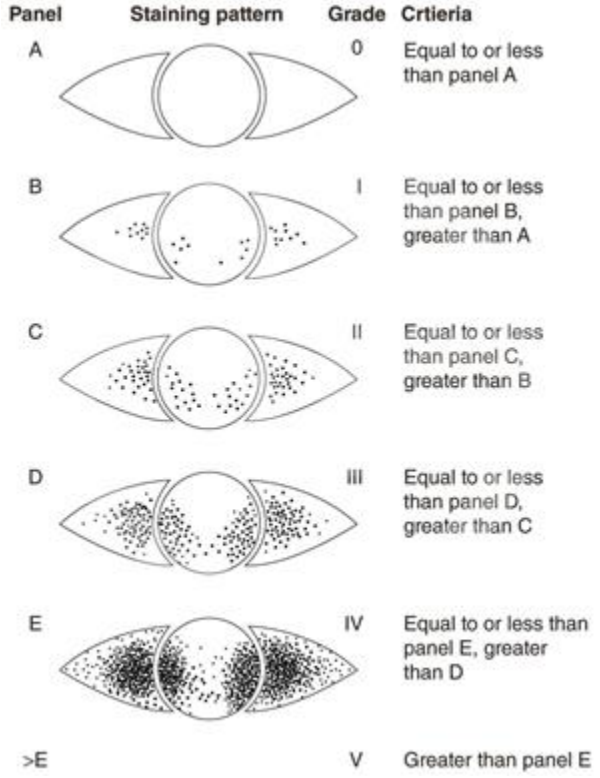
Staining Areas	Ocular Structure	Position
A – Inferior	Cornea	4-8 o'clock, extending 2 mm onto the conjunctiva
	Limbus/Conjunctiva	4-8 o'clock, extending 2 mm towards the center
B – Superior	Cornea	8-4 o'clock, extending 3 mm onto the conjunctiva
	Limbus/Conjunctiva	8-10 o'clock and 2-4 o'clock, extending 1 mm onto the conjunctiva; 10-2 o'clock, extending 2 mm onto the conjunctiva
C – Central	Cornea	Central cornea
D – Temporal	Conjunctiva	Triangular wedge of temporal conjunctiva
E – Nasal	Conjunctiva	Triangular wedge of nasal conjunctiva

Lissamine Green Staining

The Investigator will instill 10 µL of lissamine green solution into the inferior conjunctival cul-de-sac or insert lissamine strips and wait approximately 30 seconds before evaluating staining. The subject will be instructed to blink several times to distribute the lissamine green. The staining will be graded with Oxford Scale.

Oxford Grading Scale

Lissamine staining is represented by punctate dots on a series of panels (A-E). Staining ranges from 0-5 for each panel and 0-10 for the total exposed inter-palpebral conjunctiva. Both nasal and temporal regions will be graded separately. The dots are ordered on a log scale. Conjunctiva: temporal zone grading performed when the subject looks nasally; grading nasally by looking temporally.



Unanesthetized Schirmer's Test

Schirmer Tear Test will be performed according to the following procedure:

- Using a sterile Tear Flo Schirmer test strip (Rose Enterprises), a bend in the strip will be made in line with the notch in the strip.
- The subject will be instructed to gaze up and in.
- The Schirmer test strip will be placed in the lower temporal lid margin of each eye such that the strip fits tightly. Subjects will be instructed to close their eyes.
- After 5 minutes have elapsed, the Schirmer strip will be removed. The length of the moistened area will be recorded (mm) for each eye.

Visual Analog Scale (VAS)

Subjects will be asked the following questions regarding ocular discomfort (unrelated to study drug instillation) at all visits. The assessment period will include the previous week as well as Post-CAE.

The subject will be asked to rate each ocular symptom due to ocular dryness by placing a vertical mark on the horizontal line to indicate the level of discomfort. 0% corresponds to "no discomfort" and 100% corresponds to "maximal discomfort".

Burning/Stinging	0%	50%	100%
Itching	0%	50%	100%
Foreign body sensation	0%	50%	100%
Blurred Vision	0%	50%	100%
Eye Dryness	0%	50%	100%
Photophobia	0%	50%	100%
Pain	0%	50%	100%

Symptom Assessment in Dry Eye (SANDE)

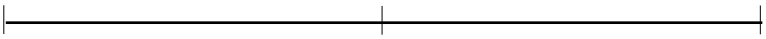
Subjects will be asked to:
PLEASE COMPLETE THE FOLLOWING QUESTIONS RELATED TO YOUR DRY EYE SYMPTOMES:

Place a vertical line to indicate how often, on average, your eyes feel dry and/or irritated.

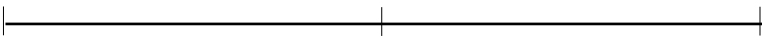
Place a vertical line to indicate how severe, on average, you feel your symptoms of dryness and/or irritation.

The assessment line length of the scale will be 100 mm and will be similar to the following depiction:

1. Frequency Rarely All the time



2. Severity Very Mild Very Severe



APPENDIX 3: HANDLING OF BIOLOGICAL SPECIMENS

Blood sample will be collected for hematology (3 mL per draw), serum chemistry (4 mL per draw), pharmacokinetics (6 mL per draw + 5mL for heparin/saline flush for post-dose draws, 6 mL per draw for the pre-dose V2 and V4 draw. The approximate collection amounts per visit are described below:

	Approximate Blood Collection Amounts per Visit			
	Visit 1	Visit 2	Visit 4	Visit 5
Hematology	3mL	N/A	N/A	3mL
Serum Chemistry	4mL	N/A	N/A	4mL
PK	N/A	6mL	71mL	N/A
Total	**7mL**	**6mL**	**71mL**	**7mL**

- Hematology includes leucocytes, erythrocytes, Hgb, Hematocrit (Hct), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), thrombocytes, and partial automated differentiation: lymphocytes, neutrophils, monocytes, eosinophils, and basophils.
- Serum Chemistry includes sodium (Na), potassium (K), chloride (Cl), bicarbonate (CO_2), calcium (Ca), phosphorus (P), blood urea nitrogen (BUN), creatinine, albumin, globulin, glucose, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (Alk Phos), gamma GT, and total bilirubin (any total bilirubin > ULN should be fractionated for direct bilirubin).
- Urinalysis includes appearance, glucose, ketones, Hgb, protein, nitrate, bilirubin, specific gravity, hydrogen ion concentration (pH), urobilinogen, and leucocytes. If positive for blood or protein trace, then microscopy will be included.
- Pharmacokinetics testing for AXR-159.

APPENDIX 4: PROTOCOL AMENDMENT SUMMARY

Not applicable.

APPENDIX 5: SPONSOR AND ORA APPROVALS

Protocol Title:	A Multicenter, Vehicle-controlled, Randomized Study to Evaluate the Safety, Tolerability, Systemic Pharmacokinetics, and Pharmacodynamics of AXR-159 Ophthalmic Solution 3 mg/mL, 30 mg/mL, and 50 mg/mL in Patients with Dry Eye Disease (DED)
Protocol Number:	AXR201701
Final Date:	February 17, 2018

This clinical study protocol was subject to critical review and has been approved by the sponsor. The following personnel contributed to writing and/or approving this protocol.

Signed: _____ Date: 6/19/2018
Achim Krauss, Ph.D.
Chief Executive Officer
AxeroVision Inc.

Signed: _____ Date: 19 JUNE 18
Charles Bosworth Ph.D.
Vice President, Head of Clinical Development
AxeroVision Inc.

Signed: _____ Date: 16 May 18
George Ousler
Vice President, Dry Eye
Ora Inc.

DocuSigned by David Hollander
I approve this document
6/19/2018 10:56:34 AM PDT
Signed: _____ Date: 6/19/2018
David Hollander, M.D., MBA
2D79ACABD19D58AD442
Chief Medical Officer
Ora Inc.

APPENDIX 6: INVESTIGATOR'S SIGNATURE

Protocol Title:	A Multicenter, Vehicle-controlled, Randomized Study to Evaluate the Safety, Tolerability, Systemic Pharmacokinetics, and Pharmacodynamics of AXR-159 Ophthalmic Solution 3 mg/mL, 30 mg/mL, and 50 mg/mL in Patients with Dry Eye Disease (DED)
Protocol Number:	AXR201701
Final Date:	February 17, 2018

I agree to implement and conduct the study diligently and in strict compliance with the protocol, good clinical practices and all applicable laws and regulations. I agree to maintain all information supplied by Ora and the sponsor in confidence and, when this information is submitted to an Institutional Review Board (IRB), Ethical Review Committee (ERC) or another group, it will be submitted with a designation that the material is confidential.

I have read this protocol in its entirety, including the above statement, and I agree to all aspects.

Signed: _____ Date: 10 MAY 18

Gail Torkildsen, M.D
Principal Investigator
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Doctor's Park II Suite 104
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